Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level 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]

CLH-O-0000007380-79-01/F

Adopted

30 November 2023

30 November 2023

CLH-O-0000007380-79-01/F

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

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted **on 30 November 2023 by consensus** an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **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]**

Rapporteur, appointed by RAC: **Gerlienke Schuur**

Co-Rapporteur, appointed by RAC: **Michael Neumann**

Administrative information on the opinion

Poland has submitted on **17 November 2022** a CLH dossier containing a proposal together with the justification and background information documented in a CLH report.

The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **28 November 2022**.

Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **27 January 2023**.

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

The following table provides a summary of the Current Annex VI entry, Dossier submitter proposal, RAC opinion and potential Annex VI entry if agreed by the Commission.

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

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	Melaleuca alternifolia, ext. [1]*Melaleuca alternifolia*, essential oil; tea tree oil [2]	285-377-1 [1] -	85085-48-9 [1]68647-73-4 [2]	**Add** Flam. Liq. 3 Repr. 2 Acute Tox. 4 Acute Tox. 4 Asp. Tox. 1 Skin Irrit. 2 Skin Sens. 1B Aquatic Acute 1 Aquatic Chronic 3	**Add** H226 H361f H332 H302 H304 H315 H317 H400 H412	**Add** GHS02 GHS08 GHS07 GHS09 Dgr	**Add** 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	Proposed notes or nothing
RAC opinion	TBD	Melaleuca alternifolia, ext. [1]*Melaleuca alternifolia*, essential oil; tea tree oil [2]	285-377-1 [1] -	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	
Resulting Annex VI entry if agreed by COM	TBD	Melaleuca alternifolia, ext. [1]*Melaleuca alternifolia*, essential oil; tea tree oil [2]	285-377-1 [1] -	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	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Tea Tree Oil (TTO) is used as a fungicide, for example on grapes and tomatoes. It is also used for example in health care products, as fragrances and as food flavors. TTO had a long history of safe use in a wide range of cosmetic and human and animal care products (e.g. mouthwash, toothpaste, shampoo, deodorants, lotions, and antifungal treatment).

TTO is a liquid. The mixture has a maximum vapour pressure of 2100 Pa at 25°C. The water solubility is 1420 mg/L. The Log P_{ow} is 3.4-5.5 at 30°C.

Substance identity

Tea tree oil consists of terpene hydrocarbons, mainly monoterpenes, sesquiterpenes and their associated alcohols. Tea tree oil is a UVCB substance and has a complex composition with specification according to ISO 4730:2004 or 2017 (in table below):

Name	CAS No.	EC No.	Min. %	Max. %
Terpinen-4-ol	562-74-3	209-235-5	30	48
γ-Terpinene	99-85-4	202-794-6	10	28
α-Terpinene	99-86-5	202-795-1	5	13
α-Terpineol	98-55-5	202-680-6	1.5	8
α-Terpinolene	586-62-9	209-578-0	1.5	5
α-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
δ-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

Toxicokinetics

No data are available on TTO itself. Based on ADME data on constituents, it might be assumed that TTO is metabolised and excreted in animals within 2-3 days, mainly via urine. No bioaccumulation is expected.

RAC evaluation of physical hazards

Explosives

TTO was examined by Differential Scanning Calorimetry (DSC) over the range 30°C to 400°C, programmed at a rate of 10°C/min. No significant exothermic events occurred during this test, which would indicate that it is very unlikely that a thermally induced explosive reaction is likely to occur with this material. The dossier submitter (DS) has screened the known constituents on the present of chemical groups with explosive properties. None of the constituents contain any of these groups.

The DS proposed no classification based on no likely or realistic possibility of TTO being an explosive hazard.

Flammable liquids

The flash point of TTO was determined according to EC Method A.9 as being 54-55°C at 100.7-102.1 kPa. This temperature is ≥23°C and ≤60°C and the pressure for determination is close to 101.3 kPa, therefore the DS proposed category 3 for flammable liquids, and H226.

Self-reactive substances

No significant exothermic events occurred during DSC testing. The auto-ignition temperature of TTO was tested in two different studies and found to be 252°C and 269 °C according to EC method A.15. The DS proposed no classification based on these data.

Pyrophoric liquids

Pyrophoric properties were tested as part of the Auto flammability test EC A.15. No ignition of TTO or charring of filter paper was observed within 5 min of the TTO. The DS proposed no classification based on these data.

Self-heating substances

Based on the auto-ignition temperature (252°C, 269°C) measured with method EC A.15, DS proposed no classification.

Substances which in contact with water emit flammable gases

Based on the chemical structure of the substance, and the experience in manufacture and handling, the substance does not react with water. No study was performed. DS concluded that the hazard class was not applicable.

Oxidising liquids

Considering each of the individual ingredients of TTO, DS notes that there is no indication of an oxidation hazard. This is supported with the following arguments:

- The ingredients do not contain any oxygen, or the oxygen is chemical bonded to carbon or hydrogen only.
- The ingredients do not contain a group indicating potential oxidising properties (such as peroxide, chlorate, perchlorate, nitrate, bromate, chromate, etc.).
- The major ingredients have a large deficiency in oxygen present, so it is less likely that the mixture will be an oxidising agent.
- In the DSC experiment, with regard to explosive properties, no signs of significant reactions were observed.

Based on the above, the DS concluded that TTO is not an oxidising substance.

Organic peroxides

The hazard class is not applicable as TTO does not contain an organic peroxide.

Corrosive to metals

There is no test available. The DS proposed no classification based on experience in manufacture and handling.

Comments received during consultation

No comments were received regarding physical hazards.

Assessment and comparison with the classification criteria

Explosives

For Explosives, RAC agrees with the assessment of the DS, with the addition that for the TTO constituents containing oxygen it can be reported that their oxygen balance is well below the trigger of -200. RAC concludes that the substance **does not warrant classification** for explosive properties.

Flammable liquids

RAC concurs with the DS's assessment of the physical hazards for Flammable liquids. Based on a flash point of TTO of 54-55 °C at 100.7-102.1 kPa, being ≥23 °C and ≤60 °C and the pressure for determination is close to 101.3 kPa, RAC concludes that the substance warrants a classification **as Flammable liquid category 3, H226**.

Self-reactive substances

DS proposes no classification on the basis of no significant exothermic events occurred during DSC testing as well as on a test according to EC method A.15. According to CLP Regulation, self-reactive properties are tested using UN test series A to H. Based on DSC test as well as on the absence of chemical groups associated with explosives and self-reactive properties, as noted in Annex I 2.8.2.1, there is no need to classify TTO for Self-reactive substances. RAC concludes that the substance **does not warrant classification** for self-reactive properties.

Pyrophoric liquids

DS concluded on no classification on the basis of an EC A.15 test, but according to the CLP regulation this endpoint needs to be assessed with UN RTDG test N.3 test (equivalent to EU A.13). However, the screening procedure (based on experience in manufacturing or handling) can be used to conclude on no classification. Therefore, RAC concludes that the substance **does not warrant classification** for pyrophoricity.

Self-heating substances

Considering the classification, an auto-ignition temperature of 252 to 269°C does not exclude self-heating of a substance. However, in the CLP-guidance, it is indicated that "*In general, the phenomenon of self-heating applies only to solids.*" and that "*Substances or mixtures with a low melting point (< 160 °C) should not be considered for classification in this class since the melting process is endothermic and the substance-air surface is drastically reduced.*". With a melting point of -22°C this applies for TTO and therefore RAC concludes that the substance **does not warrant classification** for self-heating properties.

Substances which in contact with water emit flammable gases

The DS does not indicate how the experience in manufacture and handling leads to the conclusion that the substance does not react with water. However, the OECD TG 105 (Water solubility study, 2007; available in the draft renewal assessment report (DRAR) (Volume 3, B.2)) reports no decomposition for any of the constituents tested when they come in contact with water and

therefore RAC concludes that the substance **does not warrant classification** for this hazard class.

Oxidising liquids

RAC agrees with the argumentation of the first bullet noted by the DS that "*The ingredients do not contain any oxygen, or the oxygen is chemical bonded to carbon or hydrogen only.*" and no classification is warranted. However, the argumentation in the other bullets is not in line with the CLP guidance and is not used by RAC for conclusions. RAC concludes that the substance **does not warrant classification** for oxidising properties.

Corrosive to metals

Considering the classification for corrosive to metals, experience in manufacture and handling is not part of the screening procedure. According to the CLP Guidance (2.16.4.1), "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."

As TTO does not have these properties, RAC concludes that the substance **does not warrant classification** for corrosive properties to metals.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Oral acute toxicity

Three acute oral toxicity studies with TTO are available:

- An OECD TG 425 oral acute toxicity study under GLP was performed in Wistar rats (3 females per group) which resulted in no mortality at 550 mg/kg and death of 3/3 animals at 2000 mg/kg resulting in an LD_{50} of 1049 mg/kg bw (Anonymous, 2015a).
- Anonymous (1989a) is an OECD TG 401 study in SD rats (5 males and 5 females per group), including a SPF (Specific Pathogen-free) and a non-SPF group. The resulting LD_{50} values are 2.6 mL/kg bw in SPF rats and 1.9 mL/kg bw (≈1682-1721 mg/kg bw) in non-SPF rats.
- The registration dossier contains another study (ECHA dissemination site; Anonymous, 2010) performed according to OECD TG 423 and GLP in mice (3 female/group). This resulted in no TTO-related mortalities and an LD_{50} of >2000 mg/kg bw.

LD_{50} values reported for TTO are 1049 mg/kg bw and 1682-1721 mg/kg bw/d in rats, and >2000 mg/kg bw in mice. DS also provided data on acute oral studies with TTO components, namely several monoterpenes. LD_{50} values ranged from 1280–4750 mg/kg bw.
DS concluded on Acute toxicity oral category 4 with an ATE of 1049 mg/kg bw, in accordance with the criteria (300<ATE≤2000 mg/kg bw) based on the LD_{50} of 1049 mg/kg bw.

