Scientific Committee on Consumer Safety

SCCS

SCIENTIFIC OPINION on

Tea Tree Oil

(CAS/EC No. 68647-73-4 /285-377-1)

The SCCS adopted this document
by written procedure on 28 May 2025

ACKNOWLEDGMENTS

SCCS members listed below are acknowledged for their valuable contribution to the finalisation of this Scientific Advice.

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1. ABSTRACT

The SCCS concludes the following:

1. In light of the data provided and taking under consideration the possible classification as 'Repr.1B' under Regulation (EC) No 1272/2008 (CLP Regulation) and the conditions laid out in Article 15 (2) (d) of the Regulation (EC) No 1223/2009, does the SCCS consider TTO safe when used as an anti-seborrheic and anti-microbial agent in rinse-off and leave-on cosmetic products up to the maximum concentrations provided by the applicant?

The SCCS considers the use of Tea Tree Oil (TTO) as an anti-seborrheic and anti-microbial agent safe up to the maximum concentration of 2.0% in shampoo, 1.0% in shower gel, 1.0% in face wash and 0.1% in face cream. The assessment has considered all available data, a possible classification as 'Repr.1B' under Regulation (EC) No 1272/2008, the conditions laid out in Article 15 (2) (d) of the Regulation (EC) No 1223/2009, as well as the aggregated exposure from cosmetics and non-cosmetics uses of TTO.

This Opinion is only applicable to:

- TTO with chemical composition that conforms to the updated International Standard (ISO 4730:2017) in the intended final cosmetic products.
- the use of TTO in the intended dermally applied cosmetic products, and not in aerosolised or sprayable products that may give rise to inhalation exposure of the consumer.

2. Alternatively, what is according to the SCCS, the maximum concentration considered safe for use of TTO in cosmetic products?

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3. Does the SCCS have any further scientific concerns regarding the use of TTO in cosmetic products?

Based on the data provided, TTO is a moderate skin sensitiser.

The submission did not provide any data on the stability of TTO under the conditions of storage and use. Since the chemical composition of TTO may change due to exposure to light, heat, air and /or moisture, it is not clear how TTO will be stabilised in the final cosmetic products to prevent degradation/transformation of the components. The SCCS is therefore of the opinion that stability of TTO must be maintained in the final cosmetic products so that the components remain within the specifications of the updated ISO 4730:2017 standard.

The SCCS mandate does not address environmental aspects. Therefore, this assessment has not covered the safety of TTO for the environment.

Keywords: SCCS, scientific Opinion, Tea Tree Oil, Regulation 1223/2009, CAS/EC No. 68647-73-4 /285-377-1

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In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Agency (EMA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCS

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2. MANDATE FROM THE EUROPEAN COMMISSION

Background

Tea Tree Oil (TTO) (CAS/EC No. 68647-73-4 /285-377-1) is known with the INCI name 'Melaleuca Alternifolia Leaf Oil' and is reported in CosIng database to have multiple functions including antioxidant, skin conditioning, anti-microbial and perfuming. TTO is currently not regulated under the Cosmetic Regulation (EC) No. 1223/2009, however, TTO is used in various skin and hair care products as well as oral care products.

It should be noted that TTO has also non-cosmetic uses, in particular as a fragrance ingredient in household cleaning products. These non-cosmetic consumer and professional uses are included in the EU REACH registration dossier. In addition, TTO is used as active ingredient in plant protection for the prevention and control of plant diseases on horticultural and agricultural crops, while the active ingredient is currently in a re-approval process. Lastly, TTO is also used as herbal medicine and as food flavouring ingredient.

The Scientific Committee on Consumer Product (SCCP) previously assessed the safety of Tea Tree Oil in 2004[1] and in 2008[2], remarking that TTO is a skin sensitiser and could induce skin and eye irritation and contact allergy. However, the SCCP was not able to calculate a margin of safety in the absence of reliable data on dermal absorption studies.

The European Risk Assessment Committee (RAC) of ECHA issued in February 2024 (adopted in November 2023) an Opinion recommending among others a classification for TTO as 'Reprotoxic of Category 1B (H360Fd)'. Following the RAC Opinion, the European Commission may propose a classification for TTO as a 'Repr.1B' (CLP Regulation Annex VI entry).

According to Article 15(2) of the Cosmetics Regulation, 'The use in cosmetic products of substances classified as CMR substances, of category 1A or 1B under Part 3 of Annex VI to Regulation (EC) No 1272/2008 shall be prohibited.

However, such substances may be used in cosmetic products by way of exception where, subsequent to their classification as CMR substances of category 1A or 1B under Part 3 of Annex VI to Regulation (EC) No 1272/2008, all of the following conditions are fulfilled:

(a) they comply with the food safety requirements as defined in Regulation (EC) No 178/2002[3] of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety;
(b) there are no suitable alternative substances available, as documented in an analysis of alternatives;
(c) the application is made for a particular use of the product category with a known exposure; and
(d) they have been evaluated and found safe by the SCCS for use in cosmetic products, in particular in view of exposure to these products and taking into consideration the overall exposure from other sources, taking particular account of vulnerable population groups.'

In view of the above, regulatory measures must be adopted by the Commission services within 15 months of the classification as CMR 1A or 1B of the substance(s) concerned in Part 3 of Annex VI to Regulation (EC) No 1272/2008.

[1] https://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_00c.pdf
[2] https://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_160.pdf
[3] https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=OJ:L:2002:031:FULL&from=EN

In August 2024, the Commission services received a dossier to defend the safe use of Tea Tree Oil (CAS/EC No. 68647-73-4 /285-377-1) as an ingredient with anti-seborrheic and anti-microbial function in rinse-off and leave-on cosmetic products according to Article 15(2) of the Cosmetics Regulation (EC) No. 1223/2009. The Commission, therefore, requests the SCCS to carry out a safety assessment on this ingredient in view of the information provided.

Terms of reference

1. *In light of the data provided and taking under consideration the possible classification as 'Repr.1B' under Regulation (EC) No 1272/2008 (CLP Regulation) and the conditions laid out in Article 15 (2) (d) of the Regulation (EC) No 1223/2009, does the SCCS consider TTO safe when used as an anti-seborrheic and anti-microbial agent in rinse-off and leave-on cosmetic products up to the maximum concentrations provided by the applicant?*

2. *Alternatively, what is, according to the SCCS, the maximum concentration considered safe for use of TTO in cosmetic products?*

3. *Does the SCCS have any further scientific concerns regarding the use of TTO in cosmetic products?*

3. SCIENTIFIC OPINION

3.1 CHEMICAL AND PHYSICAL SPECIFICATIONS

3.1.1 Chemical identity

3.1.1.1 Primary name and/or INCI name

Tea Tree Oil
Melaleuca alternifolia (Tea Tree) Leaf Oil (INCI)

3.1.1.2 Chemical names

IUPAC Name:
Essential oil of Melaleuca alternifolia (from ECHA registration dossier)

3.1.1.3 Trade names and abbreviations

Australian Tea Tree Oil

3.1.1.4 CAS / EC number

CAS No.: 68647-73-4
EINECS No.: 285-377-1

Name	EC no	CAS no
Extract from tea tree	614-679-1	68647-73-4
Melaleuca Alternifolia (Tea Tree) Leaf Oil	641-387-1	68647-73-4
Melaleuca alternifolia, ext. Extractives and their physically modified derivatives such as tinctures, concretes, absolutes, essential oils, oleoresins, terpenes, terpene-free fractions, distillates, residues, etc., obtained from Melaleuca alternifolia, Myrtaceae.	285-377-1	85085-48-9 68647-73-4

https://echa.europa.eu/documents/10162/aa659a39-7ad6-cc4c-417f-904131581d03

3.1.1.5 Structural formula

Tea tree oil is the essential oil obtained by steam distillation of the leaves and terminal branchlets of *Melaleuca alternifolia* (Maiden *et* Betche) Cheel or of *Melaleuca linariifolia* (Smith) and conform to the requirements given in the updated International Standard (ISO 4730:2017). Minimum and maximum concentrations of the major constituents of TTO are presented in Table 1.

Table 1. Main constituents of tea tree oil (from ISO 4730:2017)

Constituent	Minimum (%)	Maximum (%)
Terpinolene	1.5	5
1,8-Cineole	Traces	10
α-Terpinene	6	12
γ-Terpinene	14	28
p-Cymene	0.5	8
Terpinen-4-ol	35	48
α-Terpineol	2	5
Limonene	0.5	1.5
Sabinene	Traces	3.5
Aromadendrene	0.2	3
δ-Cadinene	0.2	3
Globulol	Traces	1
Viridiflorol	Traces	1
α-Pinene	1	4
Ledene (syn. Viridiflorene)	0.1	3

traces: <0,01 %

SCCS comment

The essential oil extracted from Tea Tree Oil (TTO) has been associated with more than one CAS identifiers: 68647-73-4 and 85085-48-9 (ECHA Registered Substance). According to the Applicant, it conforms to the updated International Standard (ISO 4730:2017). However, whilst ISO 4730:2017 relates to essential oil of Melaleuca alternifolia (Maiden et Betche) Cheel or Melaleuca linariifolia (commonly known as narrow-leaved paperbark), it does not provide any CAS number. Instead, it refers to ISO/TR 20192:2004, where the CAS number given (85085-43-4) is for a different species (Melaleuca citronnée – *i.e.* Lemon-scented tea tree).

In view of the discrepancies in the CAS identifiers, the SCCS has considered that the essential oil assessed in this Opinion is that extracted from Melaleuca alternifolia (Maiden et Betche) Cheel or Melaleuca linariifolia with CAS identifiers as either 68647-73-4 or 85085-48-9, and that it conforms to the specifications given in the updated standard ISO 4730:2017.

It is noted that TTO consists of terpene hydrocarbons, mainly monoterpenes, sesquiterpenes and their associated alcohols, out of which fifteen (15) have been identified by the Applicant as the main components, the concentrations of which are within the ranges given in the updated ISO Standard (ISO 4730:2017).

3.1.1.6 Empirical formula

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3.1.2 Physical form

Colourless to pale-yellow liquid possessing a characteristic odour.

3.1.3 Molecular weight

Tea tree oil is a mixture of several constituents. The main constituents (given in Table 1) have MW ranging from 134 to 222 g/mol.

3.1.4 Purity, composition and substance codes

According to the Applicant, the composition and requirements for essential oil of Melaleuca, terpinen-4-ol type (Tea Tree oil) are given in the updated ISO 4730:2017 - Oil of Melaleuca, terpinen-4-ol type (Tea Tree oil), the updated Australian Standard AS 2782:2021: Oil of Melaleuca, terpinen-4-ol type (Tea Tree oil) and in European Pharmacopoeia (Ph. Eur. Monograph 1837). For the European Pharmacopoeia a submission for revision was made to the European Pharmacopoeia Department EDQM, Strasbourg, France in July 2019.

3.1.5 Impurities / accompanying contaminants

According to the Applicant, as described in the SCCP/1155/08, the composition of Tea Tree Oil changes particularly in the presence of atmospheric oxygen but also when the oil is exposed to light and higher temperatures. SCCP confirmed that according to the Code of Practice and the Guidance document introduced by ATTIA, safe processing and storage can be achieved which can be controlled by the p-cymene content. Methyl eugenol (ME) is reported as a minor constituent of TTO.

Methyl eugenol content:
The former dossiers submitted to SCCS did not contain data on methyl eugenol (ME) content in TTO. This compound is a natural constituent of essential oils. It is identified in many other types of plants in varying levels and is described to be involved in the chemical defense against pathogens (Tan and Nishida, 2012).

Methyl eugenol is listed in Annex III (III/102) of the Cosmetic Regulation (EC) No. 1223/2009. It is restricted to 0.001% in rinse-off products, 0.0002% in leave-on and oral care products, 0.01% in fine fragrance and 0.004% in Eau de toilette.

ME levels have been detected in Australian plantation-grown samples of TTO covering five seasons (2007-2001) and five production areas. The lowest level detected was 0.016% (160 ppm) and the highest 0.0552% (552 ppm) with a mean of 337 ppm. The method used for detection of ME was GC-MS, which was shown to be precise and measured also low levels of ME in a reproducible manner (Raymond *et al.*, 2017). For all Australian plantation grown TTO samples used in this publication, the plantation species was confirmed as Melaleuca alternifolia and conformed to the former ISO 4730:2004. It was also shown that the mean methyl eugenol level for Melaleuca linariifolia species is 450 ppm and falls within the range

measured for Melaleuca alternifolia. Significant differences were apparent between samples from different production regions for the Australian planation samples. Furthermore, it was shown that the ME level in another species (Melaleuca dissitiflora) is significantly higher (mean 2315 ppm). In the European Commission position paper regarding herbal products containing methyl eugenol the species Melaleuca bracteata was reported to contain levels between 2800 and 9000 ppm ME (European Commission, 2004). These are about 5-16 times higher compared to the highest levels detected in Melaleuca alternifolia reported above. Melaleuca bracteata as well as Melaleuca dissitiflora are not used in the production of Australian TTO. Therefore, in the updated standard ISO 4730:2017 the only named species for production of TTO are the terpinen-4-ol chemotypes of Melaleuca alternifolia (Maiden *et* Betche) Cheel and Melaleuca linariifolia (Smith).

In Table 14 [of the Applicant's submission] ME levels for three TTO batches are presented. The mean level of ME from these TTO samples is 201 ppm (0,02%), which was used for the calculations of the ME content in the finished cosmetic products. Inclusion of TTO at 2% in rinse-off products and 0.1% in leave-on products will result in levels of ME presented in Table 15 [of the Applicant's submission] in the finished products. In conclusion, the calculated ME levels in finished cosmetic products do not exceed the allowed concentrations according to Annex III of the Cosmetic Regulation (EC) No 1223/2009.

Table 14. ME levels from 3 TTO batches (CoA in Appendix 5)

	Results (GC-MS detection)	
TTO sample (CoA from 2nd Sept 2019)	0.02% (w/w)	201 ppm
TTO sample (CoA from 17th Oct. 2019)	0.02% (w/w)	200 ppm
TTO sample (CoA from 29th July 2020)	0.02% (w/w)	204 ppm

Table 15. Calculated levels of ME in finished cosmetic products

	Level of ME in finished product	**Allowed according 1223/2009 Regulation, Annex III**
Rinse-off (2% TTO)	0.0004%	0.001%
Leave-on (0.1% TTO)	0.00002%	0.0002%

From the publication stated above the ME levels in TTO are variable between plantation areas, with sometimes higher ME levels of 0.05%. However, the final concentration of TTO in a cosmetic formulation must be such that ME is <= 0.0002% for leave-on formulations and <=0.001% for rinse-off formulations. It is up to the manufacturer and formulator to assure that the TTO sourced and used in the cosmetic formulation will not exceed the allowed concentration to safely comply with Annex III. Therefore, the ME content will be provided in the CoA for the EU formulators and manufacturers.

SCCS comment

Methyl eugenol, which is a minor component of TTO, is listed in Annex III (III/102) of the Cosmetic Regulation (EC) No. 1223/2009, with its levels in cosmetic products restricted to 0.001% in rinse-off products, 0.0002% in leave-on and oral care products, 0.01% in fine

fragrance and 0.004% in Eau de toilette. From the provided data, the SCCS has noted that the levels of methyl eugenol will be much lower in the final cosmetic products than those allowed according to Annex III of Regulation 1223/2009.

3.1.6 Solubility

According to the Applicant, [the following] data on solubility of TTO is available from the REACH Dossier for Melaleuca alternifolia, ext. (EC 285- 377-1/CAS 85085-48-9).

Water solubility: 1420 mg/L, Guideline: OECD TG 105 (2007), flask method, GLP

(ECHA disseminated dossier, EC 285-377-1, https://echa.europa.eu/de/registration-dossier/-/registered- dossier/20921/4/9)

3.1.7 Partition coefficient (Log Pow)

According to the Applicant, [the following] data on Partition coefficient is available from the REACH Dossier for Melaleuca alternifolia, ext. (EC 285-377-1/CAS 85085-48-9).

Partition coefficient: >= 3.4 - <= 5.5 at 30°C, Guideline: OECD TG 117, HPLC method, GLP

(ECHA disseminated dossier, EC 285-377-1, https://echa.europa.eu/de/registration-dossier/-/registered- dossier/20921/4/9)

3.1.8 Additional physical and chemical specifications

Chiral ratios:
Three of the major components of TTO are enantiomers (terpinen-4-ol, α-terpineol, limonene). The following ratios, which are depicted in Table 2 [of the Applicant's submission] for the enantiomers have been reported for a TTO sample using the test method ISO 22972.

Table 2. Isomeric ratios for a typical TTO sample

	Isomeric Ratio					
	limonene (-)	limonene (+)	terpinen-4-ol (+)	terpinen-4-ol (-)	α-terpineol (-)	α-terpineol (+)
TTO sample (CoA from 27th July 2022)	38.85	61.15	68.31	31.69	24.64	75.36

The enantiomeric distribution for terpinen-4-ol as stated in the updated ISO 4730:2017 (corrected amendment) is (S)(+) 67% - 71% and (R)(-) 29% - 33%. Based on this, the TTO sample conforms to the enantiomeric distribution of the ISO 4730:2017.
Optical Rotation:
The ranges for the optical rotation have been tightened in the updated ISO 4730:2017 to between +7° and +12°. The optical ratios from 4 batches (production year 2019-2022) of TTO, which have been obtained using the test method Ph.Eur. 2.2.7 are depicted below in Table 3 [of the Applicant's submission]. The results are in the specification of the updated ISO 4730:2017.

Table 3. Optical rotation for typical TTO samples (CoA in Appendix 2)

	ISO 4730:2017 Specification	Optical rotation
TTO sample (CoA from 20th August 2019)	between +7° and +12°	+9.98°
TTO sample (CoA from 12th June 2020)		+9.94°
TTO sample (CoA from 8th June 2021)		+10.43°
TTO sample (CoA from 27th July 2022)		+9.82°

According to the Applicant, further physicochemical data from the REACH Dossier for *Melaleuca alternifolia, ext*. (EC 285-377-1/CAS 85085-48-9), on the following endpoints are available:

organoleptic properties (colour, odour, taste if relevant)

- melting point
- boiling point: 97 to 220°C, Guideline: OECD TG 1023 (2007), GLP (ECHA disseminated dossier,
- flash point: +54°C, Guideline: EU Method A.9 (2007), closed cup method, GLP (ECHA disseminated dossier, EC 285-377-1, https://echa.europa.eu/de/registration-dossier/-/registered- dossier/20921/4/9).
- vapour pressure: 2100 Pa at 25°C, Guideline: OECD TG 104 (2007), static method, GLP (ECHA disseminated dossier, EC 285-377-1, https://echa.europa.eu/de/registration-dossier/-/registered- dossier/20921/4/9).
- relative density: 0.89 at 20°C, Guideline: OECD 109, pycnometer method, GLP (ECHA disseminated dossier, EC 285-377-1, https://echa.europa.eu/de/registration-dossier/-/registered- dossier/20921/4/9).
- Freezing point: -22°C, Guideline: OECD TG 102 (2007), GLP (ECHA disseminated dossier, EC 285-377-1, https://echa.europa.eu/de/registration-dossier/-/registered-dossier/20921/4/9).
- Viscosity: kinematic viscosity 2.86 mm^2/s at 20°C and 1.71 mm^2/s at 40°C, Guideline: OECD TG 114, GLP (ECHA disseminated dossier, EC 285-377-1, https://echa.europa.eu/de/registration-dossier/-/registered- dossier/20921/4/9).
- pKa /
- pH /
- refractive index /
- UV/visible light absorption spectrum /

3.1.9 Homogeneity and Stability

SCCS comment

The Applicant has not provided any data on the stability of TTO under the conditions of storage and use. In this regard, it is notable from a study by Brophy *et al.* (1989) that the composition of TTO may change considerably during storage, with ρ-cymene levels increasing and α- and γ-terpinene levels declining. It has been suggested that exposure to light, heat, air, and/or moisture affect the oil stability. Instability of α-terpineol standard has also been indicated in the current submission (page 19).

Although the Applicant has suggested that TTO used in cosmetic products will conform to the updated International Standard (ISO 4730:2017), it is not clear how TTO will be stabilised to prevent degradation/transformation of the components in final products. The SCCS is therefore of the opinion that stability of TTO must be maintained in the final cosmetic products so that the components remain within the specifications of the updated ISO 4730:2017 standard.

