



Scientific Committee on Consumer Products

SCCP

OPINION ON

Tea tree oil



The SCCP adopted this opinion at its 18th plenary of 16 December 2008

About the Scientific Committees

Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.

They are: the Scientific Committee on Consumer Products (SCCP), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

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

SCCP

Questions concerning the safety of consumer products (non-food products intended for the consumer).

In particular, the Committee addresses questions related to the safety and allergenic properties of cosmetic products and ingredients with respect to their impact on consumer health, toys, textiles, clothing, personal care products, domestic products such as detergents and consumer services such as tattooing.

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1. BACKGROUND

The Scientific Committee on Consumer Product (SCCP) adopted a scientific opinion (SCCP/0834/04) on Tea Tree Oil (TTO) on its 2nd plenary meeting of 7 December 2004 with the following conclusion:

"The sparse data available suggest that the use of undiluted Tea Tree Oil as a commercial product is not safe. The safety dossier of Tea Tree Oil is incomplete. The stability of Tea Tree Oil in cosmetic formulations is questionable. A standardized method for the specification of Tea Tree Oil is needed. Industry should develop an analytical testing method based on typical degradation products to ensure and control the stability of the material. Skin and eye irritation was not assessed by adequate methods. There are relevant data gaps with regard to subchronic toxicity, percutaneous absorption, genotoxicity/carcinogenicity and reproductive toxicity. The safe use of Tea Tree Oil as cosmetic ingredient cannot be assessed. A complete dossier of a representative standardized material to all relevant toxicological endpoints is required by the end of 2005; an opinion based on the information available at that time will be given."

As a response to the above scientific opinion a complete new dossier was submitted by the end of March 2007.

Tea tree oil is the essential oil obtained from *Melaleuca alternifolia*, *Melaleuca linariifolia* and *Melaleuca dissitiflora* as well as other species of *Melaleuca* provided that the oil conforms to the requirements given in ISO 4730-2004. The following types of application for cosmetic products are given in the dossier:

Skin - care products incl. post-waxing treatments up to 1.25%
Hair - care products up to 2.0%
Nail - care products up to 20%
Oral hygiene up to 0.2%
Personal hygiene including shaving products up to 2%

The undiluted might be used for other purposes as well i.e. aromatherapy.

2. TERMS OF REFERENCE

1. *On the basis of the data provided, does the SCCP consider the use of Tea Tree Oil safe for the consumers when used in cosmetic products in concentrations as mentioned above?*
2. *Does the SCCP have any safety concerns for the use of Tea Tree Oil as an undiluted product?*

3. 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

/

3.1.1.3. Trade names and abbreviations

Australian Tea Tree Oil

3.1.1.4. CAS / EINECS number

CAS: 68647-73-4 (Oils, tea-tree)
 EINECS: 285-377-1

3.1.1.5. Major constituents, contents and chemical structures

The European Inventory of cosmetic ingredients contains 3 *Melaleuca*-type ingredients (INCI names): *Melaleuca alternifolia* oil (antimicrobial), *Melaleuca cajuputi* extract (tonic), *Melaleuca leucadendron* extract (tonic). Tea Tree Oil is the essential oil obtained by steam distillation of the foliage and terminal branchlets of *Melaleuca alternifolia*, *Melaleuca linariifolia* and *Melaleuca dissitiflora* as well as other species of *Melaleuca* provided that the oil obtained conforms to the requirements given in the International Standard (ISO 4730-2004). Tea Tree Oil from *Melaleuca alternifolia* contains various mono- and sesquiterpenes as well as aromatic compounds. The monoterpenes terpinen-4-ol, α -terpinene, γ -terpinene, 1,8-cineole, p-cymene, α -terpineol, α -pinene, terpinolene, limonene and sabinene account for 80 - 90% of the oil. The natural content of the individual terpenes in Tea Tree Oil may vary considerably depending on the *Melaleuca alternifolia* population used, the climate, the leaf maceration, the age of the leaves and the duration of distillation. Its major constituents are presented in Table 1. The chemical structures for these major constituents are shown in Figure 1.