Dermal acute toxicity

Two acute dermal toxicity studies with TTO are available. A rat study (Anonymous, 2015b) was performed according to OECD TG 402 and GLP, with 2000 mg/kg bw undiluted test item. As it resulted in no clinical signs nor deaths, the LD_{50} was >2000 mg/kg bw.

Another study (Anonymous, 1989b) was performed in New Zealand White rabbits (5 males, 5 females) according to OECD TG 402, with 2000 mg/kg bw undiluted test item. Slight diarrhoea was seen in 1 out of 10 animals. The result is also an LD_{50} of >2000 mg/kg bw.
DS concluded on no classification for dermal toxicity based on the LD_{50} values of >2000 mg/kg bw.

Inhalation acute toxicity

Two acute inhalation toxicity studies with TTO are available. A rat study (Anonymous, 2010a) was performed in Wistar rats (5 males, 5 females) according to OECD TG 403 and GLP. Nose-only exposure to aerosolised TTO diluted in DMSO resulted amongst others in lethargy and nasal discharge at the low and mid dose of 0.77 and 3.69 mg/L, respectively, and in ataxia and dyspnoea at the highest dose of 5.06 mg/L. Mortality was found for 1 animal at the lowest dose, 4 at the mid dose and 7 at the highest dose. This results in an LC_{50} of 3.64 mg/L.

The registration dossier contains another study (ECHA dissemination site; Anonymous, 2011) performed according to OECD TG 403 and GLP in Wistar rats (5 males, 5 females) with nose-only exposure to TTO. This resulted in an LC_{50} of 5.23 mg/L for males, 4.29 mg/L for females and 4.78 mg/L (for both).

DS concluded on Acute toxicity inhalation category 4 based on the LC_{50} value of 3.64 mg/L being between 1.0 and 5.0 mg/L, and with an ATE of 3.64 mg/L.

Comments received during consultation

One MS supported the classification for Acute toxicity and noted that the ATE of Acute Tox. cat. 4; H332 should address dusts and mists.

Assessment and comparison with the classification criteria

Acute oral toxicity

RAC concurs with the assessment of the DS. Based on the lowest available LD_{50} of 1049 mg/kg bw in a reliable rat study, RAC concludes that the substance warrants a classification as **Acute oral Toxicity category 4, H302** with an **ATE of 1050 mg/kg bw**.

Acute dermal toxicity

RAC concurs with the assessment of the DS. Based on the available LD_{50}s of >2000 mg/kg bw, RAC concludes that the substance **does not warrant classification** for **dermal acute toxicity**.

Acute inhalation toxicity

RAC concurs with the assessment of the DS. Based on the lowest available LC_{50} of 3.64 mg/kg bw in a reliable rat study, RAC concludes that the substance warrants a classification as **Acute inhalation Toxicity category 4, H302** with an **ATE of 3.60 mg/L (dusts and mists).**

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

Summary of the Dossier Submitter's proposal

With regards to STOT SE, DS described results from acute toxicity studies by the oral (two), dermal (two) and inhalation route (one).

In the oral acute toxicity study (Anonymous, 2015a), no clinical signs were observed at 550 mg TTO/kg bw during the 14-day observation period. Rats dosed with 2000 mg TTO/kg bw showed hypoactivity and slight tremors before dying on day 1 or 2. In the other oral acute toxicity study (Anonymous, 2010), the major reaction at 2000 mg TTO/kg bw was complete lack of muscular tone in forelimbs, which recovered after a few days.

In the acute dermal toxicity studies (Anonymous, 2015b, 1989b) no clinical signs, effects on body weight or mortality were observed.

The LC_{50} in the acute inhalation study (Anonymous, 2010a) was determined to be 3.64 mg/L. All treated rats (0.77, 3.69 and 5.06 mg/L) showed toxic signs such as nasal discharge, slight salivation, lethargy, tremors, ataxia, dyspnoea, perineum wet with urine, dullness and recumbency. Further, body weight loss was noted for all dead animals. In one pre-terminally dead rat in the highest dose group, lung congestion was observed at necropsy, no histopathological abnormalities were found in other animals.

DS discussed that the described clinical signs after inhalation exposure were observed in rats treated with 3.69 mg/L which is close to (above) the LC_{50}. For that reason, the DS concluded that no classification for STOT SE category 1 or 2 is needed.

With regards to category 3 (respiratory tract irritation or narcotic effects), the DS noted that no human data is available for TTO. The dyspnoea that was seen was closely related to the general acute inhalation toxicity, and therefore covered by Acute Tox cat. 4. So, no classification is proposed for STOT SE.

Comments received during consultation

No comments with regards to STOT-SE were received.

Additional key elements

In the Table below an overview is provided on reported clinical signs in the available acute toxicity studies. Rows in italics are data present in the DRAR but not in the CLH report. The reported clinical signs are not consistent between the different studies.

Route/ species	Clinical signs	Mortality	LD_{50} or LC_{50}	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

Route/ species	Clinical signs	Mortality	LD_{50} or LC_{50}	Reference
Oral, rat	*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*	*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*	*(2.6 in SPF and 1.9 mL/kg bw in non-SPF rats)* *1682-1721 mg/kg bw*	*Anonymous, 1989a*
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
Inhalation, rat	*1.94, 3.70 and 5.04 mg/L: wet fur*		*4.78 mg/L*	*Anonymous, 2011*

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

No human data are discussed in the CLH report. However, the DRAR (Volume 3-B.6, 2022) summarizes a review by Larsen & Borling (2000). Human poisoning cases, relevant for STOT SE, were noted, see Table below. Additionally, two cases from the Swiss Toxicological Information Centre on accidental intakes of remedies from alternative medicine in children (Zuzak et al., 2010) were added.

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

In the DRAR, a publication by Villar et al. (1994) was mentioned, noting TTO toxicity in dogs and cats with as main symptoms depression, weakness, incoordination and muscle tremors. The European Medicines Agency (EMA, 2014) further explained regarding this study that the cats and dogs were treated with TTO for a dermatologic condition at inappropriately high doses. Treatment of the clinical signs was sufficient to achieve recovery. EMA noted another report of TTO poisoning of cats. Three cats were treated with 120 mL of undiluted TTO to eliminate fleas. All animals exhibited hypothermia, incoordination, dehydration and trembling. One of them died, possibly related to a pre-existing renal condition, two recovered after 1 to 2 days.

Assessment and comparison with the classification criteria

With regard to STOT SE category 3 - respiratory tract irritation, dyspnoea is reported in the acute inhalation study. These effects are found at or above the ATE of 3.64 mg/L and are therefore covered by the Acute toxicity classification. Moreover, there are no known cases of respiratory tract irritation in humans to justify classification.

With regard to STOT SE category 3 – narcotic effects, relevant effects including lethargy, tremors and ataxia occurred in one acute inhalation toxicity (animal) study at and below the LC_{50}. In addition to the relevant effects found in the inhalation study, lack of tonus in the forelimbs was observed in two oral acute toxicity studies (mice and rats). The clinical signs are reported at doses with mortality (rats) and without mortality (mice).

Human data should be considered for STOT SE as well, which was not done by the DS. In several cases of acute poisoning symptoms as ataxia, drowsiness, up to a coma were reported. These effects can be considered as depression of the central nervous system. They were however reversible within a short period of time (about 5 hours up to 36 hours), and therefore considered to be transient.

CLP guidance provides useful elements on transient target organ effects and narcotic effects[1]. In acute poisoning cases, drowsiness, lack of coordination and ataxia were noted. Similar effects were reported in poisoning cases in cats and dogs. The symptoms disappear within a short time period. Considering these effects are noted in the CLP guidance as examples of narcotic effects and they are transient in nature, STOT SE 3 is considered more appropriate.

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[1] **Annex I:** *Table 3.8.1 (continued)* ***Categories for specific target organ toxicity-single exposure*** "*Transient target organ effects. This category only includes narcotic effects and respiratory tract irritation. These are target organ effects for which a substance does not meet the criteria to be classified in Categories 1 or 2 indicated above. These are effects which adversely alter human function for a short duration after exposure and from which humans may recover in a reasonable period without leaving significant alteration of structure or function. Substances are classified specifically for these effects as laid down in 3.8.2.2".*
And:
Annex I: ***3.8.2.2.2.*** *Criteria for narcotic effects*
The criteria for classifying substances as Category 3 for narcotic effects are: (a) central nervous system depression including narcotic effects in humans such as drowsiness, narcosis, reduced alertness, loss of reflexes, lack of coordination, and vertigo are included. These effects can also be manifested as severe headache or nausea, and can lead to reduced judgment, dizziness, irritability, fatigue, impaired memory function, deficits in perception and coordination, reaction time, or sleepiness. (b) *narcotic effects observed in animal studies may include lethargy, lack of coordination, loss of righting reflex, and ataxia. If these effects are not transient in nature, then they shall be considered to support classification for Category 1 or 2 specific target organ toxicity single exposure.*

Based on this information, RAC concludes that classification is warranted for **STOT SE 3 – narcotic effects; H336.**

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

An OECD TG 404 dermal irritation/corrosion study in rabbits (Anonymous, 2015c) with 0.5 mL TTO resulted in mean erythema scores of 2.00, 2.00 and 2.67 and mean oedema scores of 1.00 for all animals after 24-76 hours.

A second, non-guideline, non GLP study (Anonymous, 1989c) in six rabbits with undiluted TTO resulted in mean irritation scores of 3.08 and 1.83 for erythema and oedema for intact skin, and 3.25 and 2.0 for erythema and oedema for abraded skin, respectively.

Another study was a Draize skin irritation study (Lee et al., 2013) reporting Draize scores of 1 after 24 and 48 hours exposure to 5 % TTO for both oedema and erythema and 1 and 2 after exposure to 10 % TTO for oedema and erythema, respectively.

DS concluded that TTO should be classified as Skin irritation category 2 (H315) based on mean scores ≥2,3 and ≤4,0 in the second rabbit study.

Comments received during consultation

One MS supported the classification as Skin Irrit. 2; H315.