3.2 TOXICOKINETICS

3.2.1 Dermal / percutaneous absorption

Study Design:

Reference:	Lorraine Mackenzie PhD and Azadeh Alinaghi PhD, 2023.
Date of final report:	2nd October 2023 (revised 1st August 2024)
Study dates:	Study Initiation: October 2018
	Experimental Start Date: January 2019 Experimental Completion Date: October 2020 Data Analysis Completion Date: February 2021
Guidelines/Methods:	OECD Guideline for Testing of Chemicals No. 428, adopted April 13, 2004 ("Skin Absorption: *in vitro* Method").
	OECD Guidance Document No. 28 for the conduct of skin absorption studies, March 2004
	Scientific Committee on Consumer Safety (SCCS, 2010) Basic criteria for the *in vitro* assessment of dermal absorption of cosmetic ingredients, June 2010, SCCS/1358/10
	Scientific Committee on Consumer Safety (SCCS, 2012) Notes of Guidance for the Testing of Cosmetic Substances and Their Safety Evaluation, 8th Revision, December 2012, SCCS/1501/12.
Skin:	Human fresh skin obtained from ProviSkin, France (538 ± 35 µm, compliant with SCCS/1358/10 specification of 200 to 500 µm), dermatomed disks of abdominal skin female abdominoplasty patients with no stretch marks and with hair (Certificates of Analysis see full report); Skin from Caucasian female donors (40.3 ± 9.7 years (mean ± SD); Range 35 - 59 years
Number of cells:	16 cells/test group (4 donors in quadruplicate)

Test substance:	Three bespoke formulated creams containing 1.25%, 2.5% and 5% TTO w/w (prepared in-house by test laboratory; exact % of the constituents of TTO was determined); Source of Tea tree oil used: P. Guinane Pty Ltd, 9441 Tweed Valley, Chinderah, NSW 2487, Australia (GMP approved manufacturing facility).
Dose:	5 mg/cm²
Receptor fluid:	4% bovine serum albumin (BSA) dissolved in 0.9% phosphate buffered saline (PBS) pH 7.4, selected by experimentation to achieve maximum solubility in fluid without impact on dermal membrane integrity
	Receptor fluids tested were, 2% BSA in PBS and 4% BSA in PBS.
Diffusion cells:	glass vertical Franz diffusion cells assembled with the allocated disc of dermatomed skin clamped between the donor and the receptor chambers with carbon filters for the volatile components; exposed surface skin area: 1.3 cm²
Skin temperature:	32°C 1°C
Skin integrity measurement:	measurements of trans epidermal water loss (TEWL) and trans epidermal electrical resistance (TEER)
Route:	Single topical application
Exposure time:	24 h
Sampling:	At 1, 2, 4, 8, 12, and 24 h 200 µL of receptor fluid was removed via sampling spout; the volume replaced by pre-warmed receptor fluid and the spout again re-sealed with parafilm®.
	Mass balance: 24h
	Tape stripping of *stratum corneum*: 20 tape strips Two carbon filters to capture the volatile phase
Analysis	validated GC-MS/MS (Shimadzu 8049 GC-MS/MS with triple quadrupole detector) to measure the permeation of four major constituents of TTO with lower limits of quantitation (LLOQ) terpinene-4-ol, 5 ng/mL; 1,8- cineole, 1 ng/mL; terpinolene, 2.5 ng/mL and γ-terpinene, 5 ng/mL and limits of detection (LOD) of 2 ng/mL; 0.33 ng/mL; 0.83 ng/mL and 1.7 ng/mL, respectively.
Quality:	equivalent to GLP, Quality Assurance Statement available. The study report and data have been audited in accordance with Quality Medication Care Pty Ltd standard procedures.

Ref: Final Report OECD TG 428: QMC Report Tea Tree Oil (TTO), Human Skin In-Vitro Permeation Study, 2nd October 2023

Material and methods:

The dermal penetration of three representative formulations containing 1.25%, 2.5% and 5% of TTO (all w/w) was studied *in vitro* through human dermatomed skin (538 ± 35 µm). The fate of 4 major constituents of Australian TTO (terpinen-4-ol, 1,8-cineole; terpinolene and γ-terpinene) was measured over 24 hours. Each formulation was applied to the skin surface at a rate of 5 mg/cm^2 to glass vertical Franz diffusion cells (1.3 cm²) representing 4 different donors. Prior to exposure the skin integrity was determined by measurements of trans epidermal water loss (TEWL) and trans epidermal electrical resistance (TEER) across the skin. During exposure the skin was maintained at a temperature of 32°C ± 1°C. As receptor fluid 4% bovine serum albumin (BSA) dissolved in 0.9% phosphate buffered saline (PBS) pH 7.4, was used without impact on dermal membrane integrity. Carbon filters were used to capture the volatile components.

Samples of the physiological saline receptor fluid were taken at recorded intervals of 1, 2, 4, 8, 12 and 24-hours post dose over the 24-hour contact period. At the 24 h time point the Franz cells were disassembled, and the various samples collected for analysis. This included residual product on the skin surface, the carbon filters, the glass donor chamber and the dermatomed skin. The skin membrane was then removed and allowed to dry naturally before being tape stripped to remove the *stratum corneum* (SC). The concentrations of each of the designated major TTO constituents were measured in the receptor phase over time and at the conclusion of the experiment in the carbon filters (evaporation) residual on the skin surface, the Franz cell apparatus and tape strips and in the epidermis and dermis skin layers using the developed and validated GC-MS/MS assay.

Tea Tree Oil component standards:

Reference standards of pure material, for each of the principal Tea Tree Oil constituents: terpinen- 4-ol, 1,8-cineole, terpinolene, γ-terpinene and α-terpineol, were obtained for use in the development and validation of the GC-MS/MS assay used for subsequent sample analysis from the *in vitro* permeation study. Although α-terpineol was successfully included as a measurable analyte in the GC-MS/MS method that was developed and subsequently validated, the α-terpineol standard proved inconsistent throughout these experiments requiring regular replacement and ultimately became unavailable. As a consequence, for experiments included in this work the α- terpineol standard needed to be replaced three times and all skin permeation samples studies to be analysed immediately after collection. Hence, the experimental data collected for α-terpineol was sporadic and therefore considered unreliable and has been excluded from the *in vitro* permeation study in this report. Estimations of α-terpineol skin penetration are therefore derived from data for the close structural isomer terpinen-4-ol.

Rationale for Selected Surrogate Substances:

α-terpineol

During the initial analytical development, the limited availability and apparent instability of the α- terpineol standard became apparent. Given the close structural and physico-chemical similarity between α-terpineol and terpinen-4-ol the dermal penetration of α-terpineol has

been derived from the experimentally determined values for terpinen-4-ol in this study. From the Combined Draft

Renewal Assessment Report prepared according to Regulation (EC) N° 1107/2009 for TTO used in plant protection (Timorex) submission to EFSA, terpinen-4-ol and α-terpineol have similar Log P values (2.643 and 2.98 respectively). Consequently, estimation of the dermal absorption of α- terpineol from that of terpinen-4-ol is reasonable.

α-terpinene

Analysis of the TTO used for the production of the bespoke creams indicated a concentration for α-terpinene of 9.54 % and for ɣ-terpinene of 20.48% in the pure oil (see table 2 of final report). These two compounds have near identical physico-chemical properties and similar Log P values (4.25, α-terpinene, 4.5, ɣ -terpinene). Use of the experimentally determined dermal penetration levels for ɣ-terpinene to estimate the level for α-terpinene is reasonable, appropriate and unlikely to underestimate total systemic absorption of this TTO constituent.

Results:
The exact percentage of the five constituents of TTO of interest in each of the three tested formulations were determined and are shown in Table 5 [of the Applicant's submission]. These values were crucial in interpreting the study results and for calculations of mass balance. For formulation 1 slightly higher values for terpinen-4-ol were achieved compared to the ISO Standard 4730:2017 values. In formulation 2 there was a slightly higher value detected for terpinolene when compared to the ISO Standard 4730:2017.

Table 5. TTO Constituents as % of Total TTO in tested formulations

Component	Formulation 1	Formulation 2	Formulation 3	ISO Standard 4730:2017
% TTO added to the Product	1.25	2.5	5.0	
Terpinen-4-ol	50.4	41.6	47.2	35 - 48
1,8-Cineole	3.2	2.8	3.0	Trace - 10
Terpinolene	3.2	5.6	3.2	1.5 - 5
ɣ-Terpinene	18.4	15.2	17.4	14 - 28
α-Terpineol	4.0	3.2	3.6	2 - 5

Thresholds for acceptance for skin integrity measurements were TEWL ≤ 50 g/m^{2}h and TEER ≥ 50 kΩ. Where skin had TEER measurements that did not meet acceptability but where the TEWL measurements did, the skin was accepted for the experiment.

The permeation rate and mass balance were determined separately for each TTO constituent in each diffusion cell and summarized as mean ± S.D. The overall adjusted mass balance to reflect the volatile components is shown in Table 6 [of the Applicant's submission]. For the defined creams, recovery of applied analytes and their mass balance was generally high and acceptable given the complex nature of TTO, the volatility of the components and the challenge of volatile capture without creation of occluded exposure conditions. For highly volatile negligibly absorbed analytes such as ɣ-terpinene and terpinolene acceptable mass balance required adjustment of the experimental values using data from preliminary experiments where

capture filters were changed at every sampling point as opposed to once at the end of the exposure/sampling period (that is: adjusted to account for filter saturation).

Table 6. Observed (Obs.) and adjusted (Adj.) mass balance amounts expressed as % applied dose for Terpinen-4-ol, 1,8-Cineole, Terpinolene and ɣ-Terpinene in each product. Mean ± SD; N=16; non-detectable values were excluded.

Product: Formulation Cream 1.25%								
	Terpinen-4-ol		**1,8-Cineole**		**Terpinolene**		**ɣ-Terpinene**	
Obs./ Adj.*	111.5 ± 24.1	111.5 ± 24.1	115.5 ± 12.6	106.1 ± 9.9	5.5 ± 2.5	83.3 ± 19.4	4.3 ± 2.8	92.7 ± 30.4
Product: Formulation Cream 2.5%								
	Terpinen-4-ol		**1,8-Cineole**		**Terpinolene**		**ɣ-Terpinene**	
Obs./ Adj.*	116.9 ± 29.6	116.9 ± 29.6	119.8 ± 7.9	119.2 ± 7.7	4.1 ± 1.4	115.8 ± 8.9	3.4 ± 1.4	109.5 ± 8.9
Product: Formulation Cream 5%								
	Terpinen-4-ol		**1,8-Cineole**		**Terpinolene**		**ɣ-Terpinene**	
Obs./ Adj.*	93.9 ± 18.7	93.9 ± 18.7	96.7 ± 22.8	96.7 ± 22.8	5.1 ± 5.1	101.2 ± 9.8	3.2 ± 1.2	122.5 ± 4.7

* Adjusted to reflect the volatile component collection using data from preliminary studies where volatiles capture filters were replaced at each sampling time point as opposed to the end of the 24 hour *in vitro* permeation studies.

The dermal penetration rates were not equal for the four compounds. The mean bioavailable dose (receptor fluid + epidermis + dermis) of terpinen-4-ol was 5750 ± 3413 ng/cm² (14.0 ± 7.3%) of applied dose for the 1.25% formulation. For the 2.5% formulation it was 21931 ± 10041 ng/cm^2 (31.6 ± 11.0%) and for the 5% formulation it was 37077 ± 14647 ng/cm^2 (26.4 ± 10.6%) of the dose applied (see Table 7 [of the Applicant's submission]). From the 4 components which were measured terpinen-4-ol showed the highest dermal absorption in all three formulations.

Table 7. Cumulative amount of Terpinen-4-ol in in Receptor Fluid and Skin Layers at 24 hours

Amount of **Terpinen-4-ol** (ng/cm^2) and expressed as a percentage w/w of the applied dose in receptor phase and skin layers **Mean ± SD; N=16**			
	Formulation 1.25%	**Formulation 2.5%**	**Formulation 5%**
Receptor Phase Cumulative 24 h	5623 ± 3417	21745 ± 10030	37060 ± 14653
TS 4-20	273 ± 108	271 ± 71	50 ± 8
Epidermis	38 ± 9	27 ± 11	<LLOQ
Dermis	89 ± 45	150 ± 50	17 ± 48
Dermal Absorption (epidermis, dermis, receptor fluid)	5750 ± 3413	21931 ± 10041	37077 ± 14647
Amount Expressed as % Applied Dose of Terpinen-4-ol in each Product			
Receptor Phase Cumulative 24 h	13.7 ± 7.3	31.4 ± 11	26.3 ± 10.6
TS 4-20	0.7 ± 0.4	0.4 ± 0.2	0.03 ± 0.06
Epidermis	0.1 ± 0.0	0.04 ± 0.02	N/A
Dermis	0.3 ± 0.2	0.2 ± 0.1	0.01 ± 0.03
Dermal Absorption (epidermis, dermis, receptor fluid)	14.0 ± 7.3	31.6 ± 11.0	26.4 ± 10.6

TS 4-20 = tape strips analysed collectively number 4-20; < LLOQ = below the limit of quantitation for terpinen-4-ol = 3.86 ng/cm^2

N/A = not applicable; Dermal Absorption = cumulative amount in receptor phase at 24 h + in Epidermis + in Dermis.

The mean bioavailable dose (receptor fluid + epidermis + dermis) of 1,8-Cineole was 69 ± 22 ng/cm² (2.7 ± 0.9%) of applied dose for the 1.25 % formulation. For the 2.5% formulation it was 212 ± 81 ng/cm^2 (4.6 ± 1.4%) and for the 5% formulation it was 358 ± 155 ng/cm^2 (4.0 ± 1.8%) of the dose applied (see Table 8 [of the Applicant's submission]).

Table 8. Cumulative amount of 1,8-Cineole in in Receptor Fluid and Skin Layers at 24 hours

Amount of **1,8-Cineole** permeated (ng/cm^2) and expressed as a percentage of the applied dose in receptor phase and skin layers **Mean ± SD; N=16**			
	Formulation 1.25%	**Formulation 2.5%**	**Formulation 5%**
Receptor Phase Cumulative 24 h	59 ± 22	209 ± 81	358 ± 155
TS 4-20	11 ± 2	7 ± 3	3 ± 3
Epidermis	4 ± 1	<LLOQ	<LLOQ
Dermis	6 ± 1	2 ± 1	<LLOQ
Dermal Absorption Bioavailability (epidermis, dermis, receptor fluid)	69 ± 22	212 ± 81	358 ± 155
Amount Expressed as % Applied Dose of 1,8-Cineole in each product			
Receptor Phase Cumulative 24 h	2.3 ± 0.9	4.5 ± 1.4	4 ± 1.8
TS 4-20	0.4 ± 0.1	0.2 ± 0.1	0.03 ± 0.03
Epidermis	0.2 ± 0.1	N/A	N/A
Dermis	0.2 ± 0.0	0.05 ± 0.03	N/A
Dermal Absorption (epidermis, dermis, receptor fluid)	2.7 ± 0.9	4.6 ± 1.4	4.0 ± 1.8

TS 4-20 = tape strips analysed collectively number 4-20; < LLOQ = below the limit of quantitation for 1,8-cineole = 0.77 ng/cm2 N/A = not applicable; Dermal Absorption = cumulative amount in receptor phase at 24 h + in Epidermis + in Dermis.

The mean bioavailable dose (receptor fluid + epidermis + dermis) of terpinolene was 52 ± 20 ng/cm² (2.0 ± 0.7%) of applied dose for the 1.25% formulation. For the 2.5% formulation it was 95 ± 23 ng/cm^2 (2.2 ± 0.7%) and for the 5% formulation it was 309 ± 124 ng/cm^2 (3.3 ± 1.6%) of the dose applied (see Table 9 [of the Applicant's submission]).

Table 9. Cumulative amount of Terpinolene in Receptor Fluid and Skin Layers at 24 hours

Amount of **Terpinolene** permeated (ng/cm^2) and expressed as a percentage of the applied dose in receptor phase and skin layers **Mean ± SD; N=16**			
	Formulation 1.25%	**Formulation 2.5%**	**Formulation 5%**
Receptor Phase Cumulative 24 h	43 ± 20	88 ± 23	299 ± 123
TS 4-20	38 ± 13	35 ± 8	30 ± 8
Epidermis	3 ± 1	3 ± 1	3 ± 3
Dermis	5 ± 1	4 ± 1	6 ± 4
Dermal Absorption (epidermis, dermis, receptor fluid)	52 ± 20	95 ± 23	309 ± 124
Amount Expressed as % Applied Dose of Terpinolene in each product			
Receptor Phase Cumulative 24 h	1.6 ± 0.7	2.0 ± 0.7	3.2 ± 1.5
TS 4-20	1.5 ± 0.7	0.8 ± 0.2	0.3 ± 0.1
Epidermis	0.1 ± 0.0	0.1 ± 0.02	0.03 ± 0.03
Dermis	0.2 ± 0.0	0.1 ± 0.03	0.1 ± 0.1
Dermal Absorption (epidermis, dermis, receptor fluid)	2.0 ± 0.7	2.2 ± 0.7	3.3 ± 1.6

TS 4-20 = tape strips analysed collectively number 4-20; < LLOQ = below the limit of quantitation for terpinolene = 1.92 ng/cm^2 Dermal Absorption = cumulative amount in receptor phase at 24 h + in Epidermis + in Dermis

The mean bioavailable dose (receptor fluid + epidermis + dermis) of ɣ-terpinene was 337 ± 213 ng/cm² (2.2 ± 1.4%) of applied dose for the 1.25% formulation. For the 2.5% formulation it was 654 ± 204 ng/cm^2 (2.8 ± 1.1%) and for the 5% formulation it was 1514 ± 480 ng/cm^2 (2.9 ± 1.0%) of the dose applied (see Table 10 [of the Applicant's submission]).

Table 10. Cumulative amount of ɣ-Terpinene in Receptor Fluid and Skin Layers at 24 hours

Amount of **ɣ-Terpinene** permeated (ng/cm²) and expressed as a percentage of the applied dose in receptor phase and skin layer **Mean ± SD; N=16**			
	Formulation 1.25%	**Formulation 2.5%**	**Formulation 5%**
Receptor Phase Cumulative 24 h	325.3 ± 210.5	635 ± 211.3	1513 ± 481
TS 4-20	157 ± 48	98 ± 35.0	71 ± 35
Epidermis	6 ± 4	4 ± 3	5 ± 5
Dermis	6 ± 3	15 ± 13	21 ± 19
Dermal Absorption (epidermis, dermis, receptor fluid)	337 ± 213	654 ± 204	1514 ± 480
Amount Expressed as % Applied Dose of ɣ-Terpinene in each product			
Receptor Phase Cumulative 24 h	2.2 ± 1.4	2.7 ± 1.1	2.9 ± 1.0
TS 4-20	1.1 ± 0.5	0.4 ± 0.2	0.1 ± 0.1
Epidermis	0.04 ± 0.03	0.0 ± 0.0	0.01 ± 0.01
Dermis	0.04 ± 0.02	0.1 ± 0.0	0.04 ± 0.03
Dermal Absorption (epidermis, dermis, receptor fluid)	2.2 ± 1.4	2.8 ± 1.1	2.9 ± 1.0

TS 4-20 = tape strips analysed collectively number 4-20; < LLOQ = below the limit of quantitation for ɣ-terpinene = 3.85 ng/cm2 N/A = not applicable; Dermal Absorption = cumulative amount in receptor phase at 24 h + in Epidermis + in Dermis.

Applicant's Conclusion and Interpretation:

From this dermal absorption study the extent of TTO penetration through human dermatomed skin into the receptor fluid was calculated from the sum obtained for the single components terpinen- 4-ol, 1,8- cineole, terpinolene and ɣ-terpinene. Total dermal absorption of TTO constituents over a 24-hour exposure period, expressed as a percentage of whole TTO applied is calculated in the Tables 11 to 13 [of the Applicant's submission]. The results demonstrate that the various constituents of TTO do permeate human skin layers differently and are considered as dermally bioavailable. It was shown from the experiments that the constituent terpinen-4-ol has reached the receptor fluid with very little retention in the skin layers while the terpenes are more retained in the skin layers. Terpinen-4-ol and, by read-across, α-terpineol, readily permeated through the skin to the receptor fluid. Terpinolene and ɣ-terpinene, and α-terpinene by read-across, penetrated the skin very poorly and largely were confined to the epidermis. For all components the majority of that recovered was from the carbon filters indicating the importance of evaporation of volatiles from the skin to the overall absorption profile of these types of material. The pattern of observations reflects the physicochemical properties of the analytes (primarily the Log P value and volatility). Of all TTO analytes absorbed, terpinen-4-ol represented 93% with all other components combined accounting for less than 7% absorbed TTO.

Furthermore, 70 to greater than 90% of all analytes applied to skin membranes was recovered from the carbon filters reflecting the high volatility of TTO constituents. This observation is critical to an interpretation of the dermal absorption data obtained in this study. In normal

consumer use scenarios TTO containing products will be applied to the surface of the skin with no occlusion or restriction of air flow across the skin surface. In the study detailed here the donor chamber of the Franz cells was necessarily covered with 2 carbon filters to capture volatile components. The presence of these filters will reduce air movement and convection currents across the surface of the skin membrane and produce a somewhat semi-occlusive environment. These factors are likely to enhance dermal absorption at least modestly.

The Combined Renewal Assessment Report prepared according to Regulation (EC) N° 1107/2009 reports a human skin *in vitro* study of dermal absorption of radiolabelled terpinen-4-ol from an agricultural pesticide using TTO as the active constituent (Volume 3 – B.6, page 60). In that study both an open static diffusion cell and a closed system to capture volatiles were compared. The recovery of terpinen-4-ol from receptor fluid for the open system where the product concentrate was applied (2.225% TTO) was only 2.63% compared to 11.63 % in the closed system in that study. A similar disparity was observed for the dilute crop application preparation. These results highlight the impact of volatilization restriction on dermal absorption of essential oils such as TTO, resulting in exaggerated dermal absorption factors.

The data in the study reported below has been generated using 24-hour exposure to the applied TTO product and will grossly overestimate the absorption of TTO from rinse-off products such as shampoos and face wash where dermal contact is unlikely to exceed 5 minutes. For rinse-off products an appropriate adjustment factor could be applied to reduce the dermal absorption value substantially. Based on the plant protection study the maximal dermal absorption of 20.41% for the 2.5% formulation derived from the current study might reasonably be adjusted downwards by a factor 4 for leave on products to account for the impact of carbon filters covering the donor chamber of the Franz cells and by a further factor of at least 2 for rinse off products with a dermal contact period of <5 minutes compared to the 24 hours of the current study.

Based on the close structural and physicochemical similarity between α-terpineol and terpinen- 4-ol the dermal penetration of α-terpineol has been derived from the experimentally determined values for terpinen-4-ol in this study. Furthermore, as discussed above, the experimentally determined dermal penetration levels for ɣ-terpinene can be used to estimate the level for α- terpinene. Therefore, these values have been added to the values actually measured in this study to derive a dermal penetration value for TTO representing > 80% of its constituents. Considering the current conservative SCCS procedure, the mean values plus 1SD are used for SED and subsequent MoS calculation. So, the final dermal absorption values used for calculation of the MoS were 12.84% for formulations up to 1.25% TTO and 20.41% for formulations containing up to 2.5% TTO in this dossier. As stated below this is a worst-case scenario since dermal absorption is overestimated.

Table 11. Cumulative values for dermal absorption of TTO for 5% formulation

Component	% present in TTO	% absorption from OECD 428 (± SD)	% of applied TTO absorbed	SD
Terpinen-4-ol	47.2	26.4 ± 10.6	12.5	5.0
1,8- Cineole	3.0	4 ±1.8	0.12	0.054
Terpinolene	3.2	3.3 ± 1.6	0.11	0.051
γ-Terpinene	17.4	2.9 ± 1	0.50	0.17
*α-terpineol**	*3.6*	*26.4 ± 10.6*	*0.95*	*0.38*
*α-terpinene**	*9.54*	*2.9 ± 1*	*0.28*	*0.095*
			14.46	**5.8**
Dermal absorption for MoS calculation of TTO (5% formulation)			**20.26 (mean + 1SD)**	

*read-across using the measured % dermal absorption values from terpinen-4-ol for α-terpineol or γ-terpinene for α-terpinene

Table 12. Cumulative values for dermal absorption of TTO for 2.5% formulation

Component	% present in TTO	% absorption from OECD 428 (± SD)	% of applied TTO absorbed	SD
Terpinen-4-ol	41.6	31.60 ± 11	13.15	4.6
1,8- Cineole	2.8	4.60 ± 1.4	0.13	0.04
Terpinolene	5.6	2.20 ± 0.7	0.12	0.04
γ-Terpinene	15.2	2.80 ± 1.1	0.43	0.17
*α-terpineol**	*3.2*	*31.60 ± 11*	*1.01*	*0.35*
*α-terpinene**	*9.54*	*2.80 ± 1.1*	*0.27*	*0.1*
			15.11	**5.3**
Dermal absorption for MoS calculation of TTO (2.5% formulation)			**20.41 (mean + 1SD)**	

*read-across using the measured % dermal absorption values from terpinen-4-ol for α-terpineol or γ-terpinene for α- terpinene

Table 13. Cumulative values for dermal absorption of TTO for 1.25% formulation

Component	% present in TTO	% absorption from OECD 428 (± SD)	% of applied TTO absorbed	SD
Terpinen-4-ol	50.4	14.0 ± 7.3	7.1	3.7
1,8- Cineole	3.2	2.7 ± 0.9	0.086	0.03
Terpinolene	3.2	2.0 ± 0.7	0.06	0.022
ɣ-Terpinene	18.4	2.2 ± 1.4	0.40	0.25
*α-terpineol**	*4*	*14.0 ± 7.3*	*0.56*	*0.29*
*α-terpinene**	*9.54*	*2.2 ± 1.4*	*0.21*	*0.13*
			8.42	**4.42**
Dermal absorption for MoS calculation of TTO (1.25% formulation)			**12.84 (mean + 1SD)**	

*read-across using the measured % dermal absorption values from terpinen-4-ol for α-terpineol or ɣ-terpinene for α-terpinene

Physicochemical data as weight of evidence of low dermal absorption

Log Pow

The Log Pow of TTO is 3.4-5.5 at 30°C (ECHA disseminated dossier, EC 285-377-1), https://echa.europa.eu/de/registration-dossier/-/registered- dossier/20921/4/9. According to ECHA Log Pow values between 1 and 4 favour dermal absorption (values between 2 and 3 are optimal) particularly if water solubility is high. Above 4, the rate of penetration may be limited by the rate of transfer between the *stratum corneum* and the epidermis, but uptake into the *stratum corneum* will be high (ECHA R7c, 2023).

The log Pow of individual constituents of TTO which were not dosed in the dermal absorption study are reported in the Combined Draft Renewal Assessment Report prepared according to Regulation (EC) N° 1107/2009 for TTO used in plant protection:

Alpha-terpineol: log Pow = 2.98 Alpha-terpinene: log Pow = 4.25 p-Cymene: log Pow = 6.34

Limonene: Log Pow = 4.57

The log Pow for most of the remaining components of TTO (α-Pinene, Aromadendrene, δ-Cadinene, Sabinene, Globulol, Viridiflorol, Ledene) is above 4.

EMA indicated in their Monograph on TTO that "*In vitro skin permeation studies using human skin preparations demonstrate that the extent of penetrating of TTO components is very low, with the more polar terpinen-4-ol and α-terpineol being the only components which penetrate to any appreciable levels*" (EMA, 2014).

Vapour Pressure

The maximum vapour pressure of TTO is 2100 Pa at 25°C (ECHA disseminated dossier, EC 285- 377-1, https://echa.europa.eu/de/registration-dossier/-/registered-dossier/20921/4/9). According to ECHA "*The rate at which gases and vapours partition from the air into the stratum corneum will be offset by the rate at which evaporation occurs therefore although a substance may readily partition into the stratum corneum, it may be too volatile to penetrate further. This can be the case for substances with vapour pressures above 100-10,000 Pa (ca. 0.76-76 mm Hg) at 25°C, though the extent of uptake would also depend on the degree of occlusion, ambient air currents and the rate at which it is able to transfer across the skin*."

The vapour pressure of individual constituents of TTO which were not dosed in the dermal absorption study are reported in the Combined Draft Renewal Assessment Report prepared according to Regulation (EC) N° 1107/2009 for TTO used in plant protection:

Alpha-terpineol: 5.64 Pa at 25°C Alpha-terpinene: 145 Pa at 25°C

EMA confirmed this point by indicating "*As TTO oil is a semi-volatile substance, the majority of the applied dose rapidly evaporates from the surface of the skin before it has the chance to absorb into the skin*." (EMA, 2015).

CIR indicates that in a study in which TTO was applied to filter paper, stored in an oven at 30°C, and then weighed, application of 1.4 mg/cm^2 evaporated within 1 h, and 84, 98, and 100% of a 7.4 mg/cm^2 application evaporated within 2, 4, and 8 h, respectively (CIR, 2021).

Conclusion:

Considering the high Log Pow and high vapour pressure of TTO (and its constituents not analysed in the dermal absorption study), the rate of dermal penetration of TTO may be globally limited. Therefore, the % values of absorption determined in the dermal absorption study according OECD TG 428 presented above are conservative even if not all constituents were analysed. Furthermore, 70 to greater than 90% of all analytes applied to skin membranes in the OECD TG 428 study were recovered from the carbon filters reflecting the high volatility of TTO constituents. This observation is critical to an interpretation of the dermal absorption data obtained in this study. In normal consumer use scenarios TTO containing products will be applied to the surface of the skin with no occlusion or restriction of air flow across the skin surface. In the OECD TG 428 study the donor chamber of the Franz cells was necessarily covered with 2 carbon filters to capture volatile components. The presence of these filters will reduce air movement and convection currents across the surface of the skin membrane and produce a somewhat semi-occlusive environment. These factors are likely to enhance dermal absorption at least modestly. The data in the study reported below has been generated using 24-hour exposure to the applied TTO product and will overestimate the absorption of TTO from rinse-off products such as shampoos and face wash where dermal contact is unlikely to exceed 5 minutes.

SCCS comments on dermal absorption

The Applicant has provided an OECD TG 428 study on the dermal absorption of TTO through human dermatomed skin at 3 tested concentrations: 1.25, 2.5 and 5%. As TTO is a complex mixture, dermal absorption was calculated from the sum obtained for six single components: terpinen- 4-ol, 1,8- cineole, terpinolene and ɣ-terpinene, *α-terpineoland α-terpinene*. The

Applicant has also provided rationale for the choice of the 4 substances as indicators of the dermal absorption of TTO.
The SCCS has noted that the results for the absorption of terpinene-4-ol to dermis layer of skin in this study are in line with those reported by Chooluck (2013), although dose metrics used in both studies are different.

Considering that TTO used in cosmetic products will conform to the specifications of the updated International Standard (ISO 4730:2017), the SCCS has accepted the Applicant's measured values for dermal absorption as 12.84% (Mean + 1SD) for formulations up to 1.25% TTO, and 20.41% (Mean + 1SD) for formulations containing up to 2.5% TTO.

3.2.2 Other studies on toxicokinetics

According to the Applicant, a search of the published literature did not identify any studies reporting the oral bioavailability for TTO or its compounds terpinen-4-ol and relatively few comprehensive pharmacokinetic studies (PK) are available for any of the related monoterpenoids. Terpinen-4-ol is a variant of the p-menthane (1 methyl-4-isopropyl-cyclohexane) skeleton. P-Menthane compounds closely related structurally to terpinen-4-ol on which useful pharmacokinetic data were located were α- Terpineol and menthol. Terpinen-4-ol, α-terpineol, & menthol are all mono hydroxylated p- menthane variants and have similar log P values. Terpinen-4- ol and α-terpineol in particular vary only in the location of the single hydroxyl group. Given these similarities, there is a reasonable basis for concluding that these compounds will have similar pharmacokinetics. For most compounds, iv pharmacokinetic studies, which are essential for determining absolute oral bioavailability, are not available.

Estimates of oral absorption based on urinary excretion of metabolites provide a useful lower bound to percentage of an oral dose absorbed but potentially omit the proportion of absorbed monoterpene which is excreted in the bile, exhaled from the lungs or excreted via gut drug transporters. An iv PK study is available for terpinen-4-ol and a comparatively well reported oral PK study is available for α-terpineol. The plasma AUC after iv administration of 2 mg/kg terpinen-4-ol to rats was 4.25 µg.h/mL or 2.125 µg.h/mL per mg/kg bw administered (Chooluck, *et al.*, 2013). The absorption of [isopropyl methyl-^{14}C]-alpha-terpineol were studied after single oral doses at 75, 250 and 750 mg/kg bw to male rats (ECHA disseminated dossier EC number: 701-188-3, Section 7.1, https://echa.europa.eu/de/registration-dossier/-/registered- dossier/20921/4/9). The AUC for α-terpineol administered orally at 75 mg/kg bw/day was 102 µg .h/g in plasma or 1.36 µg.h/mL per mg/kg bw administered. The AUC for this compound was reasonably dose proportional between 75 and 250 mg/kg bw but supra-proportional from 250 to 750 mg/kg bw. This pattern suggests saturation of metabolism at higher doses and that at doses below 75 mg/kg bw the relationship between the AUC and dose administered should be reasonably linear.

If the data for terpinen-4-ol and α-terpineol are combined the approximate oral bioavailability of α-terpineol and terpinen-4-ol is around 60% (dose normalised AUC oral/AUC iv = 1.36/2.125 = 64%). Recovery of α-terpineol in urine following an oral dose of 75 mg/kg bw in rats was 65% of that administered which supports the conclusion that the above calculation does not over-estimate the oral bioavailability. Similarly, for menthol the combined recovery from urine and bile following oral administration of 500 mg/kg bw, to bile duct cannulated rats, was 74% which is comparable to that obtained for α-terpineol (OECD SIDS Menthols, 2003). None of the available studies have examined loss of administered terpenes by

exhalation which is likely to account for some, albeit small, additional excretion of these volatile compounds. Overall, an assumption of 70% oral bioavailability for TTO is reasonable, conservative and consistent with the limited data available on the terpinen-4-ol and the closely related compounds α-terpineol and menthol. This value was used for MoS calculation in this dossier.

Data on ADME also have been provided in the Combined Draft Renewal Assessment Report prepared according to Regulation (EC) N° 1107/2009. Here, based on the data for the constituents, it was concluded that TTO is metabolized and excreted from experimental animals within 2-3 days, mainly via urine (d-limonene in Wistar rats cleared within 48 hours). No evidence of bioaccumulation was reported. Due to the structural similarity to the evaluated substances (same or no additional functional groups) no different metabolic pathways as described for the evaluated substances are expected for TTO and metabolism of the components was described to be comparable. The conclusion was that there are no dangerous/non-toxic metabolites synthetized.

SCCS comments

In an oral toxicokinetic study in male rats exposed to 75, 250 and 750 mg/kg bw of α-terpineol, the recovery of α-terpineol in urine was 65% of the administered dose (ECHA disseminated dossier EC number: 701-188-3, Section 7.1, https://echa.europa.eu/de/registration-dossier/-/registered- dossier/20921/4/9). The SCCS is of the opinion that, from this study, the oral bioavailability of α-terpineol can be estimated as 65%. Considering that kinetic of α-terpineol and terpinen-4-ol could be similar, this oral bioavailability value can also be applied to terpinen-4-ol.

3.3 EXPOSURE ASSESSMENT

3.3.1 Function and uses

The use of TTO in cosmetics is currently not regulated under the Cosmetic Regulation (EC) No1223/2009 of the European Union.

The primary function defended for Tea Tree Oil in the indicated cosmetic products is with natural anti-microbial function. Anti-seborrheic properties cover preventing or relieving the symptoms of seborrhea and/or seborrheic dermatitis, including dandruff (Cosing, 2024). This is achieved by preventing and/or slowing down microbial growth naturally on the skin to maintain the skin in good conditions.

Mode of Action of TTO

In the EU Regulation 96/335/EC, released on 6 Feb 1996, Melaleuca alternifolia oil is listed as anti-microbial. Cox *et al.*, 2000 reported that its anti-microbial activity results from its ability to disrupt the permeability barrier of the microbial membranes at already the low concentration of 0.125%. Damage to the microbial membranes is reported to be the most likely cause of cell death (Cox *et al.*, 2000). In their review article Carson *et al.*, 2006 stated that TTO is for its most part bactericidal in nature by compromising structural and functional integrity of the bacterial membrane. Similar mode of action by causing damage to the fungal

membranes and alteration of its permeability was reported for the fungus *Malassezia furfur*, which is associated with dandruff and seborrhea. The components of TTO have different activities, but all are considered active, and activity correlates with the presence of functional groups and the solubility of compounds in biological membranes. The component 1,8-cineole for example may not be one of the primary anti-microbial components but may facilitated the entry of other more active components (Carson *et al.*, 2006). Therefore, its unique combination of compounds facilitates the natural skin protecting properties of TTO and builds the basis for its anti-seborrheic function. Sebaceous secretions and fungal colonization are factors contributing to dandruff and seborrheic skin conditions. The fungus genus *Malassezia furfur* is reported to be involved since it requires lipids for growth and thus, primarily colonizes sebum rich areas (reported in Santos and da Silva, 2024) and is present in the normal flora of the skin. Nenoff evaluated the *in vitro* activity of TTO against 22 *Malassezia furfur* und other yeast strains. The minimum inhibitory concentrations (MIC) of TTO were measured by agar dilution technique. *Malassezia furfur* was most susceptible to TTO even at the low concentration of 0.5% (Nenoff, 1996).

In a randomized, single-blind, parallel-group trial in 126 male and female study participants using 5% TTO shampoo for 4 weeks 41% showed an improvement of seborrheic dermatitis. Significant improvement was also reported on oiliness and no adverse effects were reported (Satchell *et al.*, 2002). In a further study the use of TTO in a gel formulation (5%) demonstrated when used three times a day for 4 weeks by 42 study participants that TTO is effective on improving mild to moderated facial seborrhea skin conditions (Beheshti Roy A *et al.*, 2014). The authors also reported significant differences between the TTO and placebo group in improvement of itching and greasy crusts. The follicular uptake of TTO upon application of different 5% TTO formulations was determined using high-performance thin layer chromatography (Biju *et al.*, 2004). The authors showed that TTO reaches sebaceous follicular casts in bovine udder skin, which is of importance for providing anti-seborrheic functions of TTO on skin.

In an in-use study under dermatologist control, 25 volunteers applied a shampoo formulation with 2% TTO every day for regular hair wash for up to 56 days (REPORT No. 86195/17/JSHI, Appendix 3). The first 28 days a scalp lotion (1% TTO) was applied in addition as leave-in overnight. Volunteers present rather severe and severe dandruff, itching and irritation on the scalp and hair. From this in-use test it was concluded that the products were well tolerated on the scalp and hair and reduced dandruff, reduced itching and irritation (clinical evaluation). In Table 4 [of the Applicant's submission] it is depicted which product category, concentration and function of TTO is defended
in this dossier and used for MoS calculation. Based on the information presented above, a positive effect on skin and scalp as anti-seborrheic ingredient is anticipated at even low concentrations.

REPORT No. 86195/17/JSHI: REPORT OF USE TEST AND INSTRUMENTAL TEST UNDER DERMATOLOGIST CONTROL

Table 4. Concentration of TTO and function in product categories defended

Product category	Concentration	Function	Retention factor	Type of cosmetic product exposure
Shampoo	2.0%	anti-seborrheic anti-microbial	0.01	Rinse-off Skin & hair products
Shower gel	1.0%	anti-seborrheic anti-microbial	0.01	Rinse-off Skin & hair products
Face wash	1.0%	anti-seborrheic anti-microbial	0.01	Rinse-off Skin & hair products
Face cream	0.1%	anti-seborrheic anti-microbial	1	Leave on Skin products

TTO is not intended to be included in sprays or aerosols. The target consumer group are exclusively adults.

Non-cosmetic uses
TTO is used as a fragrance ingredient in household cleaning products. Non-cosmetic consumer and professional uses are included in the EU REACH dossier. Furthermore, TTO is used as active ingredient in plant protection for the prevention and control of plant diseases on horticultural and agricultural crops. The active ingredient is currently in a re-approval process. TTO is also used as herbal medicine (EMA monograph) and as food flavouring ingredient.

3.4 Calculation of SED/LED

3.4.1 Dermal route (cosmetic exposure)

The Applicant stated that exposure assessment was performed according to the SCCS Notes of Guidance (SCCS/1647/22). The external exposure was used to calculate the internal (or systemic) exposure (SED). For the calculation of the SED, absorption (or uptake) specific to the respective exposure route was taken into account.

The SED was calculated for face cream, shampoo and shower gel considering the daily external exposure values (E product) per kg body weight as specified in the SCCS Note of Guidance. The SED for face wash was calculated assuming a daily applied amount of 6.861 g (Ficheux *et al.*, 2016; P95 value for liquid face cleanser, adult women), a body weight of 60 kg and a retention factor of 0.01 (both from SCCS Note of Guidance). The data from the dermal absorption study presented above was used to calculate the systemic (internal) exposure. According to the SCCS requirements, the mean absorption plus 1 standard deviation (mean + 1 SD) was used.

The results of the cosmetic exposure calculations are presented below in Table 16 [of the Applicant's submission].

The systemic exposure dose of TTO for shampoo (2% use concentration) is 0.0062 mg/kg bw/day. The systemic exposure dose of TTO for shower gel (1% use concentration) is 0.0036 mg/kg bw/day, for face cream 0.0031 mg/kg bw/day and for face wash 0.0015 mg/kg bw/day.

The default value for all cosmetic products were used from the SCCS Note of Guidance. (Ref 12th revision, 2023, SCCS/1647/22)

Table 16. SED for cosmetic exposure

Shampoo			
Dermal absorption as percentage (%)	DA (%)	20.41	%
Calculated relative daily exposure to shampoo	E product (mg/kg bw/day)	1.51	mg/kg bw/day
Concentration in product at application site	C (%)	2.00	%
Systemic exposure dose (SED)	**(Eproduct x C/100 x DA/100)**	**0.0062**	**mg/kg bw/day**

Shower gel			
Dermal absorption as percentage (%)	DA (%)	12.84	%
Calculated relative daily exposure to shower gel	E product (mg/kg bw/day)	2.79	mg/kg bw/day
Concentration in product at application site	C (%)	1	%
Systemic exposure dose (SED)	**(Eproduct x C/100 x DA/100)**	**0.0036**	**mg/kg bw/day**

Face cream			
Dermal absorption as percentage (%)	DA (%)	12.84	%
Calculated relative daily exposure to face cream	E product (mg/kg bw/day)	24.14	mg/kg bw/day
Concentration in product at application site	C (%)	0.10	%
Systemic exposure dose (SED)	**(Eproduct x C/100 x DA/100)**	**0.0031**	**mg/kg bw/day**

Face wash			
Dermal absorption as percentage (%)	DA (%)	12.84	%
Calculated relative daily exposure to face wash	E product (mg/kg bw/day)	1.14	mg/kg bw/day
Concentration in product at application site	C (%)	1	%
Systemic exposure dose (SED)	**(Eproduct x C/100 x DA/100)**	**0.0015**	**mg/kg bw/day**

The aggregated exposure to cosmetic products (dermal route) is indicated in Table 17 [of the Applicant's submission] below.

Table 17. Aggregated exposure for the 4 cosmetic products

COSMETCIS	SED
rinse-off shampoo (2%)	0.0062 mg/kg bw/day
rinse-off shower gel (1%)	0.0036 mg/kg bw/day
leave-on face cream (0.1%)	0.0031 mg/kg bw/day
Rinse-off face wash (1%)	0.0015 mg/kg bw/day
Sum	**0.144 mg/kg bw/day**

3.4.2 Inhalation route (cosmetic exposure) - Exposure to vapours

TTO is not meant to be included in sprays or aerosols. However, due to the high vapor pressure of TTO (2100 Pa at 25°C) the inhalation of evaporated TTO was taken into account for the dermally applied cosmetic products under assessment. From the dermal absorption data are available indicating that 70-90% of the dermally applied TTO compounds evaporate. The One- box model described in the SCCS NoG was considered to be the most appropriate model to estimate inhalation exposure. The One-box-model considers the room volume, the time spent in the room, *i.e.*, time elapsed from the start of the emission and staying in this room, and the respiration rate of adults.

The respiration rate of 0.013 m^3/ min based on Rothe *et al.* (2011) and an inhalation absorption of 100% were used. The default room volume of a bathroom, *i.e.* 10 m^3 (Bremmer *et al.*, 2006; Biesebeek *et al.*, 2014), and an exposure time of 20 min were considered in a worst-case scenario (Rothe *et al.*, 2011). The calculation for the SED is depicted in Table 18 [of the Applicant's submission].

Table 18. SED to account for volatility

	$E_{Product}$ (mg/kg bw/day)	**Concentration of TTO in Product [%]**	**Fraction of TTO [%] as vapour**	**Amount TTO available for inhalation [mg/kg bw/day]***	**SED# of TTO by inhalation from product [mg/kg bw/day]**
Shampoo	1.51	2	79.6 (100-20.4)	0.024	0.00063
Shower Gel	2.79	1	87.2 (100-12.8)	0.024	0.00063
Face Cream	24.14	0.1	87.2 (100-12.8)	0.021	0.00055
Face wash	1.14	1	87.2 (100-12.8)	0,010	0,00026
sum					**0.0021**

* [Eproduct x % TTO in product] x [Fraction not considered dermally absorbed from OECD 428/100] #SED (vapour) = Residual mg/kg /day available for inhalation/10 m^3 x 20 min x 0.013 m^3/min

3.4.3 Aggregate exposure calculations for non-cosmetic uses

Aggregate exposure for CMR1 substances includes not only the exposure arising from the use of cosmetic products but also the exposure coming from the use of non-cosmetic products such as pesticides and industrial chemicals. Therefore, exposure data for consumers and professional workers from the REACH Dossier (CSR, Appendix 11) provided by the Lead Registrant Naturally Innovative Limited were included for the SED calculation. Furthermore, exposure data from the Combined Draft Renewal Assessment Report prepared according to Regulation (EC) N° 1107/2009 for TTO used in plant protection was publicly available and included here.