Additional key elements

Table 26 in the TTO CLH report presented additional data for skin sensitisation. In some studies with human skin patch testing, irritation effects are reported.

Sabroe et al. (2016) reported on 3 out of 2014 patients with irritant reactions (0.1 %) to 5 % TTO in petrolatum. Veien et al. (2004) reported that 44 patients (out of 217) had weak, irritant reactions to a 5 % commercial lotion (20.3 %). Veien et al. (2004) also reported 5 patients (out of 160) with irritant reactions to 5 % TTO commercial lotion.

Skin irritancy in human patch tests is reported in other reviews. In the opinion of the Scientific Committee on Consumer Products (SCCP, 2008), two skin irritation animal studies were reported (seemingly different from the ones presented in the CLH report). Four different human studies were reported, varying between no skin irritation and irritant reactions. SCCP concluded that TTO and 5 % formulations with TTO can exhibit skin irritancy. EMA (2014) reported the two animal studies from the SCCP opinion. Another study (Halcon & Milkus, 2004) 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. EMA concluded that undiluted TTO causes skin irritation in a small proportion of subjects (generally <5 %). The irritation potential of TTO may be related to the age of the oil, with aged oils (presumably containing higher levels of peroxides and degradation products) displaying a greater incidence of irritation. Finally, the Cosmetic Ingredient Review (CIR, 2021) summarizes many animal and human data with various results. CIR concluded that formulations of 5 % of more can induce skin irritation.

Assessment and comparison with the classification criteria

RAC concurs with the proposed classification by the DS as Skin Irritant category 2; H315 based on reversible acute dermal irritation effects in rabbits with mean values of ≥2,3-≤4.0 for erythema or oedema at 24-72 hours after patch removal. This is supported by reports of irritant reactions in human patch tests.
Based on this information, RAC concludes that classification is warranted for **Skin Irritant category 2; H315.**

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The eye damage/irritation potential of TTO was tested in 3 rabbits according OECD TG 405 and GLP (Anonymous, 2015d). Mean scores were 0 for iris and cornea and 1.0 for redness, chemosis and discharge in the conjunctiva (only 1.3 in one rabbit for discharge).

Another study (ECHA dissemination site; Anonymous, 2013) was performed in 2 male rabbits according OECD TG 405 and GLP. Mean scores were 0 for iris and cornea, and 0.67 and 1 for redness, 0.33 both for chemosis and 0.0 for discharge in the conjunctivae.
Further, the CLH report reported on a Bovine corneal opacity and permeability study, according to OECD TG 437 and GLP (ECHA dissemination site; Anonymous, 2012). The *in vitro* irritancy scores were 2.3 for the negative control, 2.2 for TTO and 44.5 for the positive control.

DS concluded that classification for serious eye damage is not needed as the reversible effects did not meet the criteria (corneal opacity ≥1 and/or iritis ≥1, and/or conjunctival redness ≥2 and/or conjunctival oedema (chemosis) ≥2).

Comments received during consultation

No comments were received for this endpoint.

Assessment and comparison with the classification criteria

RAC concurs with the opinion of the DS, based on the two *in vivo* and the one *in vitro* study, no classification for serious eye damage/irritation is needed.
Based on this information, RAC concludes that **classification is not warranted** for serious eye damage/irritation.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

Two guinea pig maximisation tests (GPMT) and four Local Lymph Node Assays (LLNA) with TTO are available.

In the GPMT tests, no erythema was observed in the animals tested with TTO. The four available LLNAs showed results for the stimulation index at 2 % between 1.6 and 2.4 %. EC3 values at

concentrations above 2 % up to 100 % ranged from 4.4 to 25.5 %. Therefore, DS concluded that TTO deserves to be classified as Skin Sens. 1B.

DS further noted that components in TTO, namely α-Terpinene and Limonene haven been classified as Skin Sens. 1.

Some relevant studies from open literature were summarized in the CLH report (Table 26). Amongst them are two studies with patch tests in humans. Sabroe et al. (2016) tested 5 % TTO in petrolatum in 2104 patients. This resulted in 11 (0.5 %) positive, 2 (0.1 %) doubtful, and 3 (0.1 %) irritant reactions. Veien et al. (2004) tested 10 % TTO in petrolatum and 5 % of a commercial lotion in 217 patients. Results were 65 positive patch tests (30 %). In another test, 4 commercial lotions containing 5 % TTO were tested in 160 patients. The result was no allergic reactions and 5 patients (3.1 %) had irritant reactions.

In addition, several case reports were included from a review by the Danish Toxicology center, showing skin irritation and sensitisation induced by TTO (Larsen & Borling, 2000).
DS provided also some information on animal studies or human patch test with components of TTO. It is noted that α-Terpinene (CAS no. 99-86-5) and Limonene (CAS no. 138-86-3) are classified as Skin Sens. 1.

DS concluded that TTO needs to be classified as Skin Sens. 1B, based on the positive results in the four LLNAs (EC3s above 2 %) and a stimulation index above 3.

Comments received during consultation

Two MSs supported the classification of TTO as Skin Sens. 1B, based on consistent results in four positive LLNA (GLP) studies.

Four companies and two trade associations stated that the four LLNA tests, which results suggest that TTO has a weak to moderate skin sensitising potential, are confounded because of the fact that TTO is a skin irritant. Further arguments provided by them are: LLNA tests are not suitable for all high-log K_{ow} substances (such as limonene, linalool, citronellol) and the LLNA protocol is favourable for autoxidation (OECD, 2021). Given the strengths of the GPMT method, the negative results in two studies should be used leading to no classification.
DS responded that all available data should be used for classification purposes. DS further noted that limitations of LLNA for skin irritating substances are not unique to LLNA, but are also associated with GPMTs (Basketter et al., 2010). Therefore, the four positive mouse LLNAs cannot be completely omitted. TTO used in the studies is stable under storage conditions, used in the LLNAs. In addition to limonene, also α-Terpinene (5-13 %) is classified as skin sensitiser.

Additional key elements

Several other reviews have looked into the skin sensitising properties of TTO, such as CIR (2021), EMA (2014), and SCCP (2008). For example, CIR (2021) has provided overview Tables with human patch tests (5), retrospective, multicenter and cross-sectional patch test studies (about 40), and case reports with TTO (>30). They concluded that oxidized TTO is a skin sensitiser.

Assessment and comparison with the classification criteria

The available animal studies are summarised in the Table below.

Species, strain, sex, no/group	Test substance	Dose levels / duration of exposure	Results	Reference / reliability score/ guideline
Skin Sensitization Study (Magnusson and Kligman) in Guinea Pigs				
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
LLNA test				
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-hexylcinnam-aldehyde 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-hexylcinnam-aldehyde 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,	ECHA dissemination site; Anonymous, 2006b 1 OECD TG 429 /

Species, strain, sex, no/group	Test substance	Dose levels / duration of exposure	Results	Reference / reliability score/ guideline
			above Slight ear erythema in two highest dose groups	GLP
Mouse (CBA/J) Female 5/dose/group	Melaleuca alternifolia, ext., Purity 100 % Positive control: alpha-hexylcinnam-aldehyde 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.

There were four positive LLNA tests of acceptable reliability. They are contradicted, but not overruled by two negative GPMT's. It is also noted that the positive control in the 2015 GPMT did not give a very strong result and no positive control was reported in the summary of the 1989 GPMT, raising some doubt on the sensitivity of these studies. Human data confirm that TTO induces skin sensitisation. Considering the incidence was not very high and the concentrations that induced responses not particularly low, cat. 1B seems more appropriate than cat. 1A based on the human studies.

It is noted from a literature review that oxidised TTO is a stronger sensitiser than fresh TTO (Larsen & Borling, 2000). In the LLNA tests however, TTO was reported to be stable under storage conditions for the test, i.e. considered not as oxidised. Irritation is noted in the LLNA studies, but not in all four. Irritation is also noted in human patch tests. In the CLP guidance (3.4.2.2.2) it is noted that when a substance may autooxidise to a more hazardous form, this may warrant classification of the parent substance. Further in support of classification is the fact that two components of TTO (Limonene, α-Terpinene) are classified as skin sensitisers.

Based on the available data in the CLH report and the positive results in the LLNA tests and supported by human patch test studies and case reports, RAC concludes that TTO warrants a classification as **Skin Sensitizer cat. 1B; H317**.

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

Summary of the Dossier Submitter's proposal

Specific target-organ toxicity of TTO is studied in several repeated dose toxicity studies.

A 28-day study in SD rats (5 males and females per group) dosed by gavage with doses of 0, 5, 15 and 45 mg TTO/kg bw/d was performed according to OECD TG 407 and GLP (ECHA dissemination site; Anonymous, 2017b). No effects were observed.

Another 28-day study was performed according to OECD TG 407 with some deviations and no GLP stated (Anonymous, 2010b). Wistar rats were dosed by gavage (6 males and females per

group) with 0, 62.5, 125 and 250 mg TTO/kg bw/d. No clinical signs and no effects on body weight were found. In the males, decreased organ weights (testes and epididymides) starting from the mid dose were observed. Liver and adrenal weights were increased. Histopathological changes were also found in the testes (degeneration) and epididymides (oligospermia and cell debris in lumen), also starting from the mid dose.

A 90-day study was performed according to OECD TG 408 and GLP (Anonymous, 2011b). Wistar rats (males and females) were dosed by gavage with 0, 30, 60, or 120 mg TTO/kg bw/d. Starting from the mid dose, sperm effects were found. At the high dose, also histopathological changes were found in testes and epididymides. Further effects noted were spleen vacuolation (minimal degree) and tubular dilation in kidneys (minimal degree).

Another 90-day study was performed according to OECD TG 408 and GLP (Anonymous, 2016a). Wistar rats (males and females) were dosed by gavage with 0 or 60 mg TTO/kg bw/d. At 60 mg/kg bw/d, sperm counts and motility were decreased and degeneration and atrophy of seminiferous tubules was noted.