Ref: TTO Joint CSR Part B Sections 9-10, REACh CSR for Melaleuca alternifolia, provided by the Lead registrant

TTO is also used as herbal medicine. An EU herbal monograph on *Melaleuca alternifolia (Maiden and Betch) Cheel, Melaleuca linariifolia Smith,Melaleuca dissitiflora F. Mueller and/or other species of Melaleuca, aetheroleum radix* was published by EMA. Here, treatment of small superficial wounds, insect bites, small boils, athlete's foot, as well as oromucosal use is described in detail. The Committee on Herbal Medicinal Products (HMPC) concluded that, on the basis of its long-standing use, tea tree oil preparations can be used for these indications (EMA, 2015 and EMA, Addendum 2023).

Furthermore, TTO is used as flavor ingredient in foods. It was evaluated by FEMA (Flavor and Extract Manufacturers´ Association) for its safety as flavor ingredient in food and is assessed by the Expert Panel as GRAS (generally recognized as safe) substance with the FEMA No. 3902 (GRAS, 1998).

In some member states TTO is also used as food supplement (ANSES, 2020) and this was taken into account for the calculation of the overall exposure.

An overview on the exposure data for non-cosmetic uses is provided in the Tables below. The data are presented by exposure route:

Dermal route (non-cosmetic exposure):

The exposure data for consumer and professional workers were taken from the REACh CSR (Appendix 11). The bulk production of TTO takes place outside of the EU. Worker exposure for formulation, repacking and industrial uses in EU was excluded since worker only present a small population which are obliged to use personal protective cloth like gloves and respiratory protection and exposure per PROC is low and risk is controlled under REACH.

The external values from the CSR were adapted to obtain internal exposure (SED) (Table 19 [of the Applicant's submission]) by dividing the values by the dermal absorption value of 12.84% (dermal absorption value obtained from the OECD TG 428 study for the formulation of 1.25% TTO since ≤ 1% is used in consumer applications).

Table 19. SED for non-cosmetic uses under REACh

REACH (CHEMICALS)	mg/kg bw/day (external dermal exposure) from CSR REACH	SED [mg/kg bw/day]
Consumer (sum dermal systemic long term) (Cleaners, All-purpose liquids; Cleaners, Floor cleaning liquid; Cleaners, Oven, spray; Polishes and wax blends, Shoe)	0.0067	0.0009
Consumer (sum dermal systemic short term) (Cleaners, All-purpose liquids; Cleaners, Floor cleaning liquid; Cleaners, Oven, spray; Polishes and wax blends, Shoe)	0.0121	0.0016
Professional workers (PU-1: Professional end-use of washing and cleaning product, dermal long-term)	0.0137	0.0018
Professional workers (PU-2: Professional end-use of polishes and wax blends, dermal long-term)	0.0151	0,0019
Sum		**0.0062**

The EMA EU herbal monograph on Melaleuca alternifolia describes traditional cutaneous use in treatment of small wounds or insect bites (indication 1), small boils (indication 2), irritation due to tinea pedis (indication 3) and oromucosal use (indication 4) (EMA, 2015). In the monograph four indications are described. Nevertheless, it is unlikely that the same person will use these four indications in the same year and normally not in routine daily activity. Therefore, the exposure for a more realistic scenario with yearly exposure to indication 2 (which is more common than indication 1 and 3) and 4 (Table 20 [of the Applicant's submission]) was calculated.

Table 20. SED for non-cosmetic uses as herbal medicine

EMA (HERBAL MEDICINE USE)	SED [mg/kg bw/day]
Indication 2. Traditional herbal medicinal product for treatment of small boils (furuncles and mild acne): oily liquid or semi-solid preparations containing 10% of essential oil, to be applied to the affected area 1-3 times daily or 0,7-1 ml of essential oil stirred in 100 ml of lukewarm water to be applied as an impregnated dressing to the affected areas of the skin or undiluted essential oil to be applied to the boil using a cotton bud 2-3 times daily. Duration of use: Not to be used for more than 1 month (EMA recommendation)	0.039
Indication 4. Traditional herbal medicinal product for symptomatic treatment of minor inflammation of oral mucosa: 0.17 – 0.33 ml of TTO to be mixed in 100 ml of water for rinse or gargle several times daily for symptomatic treatment of minor inflammation of oral mucosa. Duration of use: Maximal 5 days (EMA recommendation)	0.0038

Indication 2: The use of a preparation containing 10% TTO is more common that the application of pure essential oil to boils using a cotton bud and is therefore assessed below.

The following exposure scenario was used: **Oily liquid or semi-solid preparations containing 10% of essential oil, to be applied to the affected area 1-3 times daily:**

- Application: 1 mg/cm2 (following the SCCS NoG that states that the amount applied to the skin usually does not exceed 1 mg/cm2 under in-use conditions.)

- Face surface area SSA: 565 cm^2 (worst case, since not the whole face will be treated)

- Mean application frequency per day: 2 per day

- Frequency of use per year: In ECHA Guidance R15 the following is stated for consumer exposure assessment." *The large variety of consumer products corresponds to a large variety in the frequency and duration of use and exposure. Exposure may occur during use and sometimes it continues after use for a certain time. The time, in which external exposure takes place, is defined as exposure time or exposure duration and can vary from seconds to the whole day. Exposure events can occur regularly/frequently (e.g. every day) or infrequently/occasionally (only for a few times or short periods in a year). Thus, the product-specific time pattern of use and any exposure continuing after the end of the use itself need to be considered in the assessment. It will mostly be a distribution of consumer behaviour, and for many products the corresponding statistical information is not available*" […] "*It may be appropriate to adjust the assessment for long-term exposure when i) daily exposure time is clearly shorter than 24 hours and/or ii) when the exposure occurs only a few times or over a short period per year* "(ECHA, R15, 2016Th).

  According to ECHA, information on exposure duration and frequency of use/exposure can be retrieved from published surveys on habits and practices or other data sources, like for example, the function of a product may inherently determine that it is normally not used in routine daily activity. Such refinement of exposure estimates by incorporating the use frequency is also included in exposure models like ConsExpo or EPA ExpoBox (Exposure Assessment Tools by Routes - Dermal | US EPA) EMA recommended in its monograph for indication 2 to use TTO maximal 30 days. To adapt for a use of maximal 30 days per year for indication 2, a factor of 0.08 (30 days/365 days) was included for the exposure calculations.

- Dermal absorption: 26% (14.46% + 2SD (11.6%) from the OECD TG 428 study, 5% formulation tested) as worst case to reflect also the use on partially damaged skin). 26% dermal absorption is conservative given that the vast majority of TTO components are highly volatile.

- Body weight: 60 kg

Exposure = mg applied per cm^2 x face surface area x application frequency per day x TTO product concentration x use frequency per year x dermal absorption / body weight of age group

Exposure = (1 x 565 x 2 x 10% x 0.08) x 26% / 60 = **0.039 mg/kg bw/day**

Indication 4: Traditional herbal medicinal product for symptomatic treatment of minor inflammation of oral mucosa: **0.17 – 0.33 mL of TTO to be mixed in 100 mL of water**. This corresponds to a concentration of 0.29 % TTO considering the density of 0.89 g/mL.

- $E_{product}$ mouthwash value from SCCS NoG: 32.54 mg/kg bw/day
- Absorption: 100% absorption is used for passage across the oral mucosa/incidental ingestion (default value)
- Frequency of use per year: In ECHA Guidance R15 the following is stated for consumer exposure assessment." The large variety of consumer products corresponds to a large variety in the frequency and duration of use and exposure.
  Exposure may occur during use and sometimes it continues after use for a certain time. The time, in which external exposure takes place, is defined as exposure time or exposure duration and can vary from seconds to the whole day. Exposure events can occur regularly/frequently (*e.g.* every day) or infrequently/occasionally (only for a few times or short periods in a year). Thus, the product-specific time pattern of use and any exposure continuing after the end of the use itself need to be considered in the assessment. It will mostly be a distribution of consumer behaviour, and for many products the corresponding statistical information is not available" […] "It may be appropriate to adjust the assessment for long-term exposure when i) daily exposure time is clearly shorter than 24 hours and/or ii) when the exposure occurs only a few times or over a short period per year "(ECHA, R15, 2016).
- According to ECHA, information on exposure duration and frequency of use/exposure can be retrieved from published surveys on habits and practices or other data sources, like for example, the function of a product may inherently determine that it is normally not used in routine daily activity. Such refinement of exposure estimates by incorporating the use frequency is also included in exposure models like ConsExpo or EPA ExpoBox (Exposure Assessment Tools by Routes - Dermal | US EPA). EMA recommended maximal 5 days of use. It can be assumed that a person will used the mouthwash probably 3 times per year for 5 days. To adapt for this infrequent use a factor of 0.04 (15 days/365 days) was included.

  Exposure = 32.54 x 0.29% x 100% x 0.04 = **0.0038 mg/kg bw/day**

Oral route (non-cosmetic exposure):

The refined consumer Theoretical Mean Daily Intake (TMDI) of TTO of 0.0011 mg/kg bw/d from the used of plant protection products containing TTO as active substance was used as reported in the dRAR. This refined Theoretical Mean Daily Intake value was used since TTO is highly volatile and thus expected to rapidly dissipate from the plant surface on which it is applied as indicated in the Renewal Assessment. It was shown in the dossier that consumer exposure to each component of TTO from application of the plant protection product is lower than the natural exposure from food to the constituents of Tea tree oil. For calculation of the SED an oral absorption of 70% was assumed as indicated in section 2.3 (Other data on

toxicokinetic). The results of the SED calculation are presented in Table 21 [of the Applicant's submission].

Table 21. SED for non-cosmetic uses as active ingredient in plant protection

PLANT PROTECTION	mg/kg bw/day (external exposure)	SED [mg/kg bw/day]
Overall sum of exposure from application, measured Modelled TMDI (Theoretical Mean Daily Intake)	0.0011	0.00077

Tea tree oil is listed as a generally recognized as safe (GRAS) flavoring substance by the Flavor and Extract Manufacturer's Association (FEMA). Shoji Fukushima et. al. (2020) reported the estimated intake of TTO as food flavoring ingredient (FEMA number 3902) to be 330 µg/person/day. This value was used for SED calculation (Table 22 [of the Applicant's submission]).

- Default value body weight: 60 kg
- Oral absorption: For calculation of the SED an oral absorption of 70% was assumed as indicated in section 2.3 (Other data on toxicokinetic).

Table 22. SED for non-cosmetic uses as food flavouring agent

FEMA (Shoji Fukushima *et al.*, 2020)	mg/kg bw/day (external exposure)	SED [mg/kg bw/day]
Shoji Fukushima et al (2020) reported the estimated intake of TTO as food flavouring ingredient (FEMA number 3902) to be 330 µg/person/day.	0.0055	0.0039

The use as food supplement in EU is not harmonized in the different member states, in some countries TTO is banned as food supplement (for example in Belgium). In France supplements with TTO are available, while use for more than 5 days without advice of a healthcare professional is not recommended (ANSES, 2023). ANSES states that "*the use of these essential oils orally is recent, and their consumption does not seem motivated by nutritional goals. Indeed, the antimicrobial properties claimed by these essential oils through different aromatherapy works lead consumers to divert these food supplements to make them additional treatments for infections taken in self-medication. Therefore, ANSES questions the relevance of the registration of these oils essential as food supplements*". In 2020 ANSES published a report concerning the use of Melaleuca essential oil in food supplements (ANSES, 2020). Here, they reported the use of up to 178 mg TTO/day (6 drops) as food supplement for adults in France (page 22). This use as food supplement is not recommended for pregnant or breast-feeding women, people with a history of convulsions or epilepsy, asthmatics or people allergic to allergic to essential oils or for children <12 years.

In a worst-case scenario, this exposure was taken into account:

- default value body weight: 60 kg
- Oral absorption: For calculation of the SED an oral absorption of 70% was assumed as indicated in section 2.3 (Other data on toxicokinetic).
- Daily amount used: 178 mg TTO
- Recommendation to not consume any food supplement containing this oil essential for more than 5 days without the advice of a healthcare professional. It was considered that

adults use the supplement 1x per year (factor: 5 days/365 days = 0.013) Exposure = (178 mg / 60 kg) x (0.013) x 70% =

0.028 mg/kg bw/day

It seems unlikely that a consumer will use TTO for small boils or oromucosal uses (EMA uses) plus additional treatments for infections taken orally in self-medication in the same year.

Inhalation route (non-cosmetic exposure):
The external exposure data for consumers and professional workers taken from the REACH CSR have been adapted to obtain internal exposure (SED) (Table 23 [of the Applicant's submission]) using:

- inhalation volume adult: 20 m^3/day
- default body weight: 60 kg

Table 23. SED for non-cosmetic uses under REACh (inhalation)

REACH (CHEMICALS)	mg/m^3 (external exposure)	SED [mg/kg bw/day]
Consumer (sum inhalation, systemic long term) (Cleaners, All-purpose liquids; Cleaners, Floor cleaning liquid; Cleaners, Oven, spray; Polishes and wax blends, Shoe)	0.00065	0.00022
Consumer (sum inhalation, systemic short term) (Cleaners, All-purpose liquids; Cleaners, Floor cleaning liquid; Cleaners, Oven, spray; Polishes and wax blends, Shoe)	0.00275	0.00092
Professional workers (PU-1: Professional end- use of washing and cleaning product, inhalation long-term)	0.0223	0.0074
Professional workers (PU-2: Professional end- use of polishes and wax blends, inhalation long- term)	0.0312	0.0104
sum		**0.019**

The overall aggregated SED value for cosmetic and non-cosmetic exposure is depicted in Table 24 [of the Applicant's submission]. The value of **0.117 mg/kg bw/day** was used for MoS calculation.

Table 24. Aggregated SED for cosmetic and non-cosmetic uses

Exposure	SED [mg/kg/day]
Cosmetic uses	
Product categories	
Shampoo 2%	SED dermal = 0.0144 SED vapour= 0.0021
Shower gel 1%	
Face cream 0.1%	
Face wash 1%	
Non-cosmetic uses	
EMA	
use as Traditional herbal medicinal product	SED dermal = 0.039
	SED mouthwash= 0.0038
Food Supplement	
Use as food supplement (ANSES)	SED oral = 0.028
REACH	
Use in consumers and professional workers	SED dermal = 0.006 SED inhalation = 0.019
PLANT PROTECTION	
Active ingredient in plant protection formulation	0.00077
FOOD (FEMA)	
Food flavoring agent	0.0039
Aggregated Exposure	**0.117**

TTO is not intended to be used in children. However, it is expected that children and adolescent will use these cosmetic products occasionally and therefore, the exposure assessment for these vulnerable groups was performed according to the SCCS Notes of Guidance. The external exposure was used to calculate the internal (or systemic) exposure (SED). For the calculation of the SED, absorption (or uptake) specific to the respective exposure route was taken into account. The MoS is > 100 for children and adolescents. The results are presented in the Appendix 6 [MoS calculations for vulnerable groups (children)].

SCCS comment

The SCCS agrees with the Applicant's calculation of exposure from different cosmetic product categories and, on principle, with the aggregated exposure from cosmetic and non-cosmetic sources of consumer exposure. The proposed calculated SED values will be used in the calculation of margin of safety (MoS) for TTO.

However, the SCCS would like to point out that the calculation of exposure from medicines was averaged over a whole year. Therefore, the presented aggregate exposure estimate might underestimate the exposure of some users of TTO containing medicines.

3.5 TOXICOLOGICAL EVALUATION

In two former submissions on TTO relevant data on endpoints of the toxicological profile and other respective data were provided. As these data were evaluated by the SCCS and published in the SCCS Opinions in 2004 and 2008 (SCCP/0834/04, 2004; SCCP/1155/08, 2008) they are not included in this dossier.

TTO was also comprehensively evaluated according to ECHA/REACH requirements for chemicals and a disseminated ECHA dossier (https://echa.europa.eu/de/registration-dossier/-/registered- dossier/20921/4/9) with all relevant datasets is available publicly. Furthermore, toxicological data for TTO from the Combined Draft Renewal Assessment Report prepared according to Regulation (EC) N° 1107/2009 are publicly available.

In the subsequent sections only the newly available toxicological data are presented and discussed which have not been included in the former SCCS Opinions.

In addition, a discussion on the species-specific fertility effects observed in rats, which triggered the harmonized classification and labelling proposal as reproductive toxicity category 1B (H360Fd) for TTO is presented.

3.5.1 Irritation and corrosivity

3.5.1.1 Skin irritation

Two studies investigating the skin irritation potential had been presented and evaluated in SCCP/1155/08. In rabbits, the Draize irritation index for neat Tea Tree Oil was found to be 5.0, indicating a severe skin irritant while 25% Tea Tree Oil was not irritating. The test substance TTO was applied unchanged to rabbit skin for 4 hours (0.5 mL). The mean score reactions from gradings at 24, 48 and 72 hours after patch removal calculated for each individual animal were 2.67, 2.00, 2.00 for erythema and 1.00, 1.00, 1.00 for edema. No animal died and no necropsy finding have been reported. Clinical signs occurred like peeling/desquamation.

In the RAC Opinion for TTO a further Draize skin irritation study from Lee *et al.*, 2013 is reported to not induce significant skin irritation at 2.5% TTO concentration per site. At 5% and 10% TTO concentrations irritating effects were seen.

A human patch test study is available and was performed under dermatological control on a group of 50 volunteers, including 25 volunteers with positive history of allergies/atopy (TEST REPORT No. 900089318/21/GDA, 2022, Study date: 11.01.2022-14.01.2022). The preparation (leave-on deo product) in an appropriate concentration was applied onto filter paper discs of 12 mm diameter and then fixed to the arm or interscapular area with the use of a sticking patch. At the same time, in order to guarantee objective results of the study and to exclude possible reading errors connected with dermal irritations, two control samples (control sample called "blind" and control sample containing water) were used. The dermatologist removed the patch 24 hours after the application and examined the skin reaction 30 minutes after the removal. 48 hours after the application, the dermatologist examined the skin again for a reaction. While determining the skin reaction, the dermatologist

assessed the irritating and sensitising effects of the tested product. The study allowed to conclude that the tested product (leave-on product UDV090821C, 2% TTO and 1.6% 4-Terpineol) used by volunteers, who didn't report documented oversensitivity or a history of adverse reactions to individual ingredients of the tested product, is well tolerated by the skin. In the tested group of volunteers there were no irritations or allergic reactions. The full report is attached in the Appendix 7 [TEST REPORT No. 900089318/21/GDA, Dermatological test - Semi-open test (25 subjects with allergological history, 25 subjects, without allergological history)].

A 48-h occlusive patch test reported in CIR with 1% Melaleuca Alternifolia (Tea Tree) Leaf Oil in petrolatum (pet) produced no irritation in 22 human subjects (CIR, 2021).

Conclusion:
SCCP concluded in their former Opinion that TTO and 5% formulations with TTO can exhibit skin irritancy. CIR also concluded that formulations of 5% of more can induce skin irritation (CIR, 2021). From a human patch test performed in volunteers a leave-on product (2% TTO) does not induce skin irritation. To minimise the skin irritation potential, the in-use concentration for the defended products was set to ≤ 2%.

3.5.1.2 Mucous membrane irritation / eye irritation

Further toxicological data from the REACH Dossier for Melaleuca alternifolia, ext. (EC 285-377- 1/CAS 85085-48-9) are available: A new *in vitro* study according to OECD TG 437 is available and was performed under GLP conditions. Undiluted TTO was shown not to damage bovine corneas (10 min exposure/ 120 min post-treatment incubation). Positive (undiluted ethanol) and negative control (0.9% sodium chloride solution) have been included.

Study Design:

Guidelines/Methods:	OECD TG 437 Report year: 2013
Test system:	Bovine corneas (n=3 per test group) Vehicle: unchanged (0.75 mL)
Test substance:	Tea Tree Oil (Essential oil obtained from the leaves and terminal branchlets of Melaleuca alternifolia, by steam distillation)
GLP:	Yes
Reference:	ECHA disseminated Dossier EC 285-377-1.

Result: Undiluted Tea Tree Oil is not damaging to eyes.

In the REACH disseminated Dossier an *in vivo* study is also available which was performed according to OECD TG 405 and under GLP conditions in 2013. Here, 0.1 mL undiluted TTO was placed into the conjunctival sac of the right eye of New Zealand White rabbits. No corneal or iridial effects were noted, only moderate conjunctival irritation one hour after treatment. This was normal at 72 h.

Data from a further study (report year 2015) according to OECD TG 405 and GLP is available in the Combined Draft Renewal Assessment Report prepared according to Regulation (EC) No 1107/2009. The test substance (0.1 mL) was applied undiluted into the conjunctival sac of the left eye of three New Zealand White rabbits. The right eye remained untreated. The mean scores for redness, chemosis and discharge of conjunctiva were 1,.0, 1.0 and 1.3 respectively. No effect on iris and cornea were observed.

Conclusion:
In the last SCCS Opinion for TTO it was concluded from the presented data (HET-CAM assay, *in vivo* study in Japanese White rabbits), that no definitive conclusion regarding eye irritation of TTO can be drawn. Based on the newly available data it can be concluded that undiluted TTO, where the composition meets ISO Standard 4730-2004, is not irritating to eyes.

SCCS general comment on irritation and corrosivity
The SCCS considers that TTO of ISO Standard 4730-2004 is not irritating to skin and eye at the use levels in the intended cosmetic products.