A 90-day study was performed according to OECD TG 409 and GLP (Anonymous, 2018a). Beagle dogs were dosed by gavage with 0, 30, 75/60, or 180/120 mg TTO/kg bw/d. Due to signs of intoxication, the mid and top doses were reduced from test day 27 on. From the mid dose and up, the viability and motility of spermatids were decreased.

DS also included information from a 2-generation study and two prenatal developmental toxicity studies (PNDT). As no relevant effects were observed for STOT RE, these studies are summarized under Reproductive Toxicity.

DS concluded that TTO clearly has an effect on spermatogenesis. As discussed under Reproductive toxicity, this is considered to be related to the administration type (gavage vs dietary) which resulted a much higher systemic exposure than expected. DS considered no classification warranted for STOT RE with respect to sperm impairment, because gavage is a non-relevant route for humans, and no exposure as a plant protection product is expected.

Comments received during consultation

Two companies noted that no classification is warranted for STOT RE with respect to sperm impairment. Arguments provided are that effects are due to the administration type (gavage) and no exposure to TTO as plant protection product is expected as no residue is left on treated crops.

Additional key elements

With regards to the 90-day study in dogs (Anonymous, 2018a), more information was provided in the DRAR (Volume 3-B.6). It was noted that the dose reduction in the high dose group resulted in an increased body weight gain and food intake, resulting in body weights within the range of the control group values at the end of the 90-days. It was also noted that in all exposure groups the body weight and feed consumption, as well as haematological, biochemical and urine parameters were not affected.

Assessment and comparison with the classification criteria

RAC does not concur with the DS view on the non-relevance of the oral dosing by gavage for classification. Further, the argument that there is no exposure from residues on dietary products is not a relevant for hazard classification either, since classification is based on hazard and not on exposure.

TTO clearly demonstrates effects on testes and epididymides, with regards to weight (decreased), as well as histopathological changes and effects on sperm count and motility. These effects are further discussed under Reproductive toxicity.

TTO also has effects on liver and adrenals, showing increased organ weights. Minimal/mild liver vacuolation was reported in a dose-dependent way in the 28-day study, starting from the lowest dose of 62.5 mg/kg bw/d. The effect was not consistently found between studies, and mostly minimal, except in two males of the 250 mg/kg bw/d group where it increased to mild vacuolation. However, minimal vacuolation is not considered significant enough for classification. The liver weights in the 90-day studies were increased from 60 mg/kg bw/d, so below the guidance value of 100 mg/kg bw/d for STOT RE category 2. However, no corresponding histopathological effects were reported.

RAC concludes that **no classification is warranted** for TTO for STOT RE. The effects on testes and spermatogenesis are included under Reproductive toxicity.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

Mutagenicity is investigated in several *in vitro* tests, namely bacterial reverse mutation tests, mammalian cell gene mutation tests, mammalian micronucleus test, mammalian chromosomal aberration tests. Further, one *in vivo* test for DNA damage (mouse micronucleus test) is performed. Results are summarised in the Table below.

Parameter	Concentration	Results	Reference/ reliability score
***In vitro* studies**			
Bacterial Reverse Mutation Test OECD TG 471 GLP	TA 98, TA 100, TA 1535 and TA 1537 strains of Salmonella typhimurium and WP2 uvrA (pKM 101) strain of Escherichia coli.	Negative	Anonymous, 2010b 1
Bacterial Reverse Mutation Test	TA98, TA100 and TA102 strains of *Salmonella typhimurium*.	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
In vitro mammalian	Chinese hamster lung fibroblasts (V79	Negative, tested up to	ECHA dissemination site; Anonymous, 2009

Parameter	Concentration	Results	Reference/ reliability score
chromosomal aberration test OECD TG 473 GLP		cytotoxic concentrations, with and without metabolic activation	1
In vitro mammalian micronucleus test Similar to OECD TG 487	Human lymphocyte cultures	Negative	Pereira et al., 2014 2
In vitro mammalian chromosomal aberration test Similar to OECD TG 473	Human lymphocyte cultures	Negative	Pereira et al., 2014 2
***In vivo* studies**			
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

DS summarized that TTO was tested negative in bacterial gene mutation assays with and without metabolic activation and negative in a mammalian cell gene mutation assay. TTO showed no potential to induce DNA damage *in vitro*, as both a mammalian micronucleus test and chromosomal aberration tests *in vitro* were negative. *In vivo*, TTO was negative in a mouse micronucleus assay in bone marrow. Bone marrow exposure was reported to be proven by a decrease in polychromatic erythrocytes to total erythrocytes. The positive control induced an increase in micronuclei.

DS further provided several studies from open literature on TTO components, all negative.

DS concluded that TTO does not deserve to be classified for germ cell mutagenicity.

Comments received during consultation

No comments received.

Assessment and comparison with the classification criteria

RAC concurs with the DS that no classification of TTO is needed for germ cell mutagenicity, based on the negative results in *in vitro* tests (bacterial reverse mutation test, mammalian cell gene mutation test, mammalian micronucleus test, mammalian chromosomal aberration test) and an *in vivo* test for DNA damage (mouse micronucleus test).
Based on this information, RAC concludes that **classification is not warranted** for germ cell mutagenicity.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

No carcinogenicity studies with TTO are available.

DS provides several points with regards to the assessment of the carcinogenicity of TTO:

- 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).

Furthermore, information is provided on some of the components of TTO.

- A carcinogenicity study was performed in female mice with intraperitoneal injections of 1900 or 9600 mg/kg bw of α-terpineol or β-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 α2u-globulin-associated response.

DS notes that in total, the carcinogenicity studies of 1,8-cineole, terpineol and limonene cover >95 % of TTO components. Hence, TTO is unlikely to be a carcinogen.

DS concludes that there is no evidence that TTO or its components are carcinogenic in the summarized studies. A classification is therefore not required. DS concludes on no classification due to inconclusive data.

Comments received during consultation

No comments received.

Assessment and comparison with the classification criteria

RAC notes that the remarks on long history of safe use, no consumer exposure due to lack of residues on crops and natural occurrence are not relevant arguments with regards to hazard classification. Also, biotransformation, rapid clearance and lack of bioaccumulation are not relevant, as metabolites could have effects as well.

There are no carcinogenicity studies available with TTO. The negative carcinogenicity studies with components of TTO provide some information but are not enough to conclude that TTO is not carcinogenic. Therefore, RAC concludes on **no classification for carcinogenicity based on lack of data.**

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The reproductive toxicity of TTO has been assessed in four studies: in one two-generation study, and in three developmental toxicity studies (two in rats and one in rabbits).

Sexual function and fertility

The two-generation study is performed according to OECD TG 416 and GLP (Anonymous, 2017a). Wistar rats were exposed by oral gavage to 0, 10, 25 and 50 mg TTO/kg bw/d in the parental generation, and 0, 10, 25 and 38 mg TTO/kg bw/d in the F1 generation (reduced because of alterations in reproductive performance).
In the parental generation, the number of pregnancies was adversely affected by TTO dose-dependently (92, 84, 84, 56 % respectively). Male and female fertility indexes were significantly lower at the highest dose and are associated with a decrease in sperm motility, cauda epididymal sperm counts and increase in percentage of abnormal sperm counts. The maternal data such as mean number of corpora lutea and implantations were significantly lower, and percentage of pre-implantation loss was significantly higher at 50 mg/kg bw/d. The mean litter size was 10.0, 8.7, 9.0 and 7.0 in the control, 10, 25 and 50 mg/kg bw/d group.
In the F1-generation, the number of pregnancies was still affected, but not as much at the highest (lowered compared to F0-generation) dose of 38 mg/kg bw/d (100, 96, 96, 87 % respectively). At the highest dose, cauda sperm counts (number of sperms per cauda epididymis and number of sperms per gram of cauda epididymis) were lower. However, mean number of corpora lutea, number of implantations and mean litter size were not different from control.

Additionally, the adverse effects on testes and/or sperm count and motility in the repeated dose toxicity studies (28-day study in rats, two 90-day studies in rats and a 90-day study in dogs) were also considered by the DS.

DS noted the effects on fertility, testes, epididymides and sperm observed in two species (rats and dogs) in four acceptable studies at dose levels inducing slight or moderate general systemic toxicity. DS also expressed some doubt on human relevance, taking into account that such effects

were not reported in humans exposed to components of TTO at relatively high doses with food. DS proposed Repro cat. 2; H361f.

Developmental toxicity

In a PNDT study (Anonymous, 2012a) according to OECD TG 414 and GLP, Wistar rats (n=24 per group) were exposed orally by gavage to 0, 75, 150 or 300 mg TTO/kg bw/d from GD5-19. Due to severe clinical signs and mortality, doses were reduced on GD8 to 0, 30, 60 or 120 mg/kg bw/d.
Body weight gain and food consumption were reduced at 150/60 and 300/120 mg/kg bw/d. No effects by TTO were found on the mean number of corpora lutea, implantations, early resorptions, late resorptions, pre-implantation loss, and post-implantation loss. The number (and percentage) of dams with any resorption was increased at the mid and high dose compared to the control group (11/47.8 % at 60 mg/kg bw/d and 12/57.1 % at 120 mg/kg bw/d versus 6/25 % in the control group). Total number of live foetuses and mean litter size was not affected. Mean foetal weight was reduced in the mid and high dose with 4.6 and 15 %, respectively. No major external, visceral or skeletal malformations were observed. Increased incidence of delayed ossification of various bones was observed in 150/60 and 300/120 mg/kg bw/d.

In another PNDT study (ECHA dissemination site; Anonymous, 2011) according to OECD TG 414 and GLP, Wistar rats (up to 27 per group) were exposed orally by gavage to 0, 20, 100 or 250 mg/kg bw/d from GD5 to 19.
Reduced food consumption and body weight loss (with mortality of 7/27 animals) was reported in the high dose. Maternal body weight was decreased with 20 and 45 % respectively in the mid and high dose groups. Reductions in foetal body weight were seen at 100 and 250 mg/kg bw/d. Increases in external and skeletal malformations (displaced rib cartilages at the sternum, malformed vertebrae, and/or short, bent scapula, humerus or femur) were also seen in foetuses from the high dose group. These effects were considered secondary to maternal toxicity.

A PNDT study in rabbits is also available according to OECD TG 414 and GLP (Anonymous, 2018b). New Zealand white rabbits (n=24 per group) were exposed orally by gavage to 0, 15, 30 or 75 mg/kg bw/d from GD6-28. No maternal toxicity was seen. Body weight gain changes were reported, however considered non-adverse as the corrected body weight gain was comparable to control. Litter parameters were not affected. There were no signs of external, visceral and skeletal malformations. A statistically significant increase in the number of post-implantation loss was found in the high dose (1.76 versus 0.52 in control), which was considered to be treatment related as it was higher than historical control data. It was speculated that it was driven by an increase in late resorptions rather than early resorptions. DS considered that the post-implantation loss was secondary to reduced maternal food intake.

DS concluded that main developmental parameters were not affected, so no classification is warranted.

Lactation

DS noted that the reduced body weight of F1 pups in the 2-generation study during the initial days of lactation are not considered to provide clear evidence of an adverse effect in the offspring due to transfer of milk. No classification is proposed.

Comments received during consultation

One Member State supported the classification in category 2 for fertility.

Two Member States proposed to classify in category 1B for effects on fertility, because of the clear effects on male fertility observed in a 2-generation study, 28-day study and two 90-day studies in rats and a 90-day study in dogs. Next to decreased sperm count and mobility (associated sometimes with microscopic changes in testes), effects were found on the mean number of corpora lutea, implantation and a pre-implantation loss increase. One National authority requested information from the DS on the human evidence available to decrease the concern.
DS responded that no epidemiological studies are available but provided evidence on the natural occurrence of TTO components in everyday food items.
One MS proposed category 2 or 1B for developmental toxicity based on the increase in post-implantation loss in the rat 2-generation study and the rabbit PNDT.
With regards to lactation, one National authority requested data on the mean pup weight in the F2 generation.

Several companies and trade bodies disagreed with the classification as category 2 reprotoxicant for fertility. Arguments provided are:

- History of safe use of monocyclic terpenes in diet and other products.
- Non-relevant way of exposure. The effect on spermatogenesis is seen after gavage exposure with the TTO component α-Terpineol at 750 mg/kg bw/d (and not at 250 mg/kg bw/d), and not seen after dietary exposure (tested up to 623 mg/kg bw/d, with only a slight significant increase in percentage abnormal sperm). Gavage treatment is regarded as non-relevant for humans.
- No exposure of TTO as plant protection product to humans is expected because there are no residues on crops.
- Further, a mode-of-action for the spermatogenesis via a metabolite of p-cymene is proposed and noted to have a clear quantitative species difference in metabolite accumulation (p-isopropyl benzoyl Coenzyme A -conjugate was accumulating in rat hepatocytes to stable levels, but was cleared over time in human hepatocytes).

There was agreement with regards to no classification for developmental toxicity. It was noted that the small mean increase in post-implantation loss (1.76±1.84) at 75 mg/kg bw/d in comparison with controls (0.52±0.81) is rather due to one dam with resorption of all foetuses which does not seem to be treatment-related since this effect was not observed in any other dams.
DS responded that all available information should be considered for classification purposes.

Additional key elements

In the DRAR (Volume 3-B.6, 2022), additional information from the registration dossier of α-Terpineol is included. DS is referring to this information in the CLH report.

In a repeated dose study by gavage, effects were found at the highest dose of 750 mg/kg/day: decreased testes weight, lower epididymal weight, reduced numbers of spermatozoa, and histopathological changes in the seminiferous tubules. These effects were not seen at 250 mg/kg bw/d.
A comparative study was performed with two groups of male rats, one orally dosed by gavage (500 and 750 mg/kg bw/d) and the other via the diet (8000 or 120000 ppm). The result was *"Negative effects on sperm mobility clearly confirms the effects previously observed when the substance is administered by gavage while no effects are detected when administration of via diet".*

This was confirmed in a 90-day toxicity study with SD rats exposed to TTO via the diet (12000 ppm, corresponding to 623 mg/kg bw/d). A slight significant increase in the percentage of abnormal (4.8 %) sperms was noted at 12000 ppm. However, this change was considered incidental as it was well within the range of normal biological variation (HCD 0.1- 7.4 %). No effects were found on sperm motility, caudal epididymal weight/sperm count and testicular weight/spermatid count.

It was concluded that *"based on these findings, there is strong evidence that no reproductive effects are likely to occur by the realistic routes of exposure and no classification for reproductive effects is therefore warranted."*

Further, in the DRAR (and additional tables) some more information with regards to the 2-generation study (Anonymous 2017a) was provided.
It was noted that *"Treatment significantly reduced mean body weights on Days 1 and 4 in male pups and on Days 1, 4 and 7 in female pups and combined sex at 38 mg/kg bw/d."*
As well as:
"F1-Generation:
The weekly mean body weights of males rats were significantly lower on initial week and from Week 1 to 7 at 10 mg/kg bw/d dose and on initial week and week 1 at 25 mg/kg bw/d dose when compared to vehicle control.
For the females rats, the weekly mean body weights were significantly lower on initial week and week 1 at 10 mg/kg bw/d when compared to vehicle control. No significant changes in the mean body weights were observed at 25 and 38 mg/kg bw/d."

Assessment and comparison with the classification criteria

Sexual function and fertility

The 2-generation study in rats resulted in clear adverse effects on male and female mating and fertility index (Anonymous, 2017a). This is related to decreases in sperm motility, reduced sperm counts and an increase in percentage of abnormal sperm counts. Mean number of corpora lutea and implantations were significantly lower. The percentage of pre-implantation loss was significantly higher, which resulted in significantly lower mean litter and viable litter sizes (only at the highest dose). See for specific details the table below.
The CLH report provides details on food consumption and body weight gain However, absolute body weights were not directly provided in the CLH report; it should be noted that some decreasing effects were recorded in terms of the body weight gain and food consumption, they are however not dose-dependent.

Please note, body weights are added on the below table when available from the study reports provided by the DS.

Parameters	**Concentration (mg TTO/kg bw/d)**			
	0	**10**	**25**	**50**
P generation				
No. of animals per dose	25	25	25	25
Male bw on day 113 (g)	469.73	434.32*(7.54)	450.71	427.16*(9.06)
Net bw gain (Day 113-1)	326.87	294.31*	309.11	284.14*
Male Fertility Index°	84	76	80	**44***
Progressive motile sperms (%)	63.88	63.72	62.2	**54.48***(15)
Motile sperms (%)	84.80	85.68	83.32	**74.84***(12)

Normal sperms (%)	97.26	97.34	93.45	**81.34***(14)
Abnormal sperms (%)	2.74	2.66	6.55	**18.66***(486)
No. of sperms per cauda epididymis (x10^6)	196.41	181.47	**165.94***(16)	**133.71***(32)
No of sperms per gram of cauda epididymis (x10^6)	978.21	1016.84	893.76	**787.47***(19)
Female bw on GD20 (g)	334.87	326.50	325.28	310.61
Female bw change GD0-20	80.63	66.53*	68.28*	57.86*
Food consumption GD0-20 (g)	285.34	229.81*	233.25*	244.51*
Number of pregnant females	23	21	21	14
Female Fertility Index	92	84	84	**56***
Mean No. of Corpora Lutea	12.8	11.8	11.7	9.3*
Mean No. of Implantations	11.1	10.5	10.1	6.7*
Pre-implantation loss (%)	15.0	13.8	14.5	33.4*
Post-implantation loss (%)	16.3	29.6	17.6	20.7
Gestation Length (days)	22.77 ± 0.53	22.75 ± 0.55	22.55 ± 0.51	22.45 ± 0.69*
Mean Litter Size	10.0	8.7	9.0	7.0*
Mean Viable Litter Size	9.9	7.8	8.5	6.7*
Mean pup bw (g) male day 1	6.19	5.71*	5.65*	5.63
Mean pup bw (g) female day 1	5.77	5.56	5.42	5.32
Day 4 Survival Index	99.1	94.2*	91.1*	81.1*
Mean pup bw (g) day 21	26.61± 5.14	22.24*± 4.96	22.47*± 3.91	26.93*± 5.81
F1-generation				
	0	**10**	**25**	**38**
No. of Animals per dose	25	25	25	25
Male bw in week 17 (%)	367.76	352.07	355.15	386.04
Net bw gain (week 17-1)	327.81	312.25	312.06	335.17
Male Fertility Index	100	96*	96*	**87***
Progressive motile sperms %	60.92	60.4	56.96	**51.24*** (16)
Motile sperms %	84.36	82.32	80	75.68
No. of sperms per cauda epididymis (x10^6)	197.29	176.86	**168.98*** (14)	**160.40*** (19)
No. of sperms per gram of cauda epididymis (x10^6)	934.74	**837.99*** (10)	**824.66*** (12)	**766.99***(18)
Female bw on GD20 (g)	303.38	290.31	301.71	295.22
Female bw change GD0-20 (g)	85.70	78.97	84.64	83.44
Food consumption GD0-20 (g)	308.04	299.82	301.38	301.92
Number of pregnant females	25	24	24	20
Female Fertility Index	100	96*	96*	**87***
Mean No. of Corpora Lutea	12.16	11.88	12.04	12.55
Mean No. of Implantations	10.8	11.2	11.2	11.8
Pre-implantation loss (%)	11.0	6.8	6.8	6.6
Post-implantation loss (%)	17.6	19.4	17.6	17.5
Gestation Length (days)	22.92 ± 0.64	22.83 ± 0.70	22.57 ± 0.59	22.33 ± 0.49
Mean Litter Size	9.8	9.2	9.5	10.7

Mean Viable Litter Size	9.0	9.0	9.3	9.7
Mean pup bw (g) male day 1	6.01	5.74*	5.94	5.39*
Mean pup bw (g) female day 1	5.71	5.57	5.52	4.99*
Day 4 Survival Index	95.5	100.0*	98.7	97.4
Mean pup bw (g) day 21	30.93	29.76	29.32	27.45

Values in parenthesis indicate percentage change
*: Significantly different from the control group (p<0,05)

In the available repeated dose toxicity studies (see related section above for further details), similar effects are reported at testes organ weight and spermatogenesis (see short summary below), supporting the effects noted in the 2-generation study.