3.5.2 Skin sensitisation

From the Applicant:

Four LLNA studies performed according to OECD TG 429 and GLP had been presented and evaluated in SCCP/1155/08. Here, TTO concentration from 2% up to 100% had been tested and EC3 values for TTO between 4.4% and 25.5% were reported. Concentrations of 2% TTO did not induce a stimulation index above 3 and therefore no skin sensitization in these studies. Two guinea-pig maximization tests (GPMT) according to OECD TG 406 are available showing a clear negative result when using challenge concentrations of 30% TTO in guinea pigs (evaluated in SCCP/1155/08). The data from the four LLNA conducted according to OECD TG 429 under GLP conditions are summarized below:

Table 25. Results of LLNA Tests

Method	Species/Strain/ sex/group size	Vehicle	Stimulation index (SI) (Mean)	EC3 (µg/cm2)	Reference/Remark
OECD TG 429, GLP	Mouse (CBA/CaHsdRcc (SPF)) Female 5/dose/group	PEG 300	2.4 at 2% (SD=1.4) 6.9 at 20% (SD=2.0) 16 at 100% (SD=6.3)	EC3=4.4% (1100 µg/cm^2)	ECHA dissemination site (study report 2006); oxidized TTO used
OECD TG 429, GLP	Mouse (CBA/CaHsdRcc (SPF)) Female 5/dose/group	PEG 300	1.6 at 2% (SD=0.4) 2.8 at 20% (SD=0.7)	EC3=25.5 % (6375 µg/cm^2)	ECHA dissemination site (study report 2006), non-oxidized TTO used

			5.7 at 100% (SD=1.6)		
OECD TG 429, GLP	Mouse (CBA/CaHsdRcc (SPF)) Female 5/dose/group	PEG 300	1.8 at 2% (SD=0.4) 2.8 at 20% (SD=1.2) 6.5 at 100% (SD=2.3)	EC3=24.3 % (6075 µg/cm^2)	ECHA dissemination site (study report 2006), non-oxidized TTO used
OECD TG 429, GLP	Mouse (CBA/J) Female 5/dose/group	PEG 400	SI (Mean): 2.1 at 5% (SD=0.7) SI (Mean): 7.7 at 25% (SD=4.0) SI (Mean): 7.9 at 50% (SD=3.2)	EC3=8.3% (2075 µg/cm^2)	ECHA dissemination site (study report 2006), non-oxidized TTO used

The stimulation index obtained in the LLNAs at concentration of 2% was between 1.6 – 2.4. The EC3 values from the 4 studies with TTO ranged from 4.4% in PEG 300 vehicle to 25.5% in PEG 300 vehicle. The LLNA data show that oxidized TTO has a higher potency to induce skin sensitization, but still is a moderate skin sensitizer. The lowest EC3 value was 4.4%. This was converted to a dose per unit area in µg/cm^2 using an applied volume of 25 µL and an ear surface area of 1 cm^2 (*e.g.*, a 1% w/v solution delivers a dose of 250 µg/cm^2) vehicle.

Two guinea-pig maximization tests (GPMT) according to OECD TG 406 are available showing a clear negative result when using challenge concentrations of 30% TTO in guinea pigs (evaluated in SCCP/1155/08).

A new *in vitro* study is available which is part of a tiered strategy for the evaluation of skin sensitization potential (Testing Facility Study No. 48121 TIK, OECD 442D, 2020). In this study according OECD TG 442D and GLP the potential of the test item, a gel containing 2% TTO and 1.6% 4-Terpineol, to activate the Nrf2 transcription factor was evaluated. The combination of TTO and 4-Terpineol was used to increase the concentration of TTO in the formulation without breaching EU threshold for methyl eugenol. The composition of gel was the following: Aqua (CAS 7732-18-5: > 85%; PEG-40 Hydrogenated Castor Oil (CAS 61788-85-0): 5%; Glycerin (CAS 56-81-5): 2%; Melaleuca Alternifolia Leaf Oil (CAS 85085-48-9; peroxide number: 1.98 mmol/L): 2% and 4-Terpineol (EC 943-985-0; peroxide number: 8.63 mmol/L): 1.6%. This *in vitro* test uses the KeratinoSens cell line, an immortalized and genetically modified human adherent HaCaT keratinocyte cell line. These cells were plated on 96-well plates and grown for 24 hours at 37°C. Cell were exposed to the vehicle control or to different concentrations of test item and the positive control cinnamic aldehyde. The treated plates were incubated for 48 hours at 37°C and afterwards, luciferase production was measured. In parallel, cytotoxicity was measured by MTT reduction and taken into consideration in the interpretation of the sensitization results. Two independent runs were performed as part of this study. All acceptance criteria were met in both runs; the study was therefore considered as valid. This study was performed at test concentrations ranging from 0.20 to 400 µg/mL in culture medium containing 1% DMSO and 1% water for injections. At these tested concentrations, no statistically significant fold induction above the threshold of 1.5 was noted in comparison to the negative control, at any tested concentration and in either run. Since the evaluation

criteria for a negative response were met in both runs, the final outcome is negative. Under the experimental conditions, the test item was negative in the KeratinoSens assay and was therefore considered to have no potential to activate the Nrf2 transcription factor. Data from a Human Maximization Test (HMT) have been evaluated and included in a recently published database on human predictive patch test data for skin sensitization (Strickland *et al.*, 2023). In this HMT TTO failed to induce skin sensitization at a concentration of 1% (675 µg/cm^2) in 22 test subjects. However, the composition of TTO was not clear. A modified HRIPT is reported in the Cosmetic Ingredient Review for TTO (CIR, 2021): 24-h semi-occlusive induction patches (2 cm^2 absorbent pad) were applied 3x/week for 3 weeks to 102 test subjects. After a 10-d non- treatment period, 24-h challenge applications were made to the test site and a previously untreated site. Induction sites were scored 24h or 48h after application; challenge sites were scored upon patch removal and at 24 h. The tested preparation was neither an irritant nor a skin sensitizer. Aspres and Freeman (2003) reported a study based on the skin sensitization method of Draize. Tea Tree Oil products were investigated which consisted of 5, 25 and 100% TTO in cream, ointment or gel formulation. It was not possible to determine which specific concentrations were responsible for inducing sensitization. The data for human patch tests are presented in Table 26 [of the Applicant's submission]. In a further human patch test described in section 3.1.1 the tested product (leave-on deodorant, 2% TTO and 1.6% 4-Terpineol) used by 50 volunteers, is well tolerated by the skin. In the tested group of volunteers there were no irritations or allergic reactions.

Table 26. Data of TTO from Human Patch tests

Test Substance Concentration Vehicle	Dose Volume Patch area	Induction Dose (µg/cm^2)	Positive Induction response	Reference Remark Method
1% in petrolatum (patch site pre-treated with 5% aq. SDS), occlusion	0.3 mL/ 4cm^2	675	0% 0/22 test subjects	Human Skin Sensitization Data base reported in Strickland *et al.*, 2023/ Human Maximization Test composition of TTO not clear The study is also reported in CIR, 2021 and the procedure was described as following: occlusive patch applied to the forearm for 5 alternate-day 48-h periods; patch site was pretreated for 24

				h with 5%aq. SLS; for challenge, after a 10 – 14- d non-treatment period, an occlusive patch was applied to a previously untreated site; 5% SLS was applied to the test site for 30 min under occlusion on the left side of the back, and the test materials were applied without SLS treatment on the right side
Undiluted tea tree oil and 25% TTO in cream, 25% TTO in ointment, 25% TTO in gel, 5% TTO in cream and 5% TTO + 5% synergist in cream	0.1 mL (liquids) or 0.1 mg (solid preparation)/11-mm Finn chamber (ca. 1 cm2 test area)	-	1% (3/309 subjects) It was not possible to determine which specific concentrations were responsible for inducing sensitization	Aspres and Freeman (2003) Induction: 48-h occlusive applications with Finn chambers to upper arm or the back, 3x/wk for 3 wk Challenge: after a 2-wk non-treatment period, a 48-h patch was applied to a previously untreated site Formulations containing 25% or lower of tea tree oil were non-irritating.
10% in caprylic/capric Triglycerides, volatilized for 30 min	0.2 mL/2 cm^2	10000	0% (0/102)	modified HRIPT, reported in CIR, 2021 (no report available to Applicant) 24-h semi-occlusive induction patches (2 cm^2 absorbent pad) were applied 3x/wk for 3 wk;

				after a 10-d non-treatment period, 24-h challenge applications were made to the test site and a previously untreated site induction sites were scored 24- or 48-h after application, challenge sites were scored upon patch removal and at 24 h

Skin sensitisation quantitative risk assessment (QRA) was performed in line with SCCS opinion SCCS/1589/17 to receive concentrations of TTO in cosmetic products under assessment that are not expected to cause skin sensitization. This approach was recently accepted by SCCS in their Preliminary Opinion on Citral and SCCS stated that it will consider the suitability (for a population not already sensitised) of this methodology for other cosmetic ingredients on a case-by-case basis (SCCS/1666/24). A No Expected Sensitising Induction Level (NESIL) was derived based on SCCS opinion SCCS/1589/17 and adjusted by a number of uncertainty factors (Sensitisation Assessment Factors, SAFs) in order to calculate an Acceptable Exposure Level (AEL). The AEL is then compared with a consumer exposure level (CEL). In their Opinion SCCS/1589/17, SCCS stated that QRA could be a useful tool not only for safety evaluation of fragrance allergens, but potentially also for other cosmetic ingredients. Therefore, it was used for calculation concentrations of TTO for face cream (leave-on, 0.1% TTO), shampoo (rinse-off, highest concentration of 2% TTO) and shower gel (rinse-off, 1% TTO) that do not cause skin sensitisation reactions.

NESIL derivation

In deriving a NESIL, an overall WoE approach is utilized, which considers all available data. A WoE NESIL is an exposure to a skin sensitizer which should not result in the induction of sensitization in humans.

TTO is negative in the KeratinoSens assay according to OECD TG 442D. There are two clearly negative GMPT available and four positive LLNA. Data from 2 HRIPT and 1 HMT are available which gave negative results in tested volunteers. The EC3 values from the 4 studies with TTO ranged from 4.4% (1100 µg/cm^2) to 25.5% (6375 µg/cm^2). No sensitisation was induced in the HRIPT, with a NOEL of 10000 µg/cm^2. The NOEL from the HMT conducted with petrolatum was 675 µg/cm^2. However, the composition of TTO was not reported in the HRIPT and HMT studies and adjuvant tests like GPMT cannot be used as primary data sources for defining NESILs (SCCS/1666/24). RAC indicates that Human data confirm that TTO induces skin sensitisation but with an incidence not very high and the concentrations that induced

responses not particularly low. Therefore cat. 1B seems more appropriate than cat. 1A based on the human studies.
Since the composition of TTO used in the HRIPT test was unclear and either the subject size was too low or only minimal data were presented, the EC3 of 4.4% from the LLNA study was used to derive the NESIL of 1100 µg/cm^2 for TTO.

Calculation of the Acceptable Exposure Level (AEL) for use in each single product:

The AEL is essentially the NESIL divided by the overall Sensitization Assessment Factor (SAF) for the product type (SCCS opinion SCCS/1589/17).

Assessment Factors
Interindividual variability: 10 (default value)
Product: 1
Frequency/duration of product use: 3 (used frequently)
Skin site condition: 3 (face cream); 10 (shampoo and shower gel)

Overall Sensitization Assessment Factor
SAF face cream: 100 (according to SCCS/1666/24)
SAF shampoo: 300
SAF shower gel: 300

AEL= NESIL/SAF
AEL face cream = 11 µg/cm^2[8]
AEL shampoo = 3.7 µg/cm^2
AEL shower gel = 3.7 µg/cm^2

Estimation of the consumer exposure levels (CEL): The CEL for each product type is calculated according to the equations below:
CEL = Product Exposure (mg/cm^2) x Retention Factor x 1000 (mg/g). CEL face cream:
Estimated daily amount applied: 1.54 g/d
Retention factor: 1 Surface area: 565 cm^2

The CEL for face cream was derived using a scenario with a daily use amount of 1.54 g grams, applied to a skin surface area of 565 cm^2, and a retention factor of 1.

1.54 g/565 cm² x 1000 mg/g = 2.73 mg/cm^2

CEL shampoo:
Estimated daily amount applied: 10.46 g/d Retention factor: 0.01
Surface area: 1440 cm^2

10.46 g/1440 cm² x 1000 mg/g x 0.01= 0.0726 mg/cm^2

The CEL for shampoo was derived using a scenario with a daily use amount of 10.46 g grams applied to a skin surface area of 1440 cm^2, and a retention factor of 0.01.

CEL shower gel:
Estimated daily amount applied: 18.67 g/d Retention factor: 0.01
Surface area: 17500 cm^2

18.67 g/17500 cm² x 1000 mg/g x 0.01= 0.011 mg/cm^2

The CEL for shower gel was derived using a scenario with a daily use amount of 18.67 g grams applied to a skin surface area of 17500 cm^2, and a retention factor of 0.01.

Calculation of initial maximum use levels by individual product type (Unadjusted Upper Concentration Level (UCL))

Determination of the initial $UCL_{product}$ of an ingredient not to be exceeded in a finished product is calculated using the product AEL and CEL using the following equation:

(AEL µg/cm^2 x 0.001 mg/µg) / CEL mg/cm^2/day x 100 = $UCL_{product}$ % (11 µg/cm^2 x 0.001 mg/ µg) / 2.73 mg/cm^2 x 100 = 0.4% face cream The UCL for TTO in face cream is 0.4%.

(3.7 µg/cm^2 x 0.001 mg/ µg) / 0.0726 mg/cm^2 x 100 = 5% shampoo The UCL for TTO in shampoo is 5%.

(3.7 µg/cm^2 x 0.001 mg/ µg) / 0.011 mg/cm^2 x 100 = 34% shampoo The UCL for TTO in shower gel is 34%.

In the Preliminary Opinion of Citral Aggregate Adjustment factors have been introduced, which are independent from the product assessed. These factors have been applied to TTO to determine the maximum use levels considering aggregate exposure. This is a conservative approach and was applied since TTO is used in shampoo, shower gel and face cream and can also be present in non-cosmetic product applied to the skin. $UCL_{product}$ for the individual product of interest is multiplied by the appropriate Category Aggregate Adjustment Factor:

QRA Aggregate Adjustment Factor for Category Leave-on products applied to the face and body using the hands (palms): 0.33

- For a **face cream**, the maximum concentration level considering aggregate exposure is calculated as 0.4 % x 0.33 = **0.14%.**

QRA Aggregate Adjustment Factor for Category Rinse-off products with body and hand exposure: 0.5

- For a **shampoo**, the maximum concentration level considering aggregate exposure is calculated as 5% x 0.5= **2.5%.**
- For a **shower gel**, the maximum concentration level considering aggregate exposure is calculated as 34% x 0.5= **17%.**

Conclusion:

Based on the overall data and supported by QRA calculations the maximum concentration of TTO in rinse-off was set to 2% and in leave-on products to 0.1% to lower a possible risk for skin sensitizing effects. When testing leave-on products up to 2% TTO in formulations no irritating effects or sensitizing response in human volunteers were reported (see Section 3.2.1). In addition, it is recommended for manufacturers to minimize oxidization of TTO in the final cosmetic product since the potential for skin sensitizing reactions is enhanced using oxidized TTO (CIR, 2021). However, the LLNA data show that even oxidized TTO is only a moderate skin sensitizer. RAC concluded that the incidence for skin sensitization from human data was low and the concentrations that induced responses were "not particularly low" (RAC,

2023). Therefore, the concentrations used in the final products defended in this dossier are considered as safe for use in the products.

SCCS comments

In a previous assessment (SCCP/1155/08), the SCCP concluded that TTO is a sensitiser in humans, possibly due to the content of irritants such as p-cymene and 1,8-cineole, and/or the gradual accumulation of certain oxidation products.

This is supported by clinical data demonstrating that allergic contact dermatitis from TTO has been reported frequently. Most of the cases are caused by pure TTO applied on damaged skin. Cosmetics and pharmaceutical with TTO were mentioned as causes of ACD as well. Moreover, patch testing with constituents of TTO, such as ascaridole, terpinolene, ɑ-terpinene and others has confirmed the notion that several different constituents of TTO contribute to its sensitising potential (de Groot, 2019).

The Applicant performed a QRA to assess if TTO can be safely used in cosmetic products. The procedure followed for aggregate exposure assessment is not provided by the Applicant. In addition, the exposure assessment is incomplete, since face wash products were not included. For these reasons, the SCCS cannot evaluate the QRA.

To conclude, the LLNA data provided by the Applicant in the current Opinion confirm that TTO is a moderate skin sensitiser.

3.5.3 Acute toxicity

3.5.3.1 Acute oral toxicity

Data investigating the acute oral toxicity had been presented and evaluated in SCCP/1155/08. Two new studies on acute oral toxicity are available. From a study performed according to OECD TG 423 and GLP which is available in the REACH dossier the LD50 in females NMRI mice was > 2000 mg/kg bw. No mortalities or macroscopic findings were reported and no clear effects on body weight. The study design for this OECD TG 423 study was the following:

Study design:

Guideline/methods: OECD TG 423 (acute class method) Report year: 2010

Species/strain: mice/CRL:(NMRI)BA

Exposure route: oral gavage, 14 days observation period Vehicle: PEG 400

Group size: N=3/group

Test substance: Tea Tree oil, purity 100%, complies with ISO 4730:2017 specification. Concentration: 2000 mg/kg bw (group 1 and 2)

GLP: Yes

Reference: ECHA disseminated Dossier EC 285-377-1

Under the conditions of this study, the acute oral LD50 value of Tea Tree Oil was higher than 2000 mg/kg bw following administration to female CRL:(NMRI)BR mice.

In a further acute toxicity available in the REACH disseminated dossier groups of 5 male and 5 female Sprague Dawley rats (SPF and non-SPF) were treated (report year 1989). The test sample was diluted with peanut oil w/w at 3 different concentrations: 1/3, 1/4, 1/5. Four groups of SPF rats were administered 2.5, 2.6, 2.75 and 3 mL/kg of the test sample by gavage. Five groups of non-SPF rats were administered 1.70, 2.10, 2.15, 2.25 and 2.4 mL/kg of test sample by gavage. All animals were observed for any signs of toxicity or abnormal behavior during the experimental period of 14 days. The LD50 of Tea Tree Oil was determined to be 2.6 mL/kg bodyweight in SPF rats and 1.9 mL/kg bodyweight in non-SPF rats (equivalent to 1691 mg/kg bw). The sample caused weeping eyes, bloodied noses and a lack of tonus in the forelimbs of those animals which survived.
An acute oral toxicity study (report year 2015) according to OECD TG 425 and GLP is available in the Combined Draft Renewal Assessment Report prepared according to Regulation (EC) No 1107/2009. Female Wistar rats were dosed sequentially by oral gavage. The starting dose was 550 mg/kg bw and caused no mortality throughout the 14-day observation period. Rats dosed in a second group with 2000 mg/kg bw exhibited clinical signs and died on day 1 and 2. The pre- terminal dead rats lost weight when compared to their initial weight. The estimated LD50 of TTO is 1049 mg/kg bw in female rats.

From the Applicant:
TTO is classified for acute oral toxicity. However, this is of minor importance for the use of TTO in the defended, dermally applied cosmetic products.

SCCS comment
SCCCS agrees with the Applicant conclusion that acute toxicity of TTO, as indicated by oral toxicity studies, is not of importance for the intended use in cosmetic products at the proposed use levels.

3.5.3.2 Acute dermal toxicity

One study according to OECD TG 402 has been presented and evaluated in SCCP/1155/08. Here, a LD50 value of >2000 mg/kg bw in New Zealand White Rabbits was reported.

One study on acute dermal toxicity (report year 2015) is available in the Combined Draft Renewal Assessment Report prepared according to Regulation (EC) No 1107/2009. The study was conducted according to OECD TG 402 and GLP. 2000 mg/ kg bw of undiluted TTO (complies with ISO-specification) was applied to skin of Wistar rats. No clinical signs occurred and the LD50 value was set to 2000 mg/kg bw.

Conclusion:
No acute dermal toxicity for TTO is expected when included as ingredient in cosmetic products.

SCCS comment
The SCCS agrees that the levels of use of TTO in the intended cosmetic products do not raise concerns about acute dermal toxicity.

3.5.3.3 Acute inhalation toxicity

In the last SCCS Opinion for TTO in 2008 data on acute inhalation toxicity in rats was presented from a non-guideline study in which no vehicle control was included and only one control animal was evaluated histopathologically.

A new study according to OECD TG 403 performed under GLP conditions is available from the REACH disseminated dossier for Melaleuca alternifolia, ext. (EC 285-377-1/CAS 85085-48-9). Wistar rats had been exposed for 4 hours nose-only to aerosols at concentrations of 1.94, 3.70 and 5.04 mg/L air. No control group was included.

Study Design:

Guidelines/Methods:	OECD TG 403 Report year: 2011
Species/strain:	rat/Wistar (m/f)
Exposure route:	nose-only inhalation of aerosol
Vehicle:	air
Group size:	n=5
Test substance:	Tea Tree Oil (100%, Essential oil obtained from the leaves and terminal branchlets of Melaleuca alternifolia, by steam distillation)
Exposure duration:	4 h GLP: Yes
Reference:	ECHA disseminated Dossier EC 285-377-1

Seven mortalities occurred in the highest dose group. Necropsy did not reveal any test-item related findings up to the highest dose tested. The surviving animals showed body weight loss. The 4-hr LC_{50} for males and females was determined to be 4.78 mg/L air (4780 mg/m^3).

One further study (report year 2010) on acute inhalation toxicity is available in the Combined Draft Renewal Assessment Report prepared according to Regulation (EC) No 1107/2009. The study was conducted according to OECD TG 403 and GLP. The acute inhalation toxicity study with TTO was conducted in male and female Wistar rats by nose only exposure using 30%, 50% and 70% w/v aerosol of the test item diluted in dimethyl sulphoxide to 3 groups of rats. The rats were continuously exposed to the aerosol for 4 hours in an inhalation exposure chamber. The post- treatment observation period was 14 days. The analytical determined mean test item concentrations in the air inhalation sample columns were 0, 0.77, 3.69 and 5.06 mg TTO/L of chamber air. The acute inhalation LC50 of TTO in Wistar rats was 3.64 mg/L of air for both male and female rats.

Conclusion:
TTO is classified for acute inhalation toxicity. However, TTO is not intended to be used in sprayable products. Consumers using shampoo, shower gel, face wash or face cream are not exposed to high aerosol concentrations.

SCCS comment
The SCCS notes that TTO is not intended to be used in cosmetic products that could give rise to inhalation exposure of the consumer.

3.5.4 Repeated dose toxicity

3.5.4.1 Repeated dose (28 days) oral / dermal / inhalation toxicity

No repeated dose toxicity study was provided for the previous (2008) SCCS Opinion on TTO.

A new study according to OECD TG 407 performed under GLP conditions is available from the REACH Dossier for Melaleuca alternifolia, ext. (EC 285-377-1/CAS 85085-48-9). Sprague-Dawley rats had been orally administered with TTO at concentrations of 5.15 and 45 mg/kg bw/day for 28 days. Corn oil was used as vehicle control.

Study Design:

Guidelines/Methods:	OECD TG407
Report year:	2017
Species/strain:	rat/Sprague-Dawley (m/f) Exposure route:oral gavage
Vehicle:	corn oil
Group size:	5/sex/dose
Test substance:	Tea Tree Oil (Essential oil obtained from the leaves and terminal branchlets of Melaleuca alternifolia, by steam distillation)
Concentrations:	0, 5, 15, 45 mg/kg bw/day Exposure duration: 28 days
GLP:	Yes
Reference:	ECHA disseminated Dossier EC 285-377-1.

No test-item related toxicological effects have been reported. The NOAEL was determined to be 45 mg/kg bw/day for male and female rats. Based on the new available data it can be concluded that TTO, where the composition meets ISO Standard 4730-2004, induced no local or systemic effects up to 45 mg/kg bw/day after repeated oral exposure.

Further data on repeated dose toxicity are available in the Combined Draft Renewal Assessment Report prepared according to Regulation (EC) No 1107/2009: In an OECD TG 407 (non-GLP) study Wistar rats were administered TTO by oral gavage with 0, 62.5, 125 and 250 mg/kg bw/day (n=6 m/f per group). Control animals received the vehicle groundnut oil. The test item TTO which was used followed ISO 4730:2004 specifications. In the highest dose group decreased weights of testes and epididymides, degenerative changes in both organs and aspermia were observed. Liver and adrenal were increased in the highest dose. At 125 mg/kg bw/day degenerative changes in tested and epididymides and oligospermia were observed. Liver weight was increased also in the mid dose. The NOEL from this study was 62.5 mg/kg bw/day. Minimal/mild liver vacuolation was reported starting from the lowest dose of 62.5 mg/kg bw/day.

Conclusion:
RAC concluded that no classification is warranted for STOT RE regarding the liver effects (RAC, 2023). The effects on testes and epididymides, are further discussed under Reproductive toxicity paragraph 3.5.

SCCS comment
SCCS agrees with the Applicant's conclusion on the oral 28-day repeated dose study.

3.5.4.2 Sub-chronic (90 days) oral / dermal / inhalation toxicity

Data on three repeated dose toxicity studies are available in the Combined Draft Renewal Assessment Report prepared according to Regulation (EC) No 1107/2009:

A study according to OECD TG 408 and GLP was performed in male and female Wistar rats (report year 2011). Animals were dosed by oral gavage with 0, 30, 60, or 120 mg/kg bw/day. Control animals received the vehicle groundnut oil. The test item TTO was in compliance with ISO 4730:2004 specifications. Effects on sperm were reported for mid and high dose. At the high dose histopathological changes were found in testes and epididymides. Further effects noted were spleen vacuolation (minimal degree) and tubular dilation in kidneys (minimal degree). The NOAEL for males is 30 mg/kg bw/day and for females 60 mg/kg bw/day.

In another study according to OECD TG 408 and GLP Wistar rats (males and females) were dosed by gavage with 0 or 60 mg/kg bw/day (report year 2016). The test item TTO was in compliance with ISO 4730:2004 specifications. At 60 mg/kg bw/day effects on sperm were reported and degeneration and atrophy of seminiferous tubules were noted. All effects recovered after 8 weeks without dosing. A NOAEL could not be derived. The LOAEL was 60 mg/kg bw/d.

In a further study according to OECD TG 409 and GLP Beagle dogs were dosed by gavage with 0, 30, 75/60, or 180/120 mg/kg bw/day (report year 2018). Control animals received sesame oil. The test item TTO was in compliance with ISO 4730:2004 specifications. Due to signs of intoxication, the mid and top doses were reduced from test day 27 on. In the mid and high dose males the viability and motility of spermatids were decreased. The NOAEL was 30 mg/kg bw/day.

A detailed discussion on the effects observed on sperm and tested/epididymis is presented in the section 3.9 below.

SCCS comment
The SCCS agrees with the Applicant's conclusion on the 90-day repeated dose studies in rats and dogs, and that NOAEL of 30 and 60 mg/kg bw/day could be derived for male and female rats respectively from the OECD TG 408 study, and 30 mg/kg bw/day for dogs from the OECD TG 409 study.

3.5.4.3 Chronic (> 12 months) toxicity

/

3.5.5 Reproductive toxicity

No study on reproductive toxicity with TTO was provided for the previous (2008) SCCS Opinion on TTO. Data and information for this endpoint are now available and discussed below.

3.5.5.1 Fertility and reproduction toxicity

Data on the effect of TTO on sexual function and fertility are available in the Combined Draft Renewal Assessment Report prepared according to Regulation (EC) No 1107/2009:
A study according to OECD TG 416 and GLP is available (report year 2017). Wistar rats were exposed by oral gavage to 0, 10, 25 and 50 mg TTO/kg bw/day in the parental generation. The F1 generation received 0, 10, 25 and 38 mg/kg bw/day since treatment related alterations were observed in the reproductive performance at 50 mg/kg bw/day (P generation). Control animals received the vehicle groundnut oil. The test item TTO was in compliance with ISO 4730:2004 specifications. The test item was administered to male rats for 10 weeks prior to mating, during mating and after completion of mating process until the necropsy. Females received the test item for 10 weeks prior to mating, through mating, pregnancy and up to the weaning of F1 offspring, after which, parental females were sacrificed. F1 generation offspring were treated from weaning till they were sacrificed after obtaining F2 weanlings.
In their opinion RAC summarized the results from this study as following (RAC, 2023): "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." The NOAEL for reproductive toxicity is considered to be 25 mg/kg bw/day.

In the available 28 days and 90 days repeated dose toxicity studies (see section above for further details), similar effects are reported at testes organ weight and spermatogenesis supporting the effects noted in the 2-generation study.

A detailed discussion on the effects observed on sperm and tested/epididymis is presented in the section 3.9 below.

Conclusion from Applicant:
The reproductive toxicity of TTO has been assessed in a study according to OECD TG 416, in which adverse effects on fertility were reported in rats. However, these effects are considered-species specific and not relevant for humans as indicated in the discussion below

in section 3.9. Furthermore, the effects were observed at concentrations that were much higher than those used in cosmetic formulations.

Table 36: Summary of the sperm evaluation

	P-Generation			F1-Generation		
Concentration (mg/kg bwt/day)	**10**	**25**	**50**	**10**	**25**	**38**
No. of Animals per Concentration	25	25	25	25	25	25
Progressive motile sperms %	—	—	↓(15)	—	—	↓(16)
Motile sperms %	—	—	↓(12)			
Normal sperms %	—	—	↓(14)			
Abnormal sperms %	—	—	↑ (486)			
No. of sperms per cauda epididymis	—	↓(16)	↓(32)	—	—	↓(19)
No of sperms per gram of cauda epididymis	—	—	↓(19)	—	—	↓(18)

↑: Increased ↓: Decreased —: No Change
Values in parenthesis indicate percentage change

SCCS comments

The information from the OECD TG 416 two-generation fertility and reproductive toxicity study suggests that exposure to TTO can modify some parameters associated with endocrine effects at high doses of 50 mg/kg bw/d, mainly relating to sperm counts, sperm morphology and sperm motility in the F1 generation of the treated dams. In the parental generation, the number of pregnancies was adversely affected by TTO (92, 84, 84, 56% respectively). At 50 mg/kg bw/d, 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 the highest dose in the F1 generation, 38 mg/kg bw/d cauda sperm counts (number of sperms per cauda epididymis and number of sperms per gram of cauda epididymis) were lower and the number of pregnancies was affected.

However, the effect at 25 mg/kg bw/d on the number of sperms per cauda epididymis ($x10^{-6}$) and number of sperms per gram of cauda epididymis ($x10^{6}$) at 25 mg/kg bw/d is mainly due to one rat (1831), whose value is 100 times lower than the average of the other 24 rats in the same group, and is therefore clearly an outlier. Excluding this outlier from the statistical analysis, and keeping in view the historic control values, the SCCS considers that a NOAEL of 25 mg/kg bw/d could be derived for fertility and reproductive toxicity from this study.

3.5.5.2 Developmental Toxicity

In the previous SCCP Opinion for TTO (SCCP/1155/08), no data on developmental toxicity were provided for TTO.

A new study according to OECD TG 414 performed under GLP conditions is available from the REACH Dossier for Melaleuca alternifolia, ext. (EC 285-377-1/CAS 85085-48-9) The full report for this study is attached in Appendix 9 [Study Report OECD TG 414, rats, STUDY CODE: 09/016-105P, 08. November 2010]. Female Wistar rats had been orally administered from GD 5 to GD 19 with TTO at concentrations of 20,100 and 250 mg/kg bw/day, a control group without treatment was included.

Study Design:

Guidelines/Methods:	OECD Guideline for Testing of Chemicals no. 414, 22nd January 2001, OECD Guidance no. 43 on Mammalian Reproductive Toxicity Testing and Assessment, 24th July 2008
Study schedule:	Start of the Experiment: 15 November – 01 December 2009 (GD5, first dose administration)
	End of the experiment: 30 November – 16 December 2009 (GD20, final necropsy)
Report date:	8th November 2010
Species/strain:	rat/Wistar (m/f)
Exposure route:	oral gavage
Vehicle	PEG 400
Group size:	n=27
Test substance:	Tea Tree Oil (Essential oil obtained from the leaves and terminal branchlets of Melaleuca alternifolia, by steam distillation), meeting ISO Standard 4730-2004
Concentrations:	0, 20, 100, 250 mg/kg bw/day
Exposure duration:	GD 5 to GD 19
GLP:	Yes

Reference: Study Report, LAB Research Ltd, STUDY CODE: 09/016-105P, 8.11.2010

Summary

A developmental toxicity study was conducted with TTO in naturally mated, assumed pregnant Hannover Wistar female rats according to OECD Test Guideline 414, to evaluate the effect on dams and developing conceptuses after oral (gavage) administration during pregnancy. A control group which received PEG 400 only and three groups treated with TTO formulated in PEG400 at 250, 100 and 20 mg/kg bw/day were included in the study. TTO formulated in PEG 400 was administered daily from gestation day (GD) 5 to GD19, where GD0 was considered the day of mating when the sperm-positive vaginal smear, and/or the vaginal plug was identified. Clinical observations were performed on all animals twice daily. Individual body weight was recorded on GD 0, 3, 5, 8, 11, 14, 17, and/or 20 with accuracy of 1 g. For each surviving animal, the food was weighed on GD 0, 3, 5, 8, 11, 14, 17 and 20 with accuracy of 1 g. Food consumption was calculated for each measured interval, including for GD0-20. Caesarean section and maternal necropsy with macroscopic examination were performed on GD20 in all the females surviving to termination. Placentas and fetuses were examined macroscopically, and fetal body weight was measured.

Reduced maternal body weight gain and food consumption were noted at 100 and 250 mg TTO/kg bw/day. At 250 mg TTO/kg bw/day, mortality occurred in 7/27 females between GD8 and GD11. Clinical signs including noisy respiration, decreased activity and/or piloerection were observed in 2/7 animals prior to death. In 5/7 found dead animals, no test item related clinical signs were noted prior to death. In 16/20 high dose surviving females, clinical signs including decreased activity, hunched back position, noisy respiration, piloerection, red spots on the tail and/or soft feces were observed as of GD7 and were considered potentially related

to test item administration. In 4/20 high dose surviving females, no clinical signs considered related to TTO administration were noted. Treatment with TTO at a dose level of 100 mg/kg bw resulted in no mortality but clinical signs such as noisy respiration, decreased activity, hunched back position, red spots and/or soft feces were noted in 13/26 females. At 20 mg TTO/kg bw/day, there was no mortality. Clinical signs including but not limited to noisy respiration or soft feces were occasionally noted but were considered toxicologically equivocal in correlation with TTO administration. At necropsy of the females treated at 250 mg TTO/kg bw/day, bilaterally enlarged adrenals potentially associated with TTO administration were observed in all animals found dead and in 6/20 of animals that survived until scheduled euthanasia. There were no macroscopic findings considered related to TTO administration in animals treated at 20 and 100 mg TTO/kg bw/day, with the exception of 1/26 females treated at 100 mg TTO/kg bw/day that had bilaterally enlarged adrenals.

At 250 mg TTO/kg bw/day, statistically higher numbers of pre-implantation losses compared to the total number of corpora lutea was observed. As treatment started on GD5 after most implantations are complete, this is considered unlikely to be related to treatment. A higher number of late embryonic deaths and post-implantation losses, when compared to number of implantations, occurred in the high dose group, with an overall higher total intrauterine mortality compared to the number of corpora lutea. Post-implantation losses were normal in the low and mid treatment groups. When evaluated on a per litter basis, the number of corpora lutea and implantation sites was higher in the treated groups compared to the control animals. There were no significant differences in pre-implantation loss between the treated and control animals. Higher values, without statistical significance, were observed for late embryonic death, post-implantation loss and total intrauterine mortality at 250 mg TTO/kg bw/day. In view of the adverse effects related to the systemic maternal toxicity, the post-implantation mortality could be secondary to maternal toxicity.

No macroscopic findings were noted in the gravid uteri or in the appearance of the placenta in the treated pregnant dams when compared to the controls, with the exception of one high dose female that had placental fibrinoid degeneration associated with 2 malformed fetuses. Statistically lower than control mean gravid uterine weight was noted at 250 mg TTO/kg bw/day and lower terminal mean body weight when corrected for the gravid uterine weight, at 100 and 250 mg TTO/kg bw/day. The corrected mean body weight gain was lower than the controls in all the dose groups, with a dose related pattern. These adverse effects were considered to be related to TTO administration. Most fetuses examined were viable and no effects considered related to TTO were noted in the mean number of viable fetuses/group, or their sex distribution. The sex ratios were similar in the control and treated groups when evaluated per litter. Test-item related adverse effects were noted in the mean fetal weights at 100 and 250 mg TTO/kg bw/day, with a dose related pattern and mean values up to 32% lower than controls in the high dose group. Fetal external variations were observed and consisted of test item related adverse effects on the fetal body weight in the 100 and 250 mg TTO/kg bw/day dose group, considered related to the intrauterine growth retardation. The number and percentage of growth retarded fetuses were higher than the controls in the mid 100 mg TTO/kg bw/day and high 250 mg TTO/kg bw/day dose groups. In addition, local edema was noted in the cervical area in 1/159 high dose fetuses. Malformations such as generalized edema (3 litters) or short maxilla (1 fetus,1 litter) were noted in the high dose 250 mg TTO/kg bw/day group and were ascribed to TTO administration. During the visceral evaluation, a statistically higher number of variations were noted in the high dose group,

resulting in a higher number of overall abnormalities in this dose group, although there were no statistically significant differences to control in the number of malformations.

Visceral variations including dilated brain ventricles and displaced gonads potentially associated with the intrauterine growth retardation were noted. In addition, variations such as small nasal conchae, close origin of brachiocephalic and carotid, dilated ureter or dilated renal pelvis were observed during the study, with a statistically higher number at 250 mg TTO/kg bw/day. The visceral malformation noted in 1 mid dose and 2 high dose fetuses was hypoplastic thymus; due to the low incidence and in the absence of any statistical differences to the control group, a correlation with TTO administration cannot be ascertained, although it cannot be excluded. A statistically higher number of variations were noted at 100 and 250 mg TTO/kg bw/day and a statistically higher number of malformations, at 250 mg TTO/kg bw/day only. The number of abnormalities (variations and malformations) was statistically higher in both mid and high dose group fetuses. Skeletal variations including retarded calcification of the skull, 3 or less sternal bodies, missing calcification of vertebral bodies, 2.5 or less metacarpals, 3 or less metatarsals, and/or missing pubic ossification were observed with a higher incidence in the 100 and 250 mg TTO/kg bw/day groups and were considered related to the intrauterine growth retardation. Other variations such as irregular calcification of skull, displaced and/or misshapen sternal bodies, split xyphoid process, dumbbell shaped vertebral bodies (2 or more), bipartite and/or hemicentric vertebral bodies were observed, unrelated to the intrauterine growth retardation. A statistically higher incidence of skeletal malformations unrelated to the intrauterine growth retardation was noted in the high dose group and included displaced rib cartilages at the sternum, malformed vertebrae, and/or short, bent scapula, humerus or femur. All formulations proved to be homogeneous, as similar results were obtained at analysis of the top/middle/bottom samples, and the measured concentrations were found to be in the acceptable range of 100±10% (94 to 108%) of the nominal concentrations. No peak was detected in the control samples at the retention time of (+/-) Terpinen-4-ol.

Severe maternal toxicity was seen in mid and high dose dams (reduced food consumption and weight loss gains, and mortality in high dose group only). The NOAEL for maternal toxicity was 20 mg/kg bw/day. Reductions in fetal body weight were seen in the mid and high dose groups. Increases in external and skeletal malformations were also seen in fetuses from the high dose group. All effects were secondary to maternal toxicity. The NOAEL for fetuses for TTO for developmental toxicity (secondary to severe maternal toxicity) was 20 mg/kg bw/day. The NOAEL of 20 mg/kg/day for maternal toxicity was used as Point of Departure for MoS calculations because it was the lowest NOAEL from all repeated dose/reproductive toxicity studies.

Further data on developmental toxicity of TTO are available in the Combined Draft Renewal Assessment Report prepared according to Regulation (EC) No 1107/2009:

A study according to OECD 414 and GLP was conducted in which Wistar rats received TTO (reflects the ISO 4730:2004) via oral gavage on GD 5 to GD 19 (report year 2012). The initial tested doses of 75, 150 and 300 mg/kg bw/day were reduced to 30, 60 and 120 mg/kg bw/day on GD 8 due to severe effects. The NOAEL for maternal toxicity was 30 mg/kg bw/day based on reduced maternal body weight and food consumption at higher doses. No major external, visceral or skeletal malformations were observed. Delayed ossification and reduced pup body weight was secondary to maternal toxicity. The NOAEL for fetal toxicity was 60 mg/kg bw/day.

A study according to OECD 414 and GLP was conducted in which pregnant New Zealand rabbits received TTO (reflects the ISO 4730:2004) via oral gavage on GD 6 to GD 28 (report year 2018). Doses of 0, 15, 30 and 75 mg/kg bw/day were tested. The NOAEL for maternal toxicity was reported 75 mg/kg bw/day. External, visceral and skeletal examination of fetuses revealed no signs of teratogenicity or developmental toxicity. The NOAEL for teratogenicity was 75 mg/kg bw/day. Since increased post-implantation loss at the highest dose was reported the NOAEL for fetal toxicity was set to 30 mg/kg bw/day.

From the Applicant:
In the developmental toxicity studies some effects on pup weight and skeletal development in rats were seen but secondary to maternal toxicity. A dose-dependent increase in post-implantation loss in the study according to OECD TG 414 performed in rabbits was reported. The effects were observed at concentrations that were much higher than those used in cosmetic formulations.

SCCS comment
The SCCS agrees with the Applicant's conclusion regarding the OECD TG 414 prenatal developmental toxicity study in rats orally administered with TTO at concentrations of 20,100 and 250 mg/kg bw/day, and notes that a NOAEL of 20 mg/kg bw/day could be derived since all the adverse effects at higher doses were secondary to maternal toxicity.

3.5.6 Mutagenicity / genotoxicity

From the Applicant
In vitro and *in vivo* studies on mutagenicity potential had been presented and evaluated in SCCP/1155/08: In an *in vivo* mouse micronucleus study according to OECD TG 474 and GLP the test item TTO (ISO 4730:2004) was non-clastogenic. No genotoxicity was reported using S. typhimurium tester strains.

3.5.6.1 Mutagenicity / genotoxicity *in vitro*

From the Applicant
From the REACH disseminated dossier an *in vitro* chromosomal aberration study according to OECD TG 473 and GLP and an *in vitro* cell gene mutation study according to OECD TG 476 and GLP are available. The details are presented below:

Study design:	Guideline/methods: OECD TG 473
Report year	2009
Cell line:	Chinese hamster lung fibroblasts (V79)
Concentrations:	Exp. 1 (3/20 h treatment): 9.76, 19.53, 39.06 and 58.59 µg /mL (+/- S9) Exp. 2 (20/28 h treatment): 4.88, 9.76, 19.53 and 39.06 µg /mL (- S9) Exp. 3 (3/28 h treatment): 9.76, 19.53, 39.06 and 58.59 µg /mL (+ S9)
Vehicle:	DMSO and medium
Test substance:	Tea Tree oil, purity 100%, complies with ISO 4730:2017 specification.
GLP:	Yes
Results:	negative with and without metabolic activation, tested up to cytotoxic concentrations
Reference:	ECHA disseminated Dossier EC 285-377-1

TTO tested up to cytotoxic concentrations, both with and without metabolic activation, did not induce structural chromosome aberrations in this test in V79 Chinese Hamster lung cells. Therefore, TTO and its metabolite(s) are not considered to be clastogenic in this test system.