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

Therefore, with regard to sexual function and fertility, RAC considers that the above information justifies the classification of the substance for Reproductive toxicity in category 1B; H360F based on the clear effects on testes, epididymides, sperm counts and motility, resulting in decreased male fertility. It is also noted that the number of corpora lutea and implantations were lower and the pre-implantation loss higher, indicating that also female fertility could be affected.

While RAC considers that the available data is supportive of the classification for Reproductive toxicity in category 1B; H360F, it has also considered whether there are any reasons that would support a different outcome:

- The DS and stakeholders expressed some doubt on human relevance, taking into account that such effects were not reported in humans exposed to components of TTO at relatively high doses with food. However, DS and RAC note that no human data on TTO are available.
- In addition, RAC received comments regarding a hypothesis to explain the mode-of-action (MoA) for the spermatogenesis via a metabolite of p-cymene. This metabolite was shown to have a clear quantitative species differences in accumulation between rats and humans *in vitro*. RAC responded regarding the proposed MoA via a p-cymene metabolite, that the clearance of the p-cymene metabolite in human hepatocytes might be higher compared to rat hepatocytes, still the overall metabolisation *in vivo* is not known for this component, let alone that (quantitative) differences in metabolisation are known for the full TTO

mixture (p-cymene presents in the range of 0.5-8 %). Therefore, it is not possible to either exclude another MoA nor conclusively demonstrate the human non-relevance[2].

- Concerning the comment relating to the applicability of studies dosed by gavage, RAC considers such studies as relevant for hazard classification (as also noted in the STOT RE section), as this is consistent with the OECD TG under which these studies were conducted.
- RAC considers the argument that there is no exposure from residues on dietary products (e.g. crops) not relevant for hazard classification, since classification is based on the hazardous properties of the substance.
- Regarding the comments that there is history of safe use of monocyclic terpenes in diet and other products, no relevant data to address this (e.g., epidemiology studies in humans exposed to the substance) were provided to RAC to support this comment. RAC notes the findings in animal studies described above.

Therefore, in consideration of all above elements, RAC concludes that classification is warranted as **Reproductive toxicity category 1B; H360F.**

Developmental toxicity

The main findings from the three PNDT studies and the 2-generation study have been summarized in the table below.

Dose (mg/kg bw/d)	0	10	15	20	25	(75/30)	50	(150/60)	75	100	(300/120)	250
PNDT, rats, OECD TG 414 [1] (Anonymous, 2012a)												
Mean weight fetuses	3.47					3.58		3.31			2.95	
Post-implantation loss	0.58					0.39		0.91			1.05	
No. of dams with any resorption (%)	6 (25)					5 (22)		11 (48)			12 (57)	
Incomplete/poor ossification						-		-			↑	
Maternal bw (g)	317.11					323.14		308.11			291.3.2↓ 8.1 %	
Corrected bw gain (g)	18.34					20.05		9.33			-4.40	
PNDT, rats, OECD TG 414 (Anonymous, 2011)												
Mean fetal weight				-						↓ 13 %		↓ 32 %
Skeletal malformations				-								↑ 9 %
Post-implantation loss				-						-		↑
Mortality dams				-						-		7/27
Maternal bw gain				-						↓ 20 %		↓ 45 %
PNDT, rabbits, OECD TG 414 (Anonymous, 2018b)												

2 See Section 3.7.2.3.2 of CLP: "*If it is conclusively demonstrated that the clearly identified mechanism or mode of action has no relevance for humans or when the toxicokinetic differences are so marked that it is certain that the hazardous property will not be expressed in humans then a substance which produces an adverse effect on reproduction in experimental animals should not be classified*".

Mean no of post-implantation loss	0.52		0.65			0.76			1.76↑[2]			
Mean post-implantation loss	8.06		12.16			13.22			25.02			
Maternal bw gain	0.412		0.353			0.246			0.189 ↓			
Maternal bw GD 29 (kg)	3.630		3.595			3.519			3.506			
Corrected bw gain (kg)	-0.0036		-0.043			-0.137			-0.199			
Food consumption (g/rabbit/day)	133.75		129.84			96.82 *			88.85 *			
2-generation study, rats, OECD TG 416 (Anonymous, 2017a)												
% post-implantation loss	16.3	29.6			17.6		20.7					
% post-implantation loss	17.6	19.4			17.6		17.5					
F1 pup bw day 21	26.61	22.24			22.47		29.63					
F2 pup bw day 1	6.00	5.83			5.83		5.28 *					
F2 pup bw day 4	8.04	7.53			7.43		6.63 *					
F2 pup bw day 21	30.93	29.76			29.32		27.45					
Day 4 survival							↓					
Maternal bw gain		-			-		-					

[1] *Initial tested doses of 75, 150 and 300 mg/kg/day and at the reduced doses of 30, 60 and 120 mg/kg/day. The doses were reduced on GD 8 (third day of treatment) due to mortality, at a dose of 300 mg/kg.*

[2] *Historical control data for the rabbit study on post-implantation loss (N=222), number 99, mean 0.45 with SD 0.78, range 0-4.*

In the three PNDT studies available with TTO, general toxicity consisted mainly of reduction in body weight gain and food consumption (mostly only weight gain is provided in the available information). From about 100 mg/kg bw/d maternal body weight loss was observed. Mortality occurred at 250 mg/kg bw/d (7 out of 27 dams) in the rat PNDT study from 2011, also mortality occurred in the first high dosing with 150 and 300 mg/kg bw/d (PNDT study from 2012a).

The most severe developmental effect was post-implantation loss, which was observed in one PNDT in rats (2011) at 250 mg/kg bw/d and in the rabbit PNDT at 75 mg/kg bw/d. The reporting of the other PNDT in rats (2012a) was unclear on this point. On one hand there was no increase in post-implantation loss, but the number of dams with resorptions was increased.
The effect in the 2012 rat PNDT occurred at high maternal toxicity (even mortality) and is therefore not relevant for classification. The effect in the rabbit PNDT was attributed by the DS to decreased food consumption. However, the maternal body weight is not affected, and the corrected body weight gain is statistically significant reduced. Moreover, a comparison of the food consumption and post-implantation loss on the level of the individual dams did not show a relationship.
Also, the CLP criteria, Annex I 3.7.2.4.2. should be considered: "*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.*"

Skeletal variations and delayed ossification were observed in both rat PNDT studies at 100/120 mg/kg bw/d, as well as skeletal malformations at 250 mg/kg bw/d. These skeletal effects can be caused by growth retardation, which can be related to maternal toxicity. The following is stated on this in the CLP criteria Annex I: 3.7.2.4.3.: "*Classification is not necessarily the outcome in the case of minor developmental changes, when there is only a small reduction in foetal/pup body weight or retardation of ossification when seen in association with maternal toxicity*."

In conclusion, there was a statistically significant and dose-dependent increase in post-implantation loss in the rabbit study. The effect on pup survival in the rat PNDT studies and 2-generation study were more questionable, as the effects are smaller and generally accompanied by maternal toxicity. There were also effects on pup weight and skeletal development in the rat, but again only significant at high dose levels. Considering there are some doubts on the relevance of the effects in rats, category 2 (H361d) is considered more appropriate than category 1B for developmental toxicity.
Based on this information, RAC concludes that classification is warranted as **Reproductive toxicity category 2 (H361d).**

Lactation

There are no data available on the presence of TTO components in breast milk.

In the two-generation reproduction toxicity study in rats, post-natal survival of pups was not affected by TTO exposure, and no effects on lactation or viability indices in either generation were reported. During lactation, treatment with TTO significantly reduced mean body weights in the F2 on PND1 and 4 in male pups and on PND1, 4 and 7 in female pups and combined sex at the highest dose of 38 mg/kg bw/d. Body weights of the F1 were however not affected, at the higher dose of 50 mg/kg bw/d. At the end of the lactation period (21 days), body weights recovered and were no longer different from control animals indicating that the body weight reduction, even if treatment related, should not be considered as a severe toxic effect. There was no indication of impaired nursing behaviour.

With regards to lactation, no classification is proposed. This is based on the lack of evidence of TTO component present in the milk, as well as no effects on postnatal survival of pups in the 2-generation rat study.

Based on this information, RAC concludes that **classification is not warranted** for lactation effects.

RAC evaluation of aspiration toxicity

Summary of the Dossier Submitter's proposal

Kinematic viscosity of TTO (containing >10 % hydrocarbons) is 1.71 mm^2/s at 40°C. Based on that, DS proposed to classify TTO as Asp. Tox. 1; H304.

Comments received during consultation

One MS supported the classification as Asp. Tox 1; H304, since it is a hydrocarbon and has a kinematic viscosity of 1.71 mm^2/s measured at 40 °C, which is sufficient according to Regulation (EC) No 1272/2008.

Assessment and comparison with the classification criteria

RAC concurs with the DS on the classification as Asp. Tox. 1; H304 of TTO. This is based on the kinematic viscosity of 1.71 mm^2/s measured at 40 °C, which is below 20.5 mm^2/s from the classification criteria in the CLP regulation.