Study design:	Guideline/methods: OECD TG 476
Report year:	2010
Cell line:	Mouse lymphoma L5178Y cells
Concentrations:	Assay 1: 3h +S9: 100, 75, 50, 25, 10 and 5 µg/mL 3h –S9: 70, 60, 40, 20, 10 and 5 µg/mL Assay 2: 3 h +S9: 125, 112.5, 100, 75, 50, 25, 10 and 5 µg/mL/ 24 h –S9: 40, 30, 20, 10 and 5 µg/mL Assay 3: 24 h –S9: 50, 45, 40, 30, 20, 10 and 5 µg/mL
Vehicle:	DMSO
Test substance:	Tea Tree oil, purity 100%, complies with ISO 4730:2017 specification.
GLP:	Yes
Results:	negative with and without metabolic activation
Reference:	ECHA disseminated Dossier EC 285-377-1,

No mutagenic effect of TTO nor any formed metabolites was observed either in the presence or absence of a metabolic activation system under the conditions of this Mouse Lymphoma Assay.

Further *in vitro* data on mutagenicity are available in the Combined Draft Renewal Assessment Report prepared according to Regulation (EC) No 1107/2009: It was concluded that TTO is negative in a bacterial gene mutation assay according to OECD TG 471 and GLP (report year 2010) with and without metabolic activation and negative in a mammalian cell gene mutation assay according to OECD TG 476 and GLP (Chinese hamster Ovary cells, report year 2015).

RAC concludes 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).

Conclusion:
No classification of TTO for mutagenicity is warranted.

Additional mutagenicity study reports received with the submission:
The SCCS received from the Applicant original study reports on gene mutation in mammalian cells (MLA) and chromosomal aberration test in V79 cells. Analysis of the reports is presented below.

Gene mutation in mammalian cells (Mouse Lymphoma Assay)

Guideline/methods:	OECD TG 476
Test system:	L5178Y TK+/- 3.7.2 C mouse lymphoma
Replicates:	Duplicates
Test substance:	Tea Tree oil
Batch (Purity):	A352, complies with ISO 4730:2017 specification.
Vehicle:	DMSO and medium
Concentrations and exposures:	Exp. 1: 3 h treatment +S9-mix: 100, 75, 50, 25, 10 and 5 µg/mL; 3 h treatment –S9-mix: 70, 60, 40, 20, 10 and 5 µg/mL. Exp. 2: 3 h treatment +S9-mix: 125, 112.5, 100, 75, 50, 25, 10 and 5 µg/mL; 24 h treatment –S9-mix: 40, 30, 20, 10 and 5 µg/mL Exp. 3: 24 h treatment –S9-mix: 50, 45, 40, 30, 20, 10 and 5 µg/mL
Positive controls:	4-Nitroquinoline-N-oxide (0. 15 µg/mL for 3 h treatment; 0.1 µg/mL for 24 h treatment) – S9-mix Cyclophosphamide (4 µg/mL) - +S9-mix
Negative controls:	DMSO
GLP:	Yes
Date of report:	25 June 2010
Study period:	21 May 2009-25 June 2010

An *in vitro* mammalian cell assay was performed in mouse lymphoma L5178Y TK+/- 3.7.2 C cells to test the potential of tea tree oil (TTO) to cause gene mutation and/or chromosome damage.

Materials and methods:
Treatment was performed for 3 hours with and without metabolic activation (±S9 mix) and for 24 hours without metabolic activation (-S9 mix). Treatment concentrations for the mutation assay were selected based on the results of a preliminary cytotoxicity test. Dimethyl sulfoxide was used as the co-solvent in this study.

Results:

In Assay 1, TTO was tested at concentrations of 275; 250; 225; 200; 150; 100; 75; 50; 25; 10 and 5 µg/mL during a 3-hour treatment with metabolic activation and at concentrations of 120; 110; 100; 90; 80; 70; 60; 40; 20; 10 and 5 µg/mL during a 3-hour treatment without metabolic activation. In Assay 1, following a 3-hour treatment with metabolic activation, excessive cytotoxicity was observed at concentrations of 275, 250, 225, 200 and 150 µg/mL. Therefore, at these concentrations, cells were not plated for viability and TFT resistance due to the absence of surviving cells. An evaluation was made at concentrations of 100, 75, 50, 25, 10 and 5 µg/mL, although the observed cytotoxicity at 100 µg/mL (relative survival of 87%) was above 10-20% relative survival for the highest concentration, as recommended in the OECD guideline. No significant increase in mutation frequency was observed at the evaluated concentrations. No significant dose response to the treatment was indicated by the linear trend analysis. In Assay 1, following a 3-hour treatment without metabolic activation, excessive cytotoxicity was observed at concentrations of 120, 110, 100 and 90 µg/mL. At these concentrations cells were not plated for survival, viability and TFT resistance due to the low number of surviving cells. Marked cytotoxicity was observed at 80 µg/mL (3% relative survival), which is lower than the acceptance criteria of the OECD guideline (10-20% relative survival), therefore this concentration was excluded from further evaluation. The concentration of 70 µg/mL resulted in 17% relative survival, which met the acceptance criteria for the highest concentration. Therefore, an evaluation was made at concentrations of 70, 60, 40, 20, 10 and 5 µg/mL. No significant increase in the mutation frequency was observed at the evaluated concentrations. No significant dose response to the treatment was indicated by the linear trend analysis.

In Assay 2, TTO was tested at concentrations of 275; 250; 225; 200; 150; 137.5; 125; 112.5; 100; 75; 50; 25; 10 and 5 µg/mL during a 3-hour treatment with metabolic activation and at concentrations of 120; 110; 100; 90; 80; 70; 60; 50; 40; 30; 20; 10 and 5 µg/mL concentrations during a 24-hour treatment without metabolic activation.
In the 3-hour treatment with metabolic activation performed during Assay 2, three additional treatment levels (137.5, 125 and 112.5 µg/mL) were included in an attempt to fulfil the requirements regarding the acceptable level of cytotoxicity. In this experiment, excessive cytotoxicity was observed at concentrations of 275, 250, 225 and 200 µg/mL. No cells survived on the survival plates and no samples were plated for viability or TFT resistance at these concentrations due to the absence of surviving cells during the expression period. Marked cytotoxicity was observed at 150 and 137.5 µg/mL (1 and 2% relative survival, respectively), furthermore cells died in these samples during the expression period. The concentration of 125 µg/mL resulted in 12% relative survival, which met the acceptance criteria of 10-20% relative survival for the highest concentration. An evaluation was made at concentrations of 125, 112.5, 100, 75, 50, 25, 10 and 5 µg/mL. No significant increase in mutation frequency was observed. No significant dose response to treatment with TTO was indicated by linear trend analysis. In Assay 2, following a 24-hour treatment without metabolic activation, excessive cytotoxicity was observed at concentrations of 120, 110, 100, 90, 80, 70 and 60 µg/mL. At these concentrations cells were not plated for survival or maintained through the expression period due to the low number of surviving cells. The concentration of 50 µg/mL resulted in 15% relative survival, which met the acceptance criteria of 10-20% relative survival for the highest concentration. However, cells treated at 50 µg/mL died during

the expression period therefore this sample was not plated for viability or TFT resistance. The concentration of 40 µg/mL resulted in 46% relative survival, which is higher than the acceptance criteria. An evaluation was made at concentrations of 40, 30, 20, 10 and 5 µg/mL. No significant increase in mutation frequency was observed and no significant dose response to the treatment was indicated by linear trend analysis.

In Assay 3, TTO was tested at concentrations of 120, 110, 100, 90, 80, 70, 60, 50, 45, 40, 30, 20, 10 and 5 µg/mL during a 24-hour treatment without metabolic activation. In Assay 3, excessive cytotoxicity was observed at concentrations of 120, 110, 100, 90, 80, 70 and 60 µg/mL. At these concentrations cells were not plated for survival or maintained through the expression period due to the low number of surviving cells. The concentration of 50 µg/mL resulted in 30% relative survival, which is slightly higher than the acceptance criteria of 10-20% relative survival for the highest concentration. An evaluation was made at concentrations of 50, 45, 40, 30, 20, 10 and 5 µg/mL. No significant increase in mutation frequency was observed and no significant dose response to the treatment was indicated by linear trend analysis. No precipitation of the test item was observed visually in any mutation experiment at the start or end of treatment.

Conclusion:
In conclusion, no mutagenic effect of tea tree oil nor of any formed metabolites was observed either in the presence or absence of a metabolic activation system under the conditions of this Mouse Lymphoma Assay.

Ref.: Tea tree oil. *In vitro* mammalian cell gene mutation test: mouse lymphoma assay. LAB Research Ltd. 25 June 2010. Study code: 09/016-033EL.

SCCS comment
The report for this valid gene mutation in mammalian cells (MLA) shows negative results and the low concentrations tested have been appropriately justified by the cytotoxicity observed.

Chromosome aberration test in V79 cells

Guideline/methods:	OECD TG 473
Test system:	L5178Y TK+/- 3.7.2 C mouse lymphoma
Replicates:	Duplicates
Test substance:	Tea Tree oil
Batch (Purity):	A352, complies with ISO 4730:2017 specification.
Vehicle:	DMSO and medium
Concentrations and exposures:	Experiment A with 3/20 h treatment/sampling time • without S9 mix: 9.76 19.53, 39.06 and 58.59 µg TTO/mL • with S9 mix: 9.76 19.53, 39.06 and 58.59 µg TTO/mL Experiment B with 20/28 h treatment/sampling time • without S9 mix: 4.88, 9.76 19.53, and 39.06 µg TTO/mL Experiment B with 3/28 h treatment/sampling time • with S9 mix: 9.76 19.53, 39.06 and 58.59 µg TTO/mL
Positive controls:	Ethyl methanesulphonate (0.4 or 1 µL/mL for 3 h treatment) – S9-mix N-Nitrosodimethylamine (1 µg/mL) - +S9-mix

Negative controls:	DMSO
GLP:	Yes
Date of report:	02 October 2009
Study period:	27 April 2009-02 October 2009

The test item, Tea Tree Oil (TTO) was tested in a Chromosome Aberration Assay in V79 cells of the Chinese hamster lung *in vitro*.

Materials and methods:

TTO was dissolved in DMSO and a range of test concentrations were selected on the basis of cytotoxicity investigations made in a preliminary study (without and with metabolic activation). In two independent experiments (performed in duplicate) at least 200 well-spread metaphase cells were analysed at concentrations and incubation/expression intervals given below, ranging from low to maximum (< 50% survival) toxicity:

Experiment A with 3/20 h treatment/sampling time

- without S9 mix: 9.76 19.53, 39.06 and 58.59 µg TTO/mL
- with S9 mix: 9.76 19.53, 39.06 and 58.59 µg TTO/mL

Experiment B with 20/28 h treatment/sampling time

- without S9 mix: 4.88, 9.76 19.53, and 39.06 µg TTO/mL

Experiment B with 3/28 h treatment/sampling time

- with S9 mix: 9.76 19.53, 39.06 and 58.59 µg TTO/mL

Results:

In **Experiment A**, there were no increases in the number of cells showing structural chromosome aberrations without gaps, either in the absence or in the presence of metabolic activation, up to and including cytotoxic concentrations (9.76, 19.53, 39.06 and 58.59 µg TTO/mL). There were no statistical differences between TTO and negative control groups and no dose-response relationships were noted.

In **Experiment B**, the number of cells with structural chromosome aberrations without gaps was not increased when TTO was examined up to cytotoxic concentrations (4.88, 9.76 19.53, and 39.06 µg/mL) without S9 mix over a prolonged treatment period (20 h). Furthermore, a 3 h treatment with TTO up to cytotoxic concentrations (9.76 19.53, 39.06 and 58.59 µg TTO/mL) in the presence of S9 mix did not cause an increase in the number of cells with structural chromosome aberrations without gaps, confirming the negative results in Experiment A. No statistical differences between TTO and negative control groups and no dose-response relationships were noted.

There were no polyploid or endoreduplicated metaphases in either experiment in the presence or absence of metabolic activation.

The validity of the test was demonstrated using Ethyl methanesulphonate (0.4 and 1.0 µL/mL) and N-Nitrosodimethylamine (1.0 µL/mL) as positive controls.

Conclusion:

In summary, Tea Tree Oil tested up to cytotoxic concentrations, both with and without metabolic activation, did not induce structural chromosome aberrations in this test in V79 Chinese Hamster lung cells. Therefore, Tea Tree Oil and its metabolite(s) are not considered to be clastogenic in this test system.

Ref.: Tea tree oil. *In vitro* mammalian chromosome aberration test. Lab Research Ltd. 02 October 2009. Study code: 09/016-020C

SCCS comment

The report for valid chromosomal aberrations (V79 cells) study shows negative results and the low concentrations tested have been appropriately justified by the cytotoxicity observed. A limitation in the chromosomal aberration assay is that it was scored on 200 metaphases, according to previous OECD TG 473, not on 300 recommended by the current TG.

3.5.6.2 Mutagenicity / genotoxicity *in vivo*

An *in vivo* mouse micronucleus study according to OECD TG 474 and GLP the test item TTO (ISO 4730:2004) had been presented and evaluated in SCCP/1155/08. The result was negative.

Overall SCCS comment of mutagenicity/genotoxicity of TTO

TTO was tested in valid bacterial and mammalian cells gene mutation tests with negative results. TTO was also tested in one valid *in vitro* chromosomal aberration test and one *in vivo* micronucleus test, both with negative results.

Overall, the available evidence does not raise concerns about the mutagenicity of TTO, which is in line with the previous evaluations by EFSA and ECHA.

A recent EFSA evaluation (https://doi.org/10.2903/j.efsa.2024.9026) expressed concerns over the presence of methyleugenol in TTO in one of the analysed batches. However, the data presented in the current submission did not indicate methyl eugenol to be present at or above the allowed limits under Annex III (III/102) of the Cosmetic Regulation (EC) No. 1223/2009.

3.5.7 Carcinogenicity

From the Applicant:

There are no carcinogenicity studies with Tea Tree Oil available. TTO was tested negative in genotoxicity studies and it is unlikely that carcinogenicity based on genotoxic mechanisms of action appear. In the Combined Draft Renewal Assessment Report prepared according to Regulation (EC) No 1107/2009 it is stated that it is very unlikely that TTO has carcinogenic potential. This was based on data from carcinogenicity studies of 1,8-cineole, terpineol and Limonene.

SCCS comment

No data on carcinogenicity of TTO are available. However, the lack of genotoxicity potential also means that the SCCS has no concerns over genotoxic carcinogenicity of TTO.

3.5.8 Photo-induced toxicity

From the Applicant:
According to the information presented in the Combined Draft Renewal Assessment Report prepared according to Regulation (EC) No 1107/2009 none of the TTO components absorb at > 290 nm in neutral aqueous media (at pH 6). Accordingly, a phototoxicity study with TTO is not needed.

3.5.8.1 Phototoxicity / photo-irritation and photosensitisation

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3.5.8.2 Photomutagenicity / photoclastogenicity

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SCCS comment
The SCCS accepts the Applicant's reasoning that testing of TTO for photo-induced toxicity is not necessary because of the TTO components absorb at > 290 nm in neutral aqueous media (at pH 6).

3.5.9 Human data

Nutrivigilance

The ANSES report (ANSES, 2020) on the use of essential oils of Melaleuca in food supplements discusses some oral uses of TTO in dietary supplements and food. These uses include exposures of up to 178 mg/day in adults (with exclusion of pregnant and breastfeeding women). To compile adverse effects associated with the consumption of Melaleuca essential oils, ANSES has analysed the following assessments:

- The French ANSES' nutrivigilance system was requested during 2009 and 2019 to analyze reports of adverse effects potentially associated with the consumption of dietary supplements containing Melaleuca essential oils.
- To obtain more data, ANSES requested information from other health agencies of the European Union (Switzerland, Croatia, Ireland, Austria, Hungary, Greece, Finland, Czech Republic, Lithuania, Spain, Belgium, Sweden, Italy) in 2017 related to the consumption of dietary supplements containing essential oils. (Page 43, ANSES report)
- In addition, Canadian data from Canada vigilance between 1 January 1965 and 31 March 2018 were analysed for adverse effects on TTO (Page 43, ANSES report)
- In the United States data from the FDA-Medwatch database was analyzed for TTO. (Page 43, ANSES report)
- Finaly, ANSES used cases reported in the literature in humans after oral exposure of TTO (Page 44, ANSES report)

All these assessments have the following in common: Despite oral use of TTO in large doses over long periods of time, none of the described effects had an impact on fertility and development.

Pharmacovigilance

In the Assessment report on Melaleuca alternifolia from the European Medicines Agency (EMA, 2015, EMA 2023) TTO shows a consistent and long-standing use for at least 30 years, is for its undiluted form and for the following preparations and indications:

- Liquid preparation containing 0.5% to 10% of essential oil to be applied to the affected area 1-3 times daily for treatment of small superficial wounds and insect bites or 1-2 drops (0.033-0.066 ml) of the undiluted essential oil applied to the affected area using a cotton bud 1-3 times daily.
- Oily liquid or semi-solid preparation, containing 10% of essential oil, to be applied to the affected area 1-3 times daily or 0.7-1 ml of essential oil stirred in 100 ml of lukewarm water to be applied as an impregnated dressing to the affected areas of the skin for treatment of small boils (furuncles and mild acne). The undiluted essential oil is to be applied to the boil using a cotton bud 2-3 times daily.
- Oily liquid or semi-solid preparation, containing 10% of essential oil, to be applied to the affected area 1-3 times daily for the relief of itching and irritation in cases of mild athlete´s foot. The undiluted essential oil is to be applied to the affected area using a cotton bud 2-3 times daily until the condition is cleared up.
- 0.17-0.33 ml of TTO to be mixed in 100 ml of water for rinse or gargle several times daily for symptomatic treatment of minor inflammation of oral mucosa. This volume corresponds to approx. 150-300 mg of the essential oil daily.

According to EMA's report, this type of products also has a known safety profile with a long history of usage in traditional medicinal. Pharmacovigilance and individual case reports did not detect any effect on fertility and development in humans.

3.5.10 Special investigations

Applicant's Discussion on Fertility

TTO has been assigned a Reproductive Toxicity Category 1B; H360F on the basis of effects on rat and dog sperm and evidence of decreased fertility in male rats at high doses. The TTO effect on male rat fertility is clear but the relevance to humans in general, and following dermal exposure in particular, is negligible.

Approximately 87% of TTO is composed of 5 closely related compounds (Carson et. al.,2006), all of which are para-isopropyl toluene congeners with varying degrees of unsaturation of the cyclohexane ring; terpinen-4-ol, γ-terpinene, α-terpinene, α-terpineol, and α-terpinolene. All these constituents share a common carbon skeleton to a class of compounds demonstrated by extensive data and analysis in the public domain to be subject to species-specific metabolism that correlates with species specific sensitivity to testicular/sperm and liver toxicity (Laue, *et al.*, 2020; Laue, Kern, Badertscher, Ellis, & Natsch, 2017; Natsch, Nordone,

Adamson, & Laue, 2021). These unsaturated para-alkyl monocyclic terpenoids are subject to phase I metabolism to the benzoic acid, in most species, which is then subject to phase II metabolism with clear quantitative species-differences. In species such as the rat which exhibit male fertility toxicity the benzoic acid moiety is subject to conjugation with Coenzyme A and this conjugate has been shown to persist at high levels in cultures of plated rat hepatocytes. In other species that do not exhibit male fertility toxicity only lower levels of the CoA conjugate are observed and decline rapidly to negligible levels over time. CoA is an essential cofactor in lipid metabolism critical to healthy sperm formation. Prolonged testicular depletion of CoA is consistent, and closely correlates, with observed patterns of testicular/sperm toxicity. This pattern is highly structure specific, requiring a small alkyl substituent at the para position, a degree of saturation of the cyclohexane ring and an absence of meta and ortho groups.

Based on these data, the applicant together with Stockton Israel Ltd. initiated *in vitro* mechanistic studies with TTO components to evaluate the formation of CoA conjugates in hepatocytes and compare the metabolism in 4 different species (human, rat, rabbit, dog). Incubations are performed at different concentrations (*e.g.*, 1, 10, 50 µM) and time points (*e.g.*, 0, 0.5, 1, 2, 4, 8 and 24 h). An LC-MS analysis method is under development to screen for the formation of CoA conjugates in the supernatant of the cells (Appendix 10).

Because the metabolism of TTO in rats is not representative of that in humans (and a range of other laboratory species) if TTO were to be studied as a potential human oral pharmaceutical the rat would likely be ruled out for preclinical toxicological testing. As noted by (Mayer, 1995):

> "*There are many instances in which differences in metabolism across species will influence the interpretation of safety assessment data….. it is important to perform in vitro metabolic profiling before the first study in humans to select the appropriate species for toxicology studies.*"

This species-specific male fertility toxicity is also exacerbated by dosing techniques that result in high blood levels of TTO. As noted in the EFSA/ECHA report on the TTO containing fungicide Timorex, gavage and dietary studies in rats at comparable total daily doses show sperm toxicity after gavage dosing but not after dietary dosing (Regulation (EC) N° 1107/2009 for TTO used in plant protection (Timorex) submission to EFSA of 17.5% (BM608_M-CP_Sec_7_tox_sanitized.pdf). Bolus gavage dosing is a valid route of exposure in toxicology studies but can lead to aberrant, unrepresentative metabolism profiles compared to inhalation, dermal and dietary exposure where the Cmax, bioavailability and metabolite profile are not necessarily comparable (Dain & Jaffe, 1988). This issue is especially significant for cosmetic use of TTO where exposure is dermal, only one compound, terpinen-4-ol, constitutes over 90% of absorbed TTO and blood levels arising from the slow absorption of low levels of that compound are not consistent with bolus gavage dosing. Available studies demonstrate rat testicular toxicity from structurally related monocyclic terpenoids is route and pattern of exposure specific.