Based on this information, RAC concludes that classification is warranted as **Aspiration toxicity Cat. 1; H304**

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Degradation

The DS proposed to classify TTO as rapidly degradable. The reasoning behind this proposal was that in an OECD TG 310 study (Fiebig, 2010) TTO turned out to be readily biodegradable with 87 % biodegradation observed already on day 7, whereas the maximum biodegradation came to 106 % after 28 days based on CO_2 production. However, the DS stated that this result was contrary to an OECD TG 301F study (Jenner et al. 2011) in which δ-cadinene, one of TTO constituents, was proofed to be not readily biodegradable since removal of ThOD was <60 % after 28 days. Further for an OECD TG 308 study (Bloß, 2018c) and two OECD TG 307 studies (Bloß, 2018a and Bloß, 2018b) the DS concluded that the obtained results for the non-labelled test compounds were not reliable to describe degradation. It should be noted that the DS did not include an evaluation of an available OECD TG 301 F study with γ-Terpinene (one of the main components of TTO) in which only 27 % degradation was observed after 28 days nor the RAC opinion from 2019 in which α-terpinene has been assessed been not rapidly degradable for the purpose of hazard classification. No hydrolysis study was available and the photodegradation in water was also not investigated.

Bioaccumulation

The DS proposed to consider TTO as a substance with low potential for bioaccumulation. The reasoning behind this proposal was that the experimental log K_{OW} of Terpinen-4-ol, the main component of TTO, amounts to 2.643 at 23.5°C and pH 5.85 and thus did not exceed the trigger value of 4 (log K_{OW} < 4). Further there were no experimental BCF test data available. The DS reported BCF values for the TTO components estimated by QSAR (Episuite v4.11). Estimated BCF values are < 500 for all monoterpene components, which account on average for > 95 % of TTO. For the sesquiterpenes, BCF values > 500 have been estimated, however, for the majority of these was below 600, i.e. close to the trigger of 500. The sesquiterpene content of TTO was traces to max. 3.5 % (individually), and cumulatively usually < 5 %. Cumulative content of components with BCF values > 600 (Cadinene, Aromadendrene and Ledene, BCF values range 5000-7000) usually was below 2 %.

Aquatic toxicity

Reliable acute and chronic aquatic toxicity data for each trophic level (fish, invertebrates, algae and aquatic plants) on TTO were available.

Acute aquatic toxicity

The DS proposed to classify TTO in Aquatic Acute Category 1 with an M-factor of 1. The reasoning behind this proposal was that there were reliable acute toxicity values for fish, invertebrates, algae and higher aquatic plants available. Aquatic invertebrates were the most acutely sensitive trophic group with the lowest short-term (48 hour) EC_{50} value of 0.591 mg/L for *Daphnia magna*.

***Table**. Reliable acute toxicity data on TTO. All results were expressed based on measured concentrations.*

Method	Species	Results [mg/L]	Reference
Acute toxicity to fish OECD TG 203, GLP Semi static, 96 h	*Oncorhynchus mykiss*	LC_{50} = 7.45	Anonymous (2015g)
Acute toxicity to *Daphnia* OECD TG 202, GLP Semi-static, 48 h	*Daphnia magna*	EC_{50} = 0.591 NOEC = 0.106	Noack, M. (2011)
Algae growth inhibition test OECD TG 201, GLP Static, 72 h	*Pseudokirchneriella subcapitata*	E_yC_{50} = 1.76 E_rC_{50} = 2.17	Scheerbaum, D. (2017d)
Aquatic plant toxicity test OECD TG 221, GLP Semi-static, 7 d	*Lemna gibba*	E_rC_{50} = 10.3 E_yC_{50} = 10.0	Scheerbaum, D. (2017e)

Chronic aquatic toxicity

The DS proposed to classify TTO in Aquatic Chronic Category 3 with no M-factor. The reasoning behind this proposal was that TTO was considered rapidly degradable and that there were reliable chronic toxicity values for fish, aquatic invertebrates, algae and aquatic plants. Fish were the most long-term sensitive trophic group with the lowest NOEC of 0.244 mg/L from an early life stage test performed according to OECD 210 TG with *Pimephales promelas.*

***Table**. Reliable chronic toxicity data on TTO. All results were expressed based on measured concentrations.*

Method	Species	Results [mg/L]	Reference
Early life stage test with fish OECD TG 210, GLP Flow-through, 34 days (28 days post-hatch)	*Pimephales promelas*	NOEC = 0.244	Anonymous (2017c)
Reproductive and developmental toxicity to *Daphnia* OECD TG 211, GLP Semi-static, 21d	*Daphnia magna*	NOEC = 0.303 EC_{10} = 0.411	Scheerbaum, D. (2017b)
Algae growth inhibition test OECD TG 201, GLP Static, 72 h	*Pseudokirchneriella subcapitata*	NOEC = 0.912	Scheerbaum, D. (2017d)
Aquatic plant toxicity test OECD TG 221, GLP Semi-static, 7 d	*Lemna gibba*	NOEC = 1.91	Scheerbaum, D. (2017e)

Comments received during consultation

Comments were received from two Member States, which both supported the proposed classification. A third comment by a National Authority requested a clarification of the substances identity and questioned the conclusion that TTO has no potential for bioaccumulation. It was argued that in the absence of experimentally measured BCF data the experimentally measured log K_{OW} values are usually considered more reliable than estimated BCF values to determine bioaccumulation potential. The DS argued that all the available data were presented in the CLH report and that the DS did not have any additional data.

Assessment and comparison with the classification criteria

Degradation

The Guidance on the Application of the CLP Criteria recommends that the results of biodegradability tests of a complex substance (such as UVCB), should be carefully evaluated before use for classification purposes is considered.

For instance, in Section 4.1.3.2.2 d. Complex or multi-constituent substances, it clarifies 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.*"

Annex II.3.1 to the Guidance on the Application of the CLP Criteria states that, if a complex substance is not defined as "*a homologous series of substances within a certain range of carbon chain length and/or degree of substitution*", the 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.*"

As reported in the table below, all five monocyclic monoterpenes aliphatic and aromatic hydrocarbons as well as the three bicyclic monoterpenes have a vapor pressure value above 50. Taking the extreme low water solubility of three Polycyclic sesquiterpenes in account, overall 11 of the 15 known constituents of TTO have a high Henry's law constant.

Table: *relevant physical-chemical properties of TTO constituents (please refer to the CLH report for details)*

Constituent	Min. %	Max. %	Vapour pressure [Pa] at 25°C	Volatility Henry's Law Constant [Pa m3 mol-1] at 25°C	Water solubility [mg/L]	Adsorption [log K_{oc}]
Monocyclic monoterpenes						
Aliphatic and aromatic hydrocarbons						
γ-Terpinene	10	28	145	1476	8.68 at 23.5 °C	3,36
a-Terpinene	5	13	145	2485	8,68	nd
a-Terpinolene	1,5	5	222	2803	9.48 at 23.5 °C	nd
Limonene	0,5	1,5	192	4932	6.32 at 23.5 °C	nd
p-Cymene	0,5	8	219	5355	23.4 at 25 °C	3,13
Alicyclic and aromatic saturated & unsaturated tertiary alcohols						
Terpinen-4-ol	30	48	14.9 at 20°C	4,46	3280 at 20°C	1,95
a-Terpineol	1,5	8	5,64	1,13	626.7 at 23.5 °C	nd
Bicyclic monoterpenes						
1,8-Cineole (Eucalyptol)	trace	15	501	71	2760 at 20°C - 3500	1,77
a-Pinene	1	6	633	29411	2.53 at 23.5 °C	nd
Sabinene	trace	3,5	981	16300	2.494	nd
Polycyclic sesquiterpenes						
Cadinane group						
δ-Cadinene	trace	3	2,51	97500	0.04863	nd
Aromadendrene group						
Aromadendrene	0,5	3	5,27	29700	0.07057	3,24
Ledene	trace	3	2,72	23300	0.07057	nd
Globulol	trace	1	0.00495	1,24	11,98	> 5
Viridiflorol	trace	1	0.00495	1,14	11,98	nd

The DS stated on page 148 and 154 of the CLH Report "*Due to their high vapour pressure and rather low water solubility, especially of the terpene hydrocarbons, most of the TTO constituents will volatilise from surface water within a very short time period after application. This is indicated by the high Henry constants and vapor pressures of the constituents*." This observation by the DS indicates a high risk of dissipation from biodegradation testing via volatilisation.

In addition, only 2 of the 15 known constituents of TTO have a low log K_{oc} value (< 3), while 4 constituents have a high log K_{oc} value above 3.0 or even an extreme high log K_{oc} value above 5.0 which indicates a high risk of dissipation from biodegradation testing via adsorption or building of non-extractable residues (NER). RAC notes that 9 of the 15 known constituents have no log K_{oc} value reported.

As a consequence of these observations, RAC notes that some constituents of TTO are difficult to test in biodegradation test systems and any result from a biodegradation test needs to be evaluated with high scrutiny. It must be ensured that the test result is really indicating rapid biodegradation and that the calculated DT_{50} value is representing a $DegT_{50}$ and is not influenced by rapid dissipation, e.g. by volatilisation, adsorption and/or building of NER. This would cause the biodegradation test to be invalid and unreliable.

Therefore, based on the Guidance on the Application of the CLP Criteria and since TTO is not a homologous series of substances within a certain range of carbon chain length and/or degree of substitution, RAC concludes that all constituents are well identified with an extreme variation of physical-chemical properties and some constituents of TTO are considered as difficult to test substances. Therefore, the degradability needs to be assessed/measured separately for each constituent.

Hydrolysis

No hydrolysis study is available, neither for the whole UVCB TTO nor for the separate constituents.

Photodegradation

The photodegradation in water is not investigated neither for the whole UVCB TTO nor for the separate constituents.

Ready Biodegradation on the whole substance

An OECD TG 310 study (Fiebig, 2010) with the whole UVCB TTO (as defined by ISO 4730:2004) as test item is available. RAC notes that, OECD TG 310 is a ready biodegradability study (Headspace Test) and that the OECD *"Guidelines for the Testing of Chemicals, Revised Introduction To The OECD Guidelines For Testing Of Chemicals, Section 3 Part I: Principles And Strategies Related To The Testing Of Degradation Of Organic Chemicals*" (OECD, 2006) indicates that ready biodegradability tests are intended for pure substances and are generally not applicable for complex compositions containing different types of constituents, like UVCB.

Further, the test report only reports the purity for 5 of the 15 known constituents which sum up to about 77 % of the test item of TTO. Therefore, the study report does not specify the purity of the constituents accounting for the remaining 23 % of the test item.

Following OECD TG 310 (section 10) the organic carbon content (% w/w) of the test substance needs to be known, either from its chemical structure or by measurement, so that the percentage degradation may be calculated. The OECD TG 310 study (Fiebig, 2010) on page 11 of 21 reports: *"The test item stock solution (100 mg/L) was prepared and the carbon content was determined".* On page 10 of 21 it specifies the carbon content in the test vessel was 10.2 mg C/L.

While the total carbon content in the test vessel is known, it is not known which other constituents of TTO, besides the five specified, were also tested.

The DS states "*the method is considered suitable according to point 11 in OECD 310, since complete aerobic degradation could be demonstrated although some of the components of the active substance exceed the Henry's law constant criterion of maximum 50 (Pa x m³)/mol.*"

However, the OECD TG 310 in point 11 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 m³) /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^3/mol) of the majority of the constituents the head space was not adjusted and normal headspace flasks with 120 mL were used.

The 10 % degradation level was reached after 2 days. The 60 % pass level was reached within the 10-d window after 5 days. The maximum biodegradation came to 106 % after 28 days.

RAC concludes that the OECD TG 310 test (Fiebig, 2010) is not reliable to conclude that TTO is readily biodegradable. Despite that the maximum biodegradation came to 106 % after 28 days, some RAC members argued that a ready biodegradation test system is intended to test pure substances only, making the test result on a complex substance difficult to interpret. According to OECD TG 310 the substance, normally at 20 mg C/L, is the sole source of carbon and energy in the medium. Here the carbon source is with only 10.2 mgC/L rather low, however still within the required range of 2 to 40 mg C/L. Further, 11 of the 15 known constituents of TTO have a high Henry's law constant and the headspace was not adjusted as requested in the TG. In addition to this reliability issues, the purity of only 5 of the 15 known constituents of TTO were specified while those constituents which build 23 % of the test item are unknown. Consequently for 10 of 15 known constituents no conclusion can be drawn based on this test results. RAC considers the uncertainties related to these shortcomings sufficient to question the reliability of the test results.

Ready Biodegradation on single constituents of TTO

Aerobic biodegradation of δ-cadinene was measured in a standard or prolonged OECD TG 301F Manometric Respirometry test for ready biodegradability (Jenner *et al.*, 2011). For δ-cadinene, removal of ThOD was <60 % after 28 days and it was concluded that this constituent of TTO is not readily biodegradable. However, it is noted that the purity of δ-cadinene in the test material was only 63.2 % (on a total of 100 % total sesquiterpenes), therefore the reliability of the results may be questionable.

During the written consultation with RAC members further evidence came available on single constituents of TTO which has not been taken into account by the DS.

RAC notes that some constituents of TTO have their own REACH registration dossier and some are considered not readily biodegradable by the registrants itself.

RAC notes that an aerobic biodegradation study (OECD TG 301 F) for γ-Terpinene is available in the publicly available REACH registration dossier (Anonymous, 2017d). Only 27 % of degradation was observed after 28 days leading to the conclusion that γ-Terpinene is not ready degradable. Because of a high vapour pressure of γ-Terpinene the reliability of the test result might be questionable and was not assessed by RAC.

In its opinion of 2019 (https://echa.europa.eu/documents/10162/9d7a0ea5-aa37-8358-6448-4f618c45a4bf) RAC concludes to consider α-Terpinene not rapidly degradable on the basis of data from an OECD TG 301F test (40 % degradation after 28 days).

The FI CA has performed a substance evaluation on Resin acids and Rosin acids, hydrogenated, esters with glycerol (CAS No 65997-13-9). In the SEV conclusion, indications of potential persistence for some sesquiterpenes (e.g., delta-cadinene) were presented.

QSAR on ready biodegradability with BioWin

RAC has assessed all 15 constituents of TTO using the SMILE code and the software BioWin v 4.10 and for none the result was "ready biodegradable". However, the reliability and the applicability domains has not been evaluated by RAC, therefore the results from QSAR prediction are only taken into account as supporting information when concluding on the rapid degradability of TTO.

Soil and sediment degradation data

RAC concludes in line with the DS that the obtained results for the non-labelled test compounds from studies according to OECD TG 307 and TG 308 are not reliable to describe degradation. The DT_{50} values from the non-radio-labelled studies must be regarded as dissipation half-life's as 11 of the 15 known constituents of TTO have a high Henry's law constant and additionally some have a high log K_{oc} value (or the log K_{oc} value is not reported) which indicates rapid volatilisation and/or rapid adsorption or the formation of NER. Only one of the constituents ([^{14}C]Terpinene-4-ol) has been radiolabelled in a soil degradation test (Bloß, 2018a) and in a water-sediment degradation test (Bloß, 2018c). Radiolabelled Terpinene-4-ol resulted to be degradable in soil.

Therefore, on the basis of the above information and in contrast to the proposal by the DS, RAC concludes to consider TTO for the purpose of environmental hazard classification as not rapid degradable. The reasoning behind this conclusion is that section 4.1.3.2.3.2. of the Guidance on the Application of the CLP Criteria requests that *"a substance is considered to be not rapidly degradable unless"* the rapid degradability has been proven. No reliable data are available to RAC to allow to conclude on rapid degradability. In contrast, for δ-cadinene (up to 3 %), γ-Terpinene (up to 28 %) and α-Terpinene (up to 13 %) information is available which might indicate non rapid degradability. As these three constituents represent up to 44 % of TTO, the criteria of Annex II.3.1 to the Guidance on the Application of the CLP Criteria where it is requested that "*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*" might be fulfilled.

Bioaccumulation

As TTO is a Substance of Unknown or Variable composition, Complex reaction products or Biological material (UVCB) the potential to bioaccumulate needs to be assessed separately for each constituent and cannot be measured in one experimental BCF test for the whole UVCB (see Guidance on the Application of the CLP Criteria - Annex III.3.2). However, for none of the known constituents of TTO a measured BCF values is available. As experimental measured Log K_{OW} are available they are usually considered more reliable than estimated BCFs to determine bioaccumulation potential. This is in line with the comments received by a NA.

Table: *BCF and log K_{OW} values of TTO constituents.*

Constituent	Min. %	Max. %	BCF		Log K_{OW}	
Monocyclic monoterpenes						
Aliphatic and aromatic hydrocarbons						
gamma-Terpinene	10	28	433	estimated	4.50; 4.36	experimental
a-Terpinene	5	13	433	estimated	4.57; 4.38	experimental
a-Terpinolene	1,5	5	296	estimated	4.47; 4.24	experimental
Limonene	0,5	1,5	360	estimated	4.57; 4.38	experimental
p-Cymene	0,5	8	236	estimated	6.34; 4.10	experimental
Alicyclic and aromatic saturated & unsaturated tertiary alcohols						
Terpinen-4-ol	30	48	66	estimated	2,8	experimental
a-Terpineol	1,5	8	68	estimated	2.98; 3.28	experimental
Bicyclic monoterpenes						
1,8-Cineole (Eucalyptol)	trace	15	433	estimated	2,74	experimental
a-Pinene	1	6	395	estimated	4.83; 4.48	experimental
Sabinene	trace	3,5	577	estimated	4,69	estimated
Polycyclic sesquiterpenes						
Cadinane group						
d-Cadinene	trace	3	6838	estimated	6,32	estimated
Aromadendrene group						
Aromadendrene	0,5	3	5129	estimated	6,13	estimated
Ledene	trace	3	5543	estimated	6,18	estimated
Globulol	trace	1	529	estimated	4,63	estimated
Viridiflorol	trace	1	529	estimated	4,63	estimated

As 12 of the 15 known constituents of TTO have an experimental measured log K_{OW} above the trigger of 4.0 and 6 of the 12 have additionally an estimated BCF value above the trigger of 500, only 3 of the 15 known constituents have an experimental measured log K_{OW} below the trigger of 4.0 and an estimated BCF value below the trigger of 500.

In contrast to the proposal by the DS, RAC concludes to consider TTO for the purpose of environmental hazard classification as having a high potential to bioaccumulate.

Aquatic toxicity

RAC is of the opinion that reliable aquatic acute and long-term toxicity data for TTO are available for fish, invertebrates, algae and aquatic plants.

Acute toxicity

Reliable acute toxicity endpoints for fish, invertebrates, algae and higher aquatic plants are available. Aquatic invertebrates are the most acutely sensitive trophic group with the lowest short-term (48 hour) EC_{50} value of 0.591 mg/L for *Daphnia magna*.

Based on these test results, RAC concludes that TTO warrants a classification as **Aquatic Acute 1 (H400)** with an **M-factor of 1** (0.1 <L(E)C50 $\leqslant$ 1 mg/L).

Chronic toxicity

RAC concludes in contrast with the proposal by the DS to classify TTO in Aquatic Chronic Category 2 with no M-factor. The reasoning behind this conclusion is that TTO is considered not rapidly degradable and that there are reliable chronic toxicity endpoints for fish, aquatic invertebrates, algae and aquatic plants. Fish are the most long-term sensitive trophic group with the lowest NOEC of 0.244 mg/L from an early life stage test with *Pimephales promelas.* Therefore, RAC concludes that TTO warrants a classification as **Aquatic Chronic 2 (H411)** without M-factor ($0.1 < NOEC \leqslant 1$ mg/L).

Additional references

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OECD (2021). Annex 6: Analysis of LLNA reference data to conclude on predictivity of alternative methods for skin sensitisation for lipophilic chemicals. Series on Testing and Assessment No. 336 Link

SCCP (2008). SCCP opinion on Tea tree oil. Link

Zuzak TJ, Rauber-Lüthy C, Simões-Wüst AP (2010). Accidental intakes of remedies from complementary and alternative medicine in children – analysis of data from the Swiss Toxicological Information Centre. Link

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter and additional information (if applicable).

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