Administration of compounds by bolus gavage doses is well known to potentially alter metabolic profiles, in comparison to dietary or dermal administration, equally likely to result in either the masking of genuine hazards that might be manifest by more relevant dietary dosing (Dain & Jaffe, 1988) or manifesting hazards not plausibly achievable by relevant routes of administration. If gavage administration results in aberrant pharmacokinetics

unrepresentative of real-world exposure patterns, not reproducible by more representative dietary dosing the findings from the gavage dosing cannot be assumed to be relevant for risk assessment without careful consideration of the toxicokinetic and toxicodynamic consequences of that route of administration. In these circumstances greater weight should be placed on the more relevant route of administration.

The RAC has previously shown clear agreement with this principle. The assessment of Terpineol multiconstituent, a close structural congener of the majority of tea tree oil constituents, and structural isomer of terpinen-4-ol, noted that testicular effects consistent with those of TTO, were observed when administered to rats by gavage but not where administered in the diet. The TTO assessment report further notes in regard to the terpineol multiconstituent assessment that: in the *"study conducted by gavage, pharmacokinetic analysis also confirmed the systemic over- exposition of the rat after a single dose at 750 mg/kg". ……. "Overall, the AUC_{all} values at the highest dose level (750 mg/kg) were ca 2.3-fold higher than those values predicted from a linear relationship, demonstrating that oral gavage at high dose clearly resulted in much higher systemic exposure than expected, leading to biologically non-relevant effects that should not be considered for classification purposes.*

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."*

The totality of the available evidence indicates that gavage dosing of TTO in the rat is not a relevant model for human risk assessment of low-level dermal exposure. Consequently, low level TTO exposure by the dermal route does not present a risk of sperm toxicity in humans.

This conclusion is supported by the long history of safe use of all of the constituents of TTO as components of a wide range of traditional human food including herbs, spices, fruits and vegetables. Terpinen-4-ol for example present at 13.15% in thyme essential oil (Viuda-Martos, Fernandez-Lopez, & Perez-Alvarez, 2007). Data on natural uptake of Tea tree oil components with food demonstrate that the natural exposure is considerably higher than that likely to be achieved by dermal exposure to cosmetics containing TTO. A daily average uptake of > 12.5 mg/kg bw/day and a daily peak uptake of > 154 mg/kg bw/day via food is documented for the components [Combined Renewal Assessment Report prepared according to Regulation (EC) N°1107/2009 and Proposal for Harmonised Classification and Labelling (CLH Report) according to Regulation (EC) N° 1272/2008 Tea Tree Oil (TTO) Volume 3 - B.6 (AS) pg 8].

Selection of Point of Departure (PoD)

Several repeated dose and reproductive/developmental studies are available for TTO. These have been evaluation for the PoD selection in terms of quality and relevance of human heath in the context of cosmetic exposure. Effects observed in liver from repeated dose study were not considered relevant for humans, also in terms of classification. Effects on sperm were reported from one 28-day and three 90-day repeated doses studies, which were conducted according to OECD guidelines and GLP in rats and Beagle dogs. The lowest NOAEL was 30 mg/kg bw/day in Beagle dogs receiving TTO via gavage. The NOAEL for reproductive toxicity from a study performed according to OECD TG 416/GLP was 25 mg/kg bw/day based on

fertility effects. As discussed, these fertility effects are considered species-specific due to species-specific metabolism and considered not relevant for humans. In the developmental toxicity studies available some effects on pup weight and skeletal development in rats were seen but secondary to maternal toxicity. A dose-dependent increase in post-implantation loss in the study according to OECD TG 414 performed in rabbits was reported and the NOAEL was 30 mg/kg bw/d.

The lowest NOAEL for TTO from repeated-dose toxicity studies was derived from a reliable developmental toxicity study in rats. This study was conducted in the framework of the REACH regulation (OECD TG 414/GLP, see section 3.5.2) and the full report is available to the applicant for evaluation. Severe maternal toxicity was seen in mid and high dose rat dams, which was reduced food consumption, weight loss gains and mortality (high dose group only). The NOAEL for maternal toxicity was 20 mg/kg bw/day. All effects observed for the F1 pups were secondary to maternal toxicity. Using a conservative approach, the NOAEL of 20 mg/kg bw/d for maternal, systemic toxicity from this OECD TG 414 study was used as PoD for all MoS calculations.

SCCS overall assessment on fertility, reproduction and developmental toxicity

SCCS, while agreeing with the Applicant regarding the kinetic differences depending on the different exposure routes (gavage and diet), believes that no clear species differences emerge since following repeated administration for 90 days in the rat study (OECD TG 408) and in the dog study (OECD TG 409), the viability and motility of spermatids were decreased in both species with a NOAEL of 30 mg/kg bw.
As explained in section 3.4.5.1, the SCCS considers that a NOAEL of 25 mg/kg bw/d could be derived from the OECD TG 416 two-generation fertility and reproductive toxicity study. However, for this assessment, the SCCS has accepted the use of a lower PoD of 20 mg/kg bw/day by the Applicant derived from OECD 414 Prenatal Developmental Toxicity Study.

3.6 SAFETY EVALUATION (INCLUDING CALCULATION OF THE MOS)

The MoS calculation was performed according to the SCCS Notes of Guidance (12th revision, 2023). As indicated above the NOAEL of 20 mg/kg bw/day, derived from the OECD TG 414 study in rats was used as PoD. Considering an oral bioavailability of 70%, the resulting systemic Point of Departure (PoD_{sys}) is 14 mg/kg bw/day.

The **MoS for aggregated exposure for adults is 120** based on the SED of 0.117 mg/kg bw/day, which was obtained by aggregating the cosmetic and non-cosmetics exposure values for TTO.

MoS calculation (adults)			
Systemic exposure dose (aggregated SED)	sum SED		0.117 mg/kg bw/day
No observed adverse effect level (OECD 414, rat)	NOAEL		20 mg/kg bw/day
Oral absorption (source: REACH dossier)			70%
Adjusted NOAEL considering oral absorption of 70%	PoDsys		14 mg/kg bw/day
Margin of Safety	**MoS**	**PoDsys/SED**	**120**

Applicant's Overall Conclusions

Based on the safety profile of TTO and the MoS calculations presented here, it is concluded that TTO as an ingredient at 2% in shampoo, 1% in shower gel, 0.1% in face cream and 1% in face wash pose acceptable risk to human health, although several worst-case assumptions were made in the risk assessment. The MoS value of 120 for the aggregated exposure including non- cosmetic exposure will give sufficient protection for the use as cosmetic ingredient for adults in these products.

The final concentration of TTO in a cosmetic formulation must be such that levels of plant constituents like methyl eugenol, which may cause adverse health effects are avoided. Methyl eugenol is listed in Annex III (III/102) of the Cosmetic Regulation (EC) No. 1223/2009. It is restricted to 0.001% in rinse-off products, 0.0002% in leave-on and oral care products, 0.01% in fine fragrance and 0.004% in Eau de toilette. It is up to the manufacturer and formulator to assure that the TTO sourced and used in the cosmetic formulation will not exceed the allowed concentration to safely comply with Annex III.

A Code of Practice was developed, which was described in the former submission 2008, to ensure minimal oxidation of TTO from harvest on. Furthermore, it is recommended to include antioxidants in formulations to prevent oxidation of TTO in the final cosmetic product or to use specific packaging to minimize exposure to light. This will keep oxidation products, which increase the potential for skin sensitization and irritation as low as possible. Based on the evaluated data on skin sensitization and supported by QRA calculations the maximum concentration of TTO in rinse- off was set to 2% and in leave-on products to 0.1% to lower a possible risk for skin sensitizing effects. When testing leave-on products up to 2% TTO in formulations no irritating effects or sensitizing response in human volunteers were reported. Therefore, the concentrations used in the final products and defended in this dossier are considered as safe for use in these products.

TTO does not induce eye irritation or acute dermal toxicity and is not classified for mutagenicity. Acute oral and inhalation effects have been observed for TTO, but are not relevant since TTO is not intended to be used in sprayable products and consumers using shampoo, shower gel and face cream are not exposed to high aerosol concentrations and high oral uptake is neglectable for the indicated cosmetic products.

Adverse effects were reported in developmental and reproductive toxicity studies in rats. However, these effects are considered-species specific and not relevant for humans. Furthermore, the effects were observed at concentrations that were much higher than those used in cosmetic formulations. Moreover, both nutrivigilance and pharmacovigilance data obtained over many years, at significant exposure levels for significant periods of time did not show any effects on human fertility.

In conclusion, the safety of TTO as cosmetic ingredient in shampoo, shower gel, face cream and face wash has been demonstrated when used in concentrations up to 2% as the MoS are above 100 for adult and vulnerable groups. Data from the new dermal absorption study show that 70- 90% of TTO will evaporate after dermal application and the calculated dermal absorption values are very conservative.

SCCS comment

While the SCCS is of the view that considerations in regard to the kinetic differences related to the route of administration are valid, it does not agree with the suggested difference between the species since the NOELs derived from similar effects for male rats (30 mg/kg bw - TG 408) and dogs (30 mg/kg bw - TG409), both by oral gavage, are identical in the repeated dose toxicity tests.

As explained in section 3.4.5.1, the SCCS considers that the PoD for TTO could be set at 25 mg/kg bw/d from the OECD TG 416 study on fertility and reproductive toxicity. However, for this assessment, the SCCS has accepted the use of a lower PoD of 20 mg/kg bw/day by the Applicant. The SCCS has also accepted the Applicant's calculation of the Margin of Safety (MoS) as it was performed according to the SCCS Notes of Guidance (12th revision, 2023). Using oral bioavailability of 70%, the systemic Point of Departure (PoDsys) has been worked out at 14 mg/kg bw/d, resulting in the MoS of 120 for the aggregated exposure to TTO from cosmetics and non-cosmetics uses.

3.7 DISCUSSION

Physicochemical properties

The essential oil extracted from Tea Tree Oil (TTO) has been associated with more than one CAS identifiers: 68647-73-4 and 85085-48-9 (ECHA Registered Substance). According to the Applicant, it conforms to the updated International Standard (ISO 4730:2017). However, whilst ISO 4730:2017 relates to essential oil of Melaleuca alternifolia (Maiden et Betche) Cheel or Melaleuca linariifolia (commonly known as narrow-leaved paperbark), it does not provide any CAS number. Instead, it refers to ISO/TR 20192:2004, where the CAS number given (85085-43-4) is for a different species (Melaleuca citronnée – *i.e.* Lemon-scented tea tree).

In view of the discrepancies in the CAS identifiers, the SCCS has considered that the essential oil assessed in this Opinion is that extracted from Melaleuca alternifolia (Maiden et Betche) Cheel or Melaleuca linariifolia with CAS identifiers as either 68647-73-4 or 85085-48-9, and that it conforms to the specifications given in the updated standard ISO 4730:2017.

It is noted that TTO consists of terpene hydrocarbons, mainly monoterpenes, sesquiterpenes and their associated alcohols, out of which fifteen (15) have been identified by the Applicant as the main components, the concentrations of which are within the ranges given in the updated ISO Standard (ISO 4730:2017).

Methyl eugenol, which is a minor component of TTO, is listed in Annex III (III/102) of the Cosmetic Regulation (EC) No. 1223/2009, with its levels in cosmetic products restricted to 0.001% in rinse-off products, 0.0002% in leave-on and oral care products, 0.01% in fine fragrance and 0.004% in Eau de toilette. From the provided data, the SCCS has noted that the levels of methyl eugenol will be much lower in the final cosmetic products than those allowed according to Annex III of Regulation 1223/2009.

The Applicant has not provided any data on the stability of TTO under the conditions of storage and use. In this regard, it is notable from a study by Brophy *et al.* (1989) that the composition of TTO may change considerably during storage, with ρ-cymene levels increasing and α- and γ-terpinene levels declining. It has been suggested that exposure to light, heat, air, and/or moisture affect the oil stability. Instability of α-terpineol standard has also been indicated in the current submission (page 19).
Although the Applicant has suggested that TTO used in cosmetic products will conform to the updated International Standard (ISO 4730:2017), it is not clear how TTO will be stabilised to prevent degradation/transformation of the components in final products. The SCCS is therefore of the opinion that stability of TTO must be maintained in the final cosmetic products so that the components remain within the specifications of the updated ISO 4730:2017 standard.

Toxicokinetics

The Applicant has provided an OECD TG 428 study on the dermal absorption of TTO through human dermatomed skin at 3 tested concentrations: 1.25, 2.5 and 5%. As TTO is a complex mixture, dermal absorption was calculated from the sum obtained for six single components: terpinen- 4-ol, 1,8- cineole, terpinolene and γ-terpinene, α-terpineoland α-terpinene. The Applicant has also provided rationale for the choice of the 4 substances as indicators of the dermal absorption of TTO.

The SCCS has noted that the results for the absorption of terpinene-4-ol to dermis layer of skin in this study are in line with those reported by Chooluck (2013), although dose metrics used in both studies are different.

Considering that TTO used in cosmetic products will conform to the specifications of the updated International Standard (ISO 4730:2017), the SCCS has accepted the Applicant's measured values for dermal absorption as 12.84% (Mean + 1SD) for formulations up to 1.25% TTO, and 20.41% (Mean + 1SD) for formulations containing up to 2.5% TTO.

In an oral toxicokinetic study in male rats exposed to 75, 250 and 750 mg/kg bw of α-terpineol, the recovery of α-terpineol in urine was 65% of the administered dose (ECHA disseminated dossier EC number: 701-188-3, Section 7.1,

https://echa.europa.eu/de/registration-dossier/-/registered- dossier/20921/4/9). The SCCS is of the opinion that, from this study, the oral bioavailability of α-terpineol can be estimated as 65%. Considering that kinetic of α-terpineol and terpinen-4-ol could be similar, this oral bioavailability value can also be applied to terpinen-4-ol.

Exposure

The SCCS agrees with the Applicant's calculation of exposure from different cosmetic product categories and, on principle, with the aggregated exposure from cosmetic and non-cosmetic sources of consumer exposure. The proposed calculated SED values will be used in the calculation of margin of safety (MoS) for TTO.
However, the SCCS would like to point out that the calculation of exposure from medicines was averaged over a whole year. Therefore, the presented aggregate exposure estimate might underestimate the exposure of some users of TTO containing medicines.

Toxicological Evaluation

Irritation and corrosivity

The SCCS considers that TTO of ISO Standard 4730-2004 is not irritating to skin and eye at the use levels in the intended cosmetic products.

Skin sensitisation

In a previous assessment (SCCP/1155/08), the SCCP concluded that TTO is a sensitiser in humans, possibly due to the content of irritants such as p-cymene and 1,8-cineole, and/or the gradual accumulation of certain oxidation products.

This is supported by clinical data demonstrating that allergic contact dermatitis from TTO has been reported frequently. Most of the cases are caused by pure TTO applied on damaged skin. Cosmetics and pharmaceutical with TTO were mentioned as causes of ACD as well. Moreover, patch testing with constituents of TTO, such as ascaridole, terpinolene, α-terpinene and others has confirmed the notion that several different constituents of TTO contribute to its sensitising potential (de Groot, 2019).

The Applicant performed a QRA to assess if TTO can be safely used in cosmetic products. The procedure followed for aggregate exposure assessment is not provided by the Applicant. In addition, the exposure assessment is incomplete, since face wash products were not included. For these reasons, the SCCS cannot evaluate the QRA.
To conclude, the LLNA data provided by the Applicant in the current Opinion confirm that TTO is a moderate skin sensitiser.

Acute toxicity

The SCCS agrees with the Applicant's conclusion that: 1) acute toxicity of TTO, as indicated in oral toxicity studies, is not of importance for the intended use in cosmetic products at the proposed use levels. The SCCS also agrees that the levels of use of TTO in the intended cosmetic products do not raise a concern over acute dermal toxicity, and notes that TTO is not intended to be used in cosmetic products that could give rise to inhalation exposure of the consumer.

Repeated dose toxicity

SCCS agrees with the Applicant's conclusion on the oral 28-day and 90-day repeated dose studies in rats and dogs, and that NOAEL of 30 and 60 mg/kg bw/day could be derived for male and female rats respectively from the OECD TG 408 study, and 30 mg/kg bw/day for dogs from the OECD TG 409 study.

Reproductive toxicity

The SCCS agrees with the Applicant's conclusion regarding the OECD TG 414 prenatal developmental toxicity study in rats orally administered with TTO at concentrations of 20,100 and 250 mg/kg bw/day, and notes that a NOAEL of 20 mg/kg bw/day could be derived since all the adverse effects at higher doses were secondary to maternal toxicity.

The information from anther OECD TG 416 two-generation fertility and reproductive toxicity study suggests that exposure to TTO can modify some parameters associated with endocrine effects at high doses of 50 mg/kg bw/d, mainly relating to sperm counts, sperm morphology and sperm motility in the F1 generation of the treated dams. In the parental generation, the number of pregnancies was adversely affected by TTO (92, 84, 84, 56 % respectively). At 50 mg/kg bw/d 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 the highest dose in the F1 generation, 38 mg/kg bw/d cauda sperm counts (number of sperms per cauda epididymis and number of sperms per gram of cauda epididymis) were lower and the number of pregnancies was affected.

However, the effect at 25 mg/kg bw/d on number of sperms per cauda epididymis ($x10^{-6}$) and number of sperms per gram of cauda epididymis ($x10^{6}$) at 25 mg/kg bw/d is mainly due to one rat (1831), whose value is 100 times lower than the average of the other 24 rats in the same group, and is therefore clearly an outlier. Excluding this outlier from the statistical analysis, and keeping in view the historic control values, the SCCS considers that a NOAEL of 25 mg/kg bw/d could be derived for fertility and reproductive toxicity from this study.

Mutagenicity / genotoxicity

TTO was tested in valid bacterial and mammalian cells gene mutation tests with negative results. TTO was also tested in one valid *in vitro* chromosomal aberration test and one *in vivo* micronucleus test, both with negative results.

Overall, the available evidence does not raise a concern on mutagenicity of TTO, which is in line with the previous evaluations by EFSA and ECHA.

A recent EFSA evaluation (https://doi.org/10.2903/j.efsa.2024.9026) expressed concerns over the presence of methyleugenol in TTO in one of the analysed batches. However, the data presented in the current submission did not indicate methyl eugenol to be present at or above the allowed limits under Annex III (III/102) of the Cosmetic Regulation (EC) No. 1223/2009.

Carcinogenicity

No data on carcinogenicity of TTO are available. However, the lack of genotoxicity potential also means that the SCCS has no concerns over genotoxic carcinogenicity of TTO.

Photo-induced toxicity

The SCCS accepts the Applicant's reasoning that testing of TTO for photo-induced toxicity is not necessary because of the TTO components absorb at > 290 nm in neutral aqueous media (at pH 6).

Special investigation

As explained in section 3.4.5.1, the SCCS considers that a NOAEL of 25 mg/kg bw/d could be derived from the OECD TG 416 two-generation fertility and reproductive toxicity study. However, for this assessment, the SCCS has accepted the use of a lower PoD of 20 mg/kg bw/day by the Applicant derived from OECD 414 Prenatal Developmental Toxicity Study.

Safety evaluation (including calculation of the MoS)

While the SCCS is of the view that considerations in regard to the kinetic differences related to the route of administration are valid, it does not agree with the suggested difference between the species since the NOELs derived from similar effects for male rat (30 mg/kg bw - TG 408) and dog (30 mg/kg bw - TG409), both by oral gavage, are identical in the repeated dose toxicity tests.

As explained in section 3.4.5.1, the SCCS considers that the MoS for TTO could be set at 25 mg/kg bw/d from the OECD TG 416 study on fertility and reproductive toxicity. However, for this assessment, the SCCS has accepted the use of a lower PoD of 20 mg/kg bw/day by the Applicant. The SCCS has also accepted the Applicant's calculation of the Margin of Safety (MoS) as it was performed according to the SCCS Notes of Guidance (12th revision, 2023). Using oral bioavailability of 70%, the systemic Point of Departure (PoDsys) has been worked out at 14 mg/kg bw/d, resulting in the MoS of 120 for the aggregated exposure to TTO from cosmetics and non-cosmetics uses.

4. CONCLUSION

1. *In light of the data provided and taking under consideration the possible classification as 'Repr.1B' under Regulation (EC) No 1272/2008 (CLP Regulation) and the conditions laid out in Article 15 (2) (d) of the Regulation (EC) No 1223/2009, does the SCCS consider TTO safe when used as an anti-seborrheic and anti-microbial agent in rinse-off and leave-on cosmetic products up to the maximum concentrations provided by the applicant?*

The SCCS considers the use of Tea Tree Oil (TTO) as an anti-seborrheic and anti-microbial agent safe up to the maximum concentration of 2.0% in shampoo, 1.0% in shower gel, 1.0% in face wash and 0.1% in face cream. The assessment has considered all available data, a possible classification as 'Repr.1B' under Regulation (EC) No 1272/2008, the conditions laid out in Article 15 (2) (d) of the Regulation (EC) No 1223/2009, as well as the aggregated exposure from cosmetics and non-cosmetics uses of TTO.

This Opinion is only applicable to:

- TTO with chemical composition that conforms to the updated International Standard (ISO 4730:2017) in the intended final cosmetic products.
- the use of TTO in the intended dermally applied cosmetic products, and not in aerosolised or sprayable products that may give rise to inhalation exposure of the consumer.

2. *Alternatively, what is according to the SCCS, the maximum concentration considered safe for use of TTO in cosmetic products?*

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3. *Does the SCCS have any further scientific concerns regarding the use of TTO in cosmetic products?*

Based on the data provided, TTO is a moderate skin sensitiser.

The submission did not provide any data on the stability of TTO under the conditions of storage and use. Since the chemical composition of TTO may change due to exposure to light, heat, air and /or moisture, it is not clear how TTO will be stabilised in the final cosmetic products to prevent degradation/transformation of the components. The SCCS is therefore of the opinion that stability of TTO must be maintained in the final cosmetic products so that the components remain within the specifications of the updated ISO 4730:2017 standard.

The SCCS mandate does not address environmental aspects. Therefore, this assessment has not covered the safety of TTO for the environment.

5. MINORITY OPINION

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7. GLOSSARY OF TERMS

See SCCS/1647/22, 12th Revision of the SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation – from page 158

8. LIST OF ABBREVIATIONS

See SCCS/1647/22, 12th Revision of the SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation – from page 158