Table 1: Main constituents of Tea Tree Oil (From ISO 4730-2004)

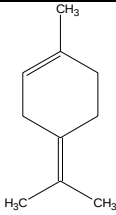
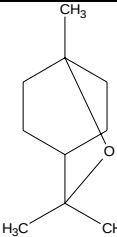
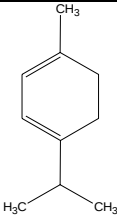
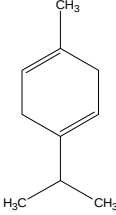
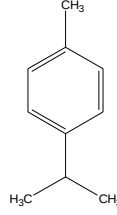
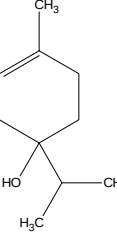
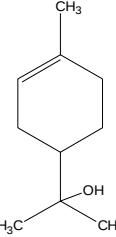
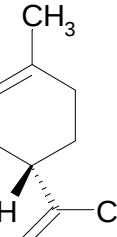
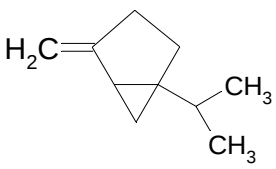
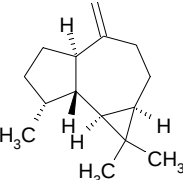
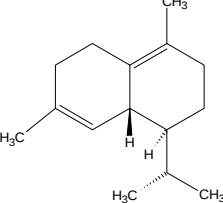
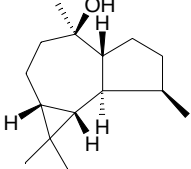
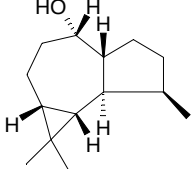
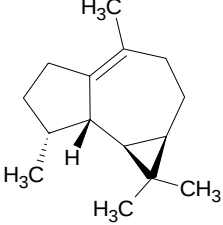
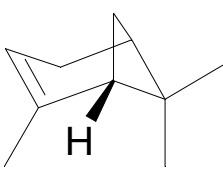
Constituent	Minimum (%)	Maximum (%)
Terpinolene	1.5	5
1,8-Cineole (eucalyptol)	Trace	15
α -Terpinene	5	13
γ -Terpinene	10	28
p-Cymene	0.5	8
Terpinen-4-ol	30	48
α -Terpineol	1.5	8
Limonene	0.5	1.5
Sabinene	Trace	3.5
Aromadendrene	Trace	3
δ -Cadinene	Trace	3

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Constituent	Minimum (%)	Maximum (%)
Globulol	Trace	1
Viridiflorol	Trace	1
α -Pinene	1	6
Ledene (syn. viridiflorene)	Trace	3

Ref.: 1

Figure 1: Chemical structures of the main constituents of Tea Tree Oil

 Terpinolene (C₁₀H₁₆) MW=136 Log P = 4.52 ± 0.22	 1,8-Cineole (C₁₀H₁₈O) MW=160 Log P = 2.82 ± 0.27	 α-Terpinene (C₁₀H₁₆), MW=136 Log P = 4.52 ± 0.22
 γ-Terpinene (C₁₀H₁₆) MW=136 Log P = 4.36 ± 0.24	 <i>p</i>-Cymene (C₁₀H₁₄) MW=134 Log P = 4.58 ± 0.24	 (+)-Terpinen-4-ol (C₁₀H₁₈O) MW=154 Log P = 4.52 ± 0.22
 (+)-α-Terpineol (C₁₀H₁₈O) MW=160 Log P = 2.73 ± 0.22	 D-Limonene (C₁₀H₁₆) MW=136.24 Log P = 4.23	 (+)-Sabinene (C₁₀H₁₆) MW=136.23 Log P = 4.13 ± 0.24
 (+)-Aromadendrene (C₁₅H₂₄) MW=204.35 Log P = 6.41 ± 0.25	 δ-Cadinene (C₁₅H₂₄) MW=204 Log P = 6.64 ± 0.24	 (-)-Globulol (C₁₅H₂₆O) MW=222.37 Log P = 4.81 ± 0.26
 (+)-Viridiflorol (C₁₅H₂₆O) MW=222.37 Log P = 4.81 ± 0.26	 (+)-ledene (syn. viridiflorene) (C₁₅H₂₄) MW=204	 α-Pinene (C₁₀H₁₆) MW=136.23 Log P = 4.37 ± 0.24

3.1.2. Physical properties

Colourless to pale-yellow liquid

3.1.3. Molecular weight

Tea Tree Oil is a mixture of several constituents. The main constituents have MW ranging from 134 to 222 g/mol.

3.1.4. Purity, composition and substance codes

The purity, composition and physico-chemical properties of Tea Tree Oil are defined in several standards and monographs. The following pharmacopoeia and composition standards are available:

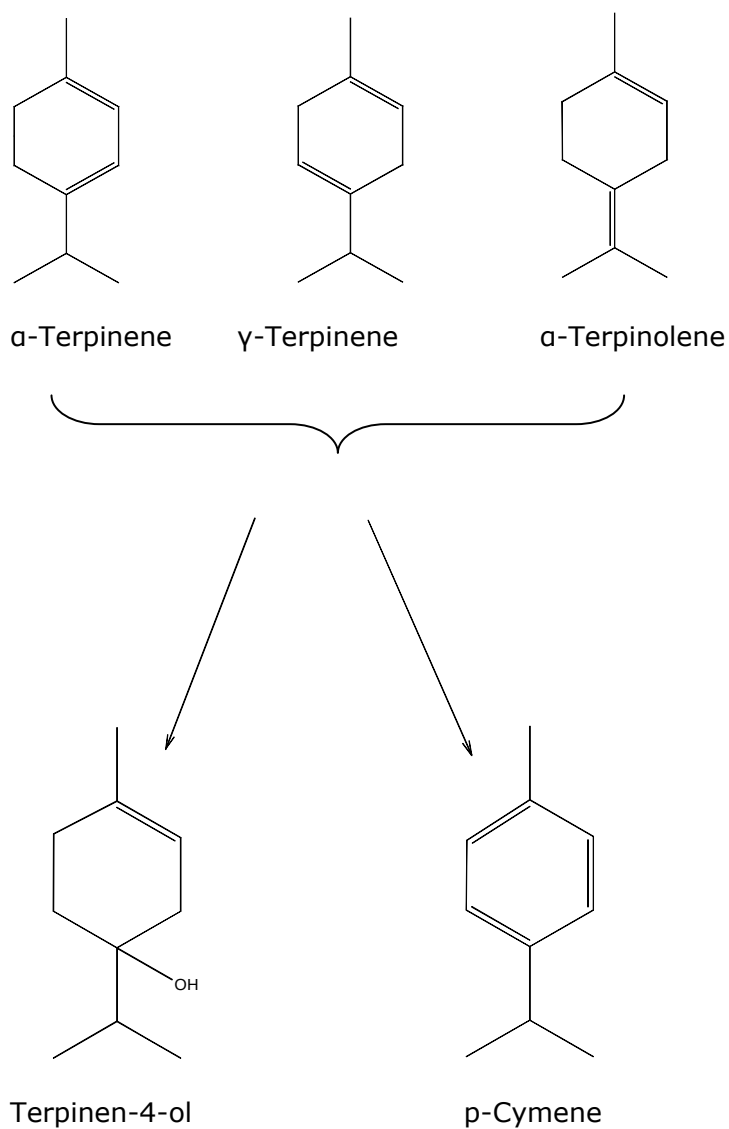
- ISO 4730:2004 - Oil of Melaleuca, terpinen-4-ol type (Tea Tree oil)
- Technical Corrigendum 1:1997 to ISO 4730:2005
- Australian Standard AS 2782-1997: Oil of Melaleuca, terpinen-4-ol type (Tea Tree oil)
- French Standard T75-358
- Deutscher Arzneimittel Codex (DAC 1986) 8th supplement, 1996
- European Pharmacopoeia
- British Pharmacopoeia
- Martindale Extra Pharmacopoeia
- Aetheroleum Melaleucae Alternifoliae, in WHO Monographs on Selected Medicinal Plants Vol. 2, P172-179, 2002¹

3.1.5. Impurities / accompanying contaminants

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. The levels of α -terpinene, γ -terpinene and terpinolene decrease whereas the level of p-cymene increases up to tenfold. Oxidation processes lead to the formation of peroxides, endoperoxides and epoxides. The main hydrolytic and oxidative degradation pathways are shown in Figure 2 (taken from Ref. 2).

Ref.: 2

¹ <http://wholibdoc.who.int/publications/2002/9241545372.pdf>

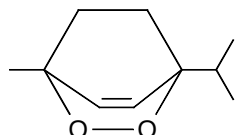
Figure 2: End products of hydrolysis and oxidation of Tea Tree Oil constituents

In Tea Tree Oil stored for 9 months under sunlight the formation of the endoperoxide ascaridole was proven using a GC-MS analytical procedure.

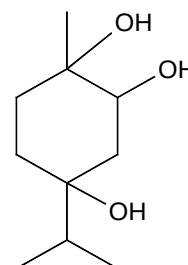
Ref.: 3

As a further oxidation product 1,2,4-trihydroxymenthane was identified.

Ref.: 4

Figure 3: Oxidation products ascaridole and 1,2,4-trihydroymenthane

ascaridole



1,2,4-trihydroymenthane

3.1.6. Solubility

Insoluble in water

Soluble in two volumes of 85% ethanol at 20 °C

3.1.7. Partition coefficient (Log P_{ow})

Log $P_{o/w}$: Tea Tree Oil is a complex mixture of chemicals. Some measured and/or calculated values are presented in Figure 1. The Log $P_{o/w}$ values for Tea Tree Oil constituents range from 2.82 to 6.64.

3.1.8. Additional physical and chemical specifications

Organoleptic properties:	Myristic odour
Melting point:	Not applicable (liquid at room temperature)
Boiling point:	approximately 176°C
Flash point:	
Vapour pressure:	
Density:	885 – 906 kg/m ³
Refractive index:	1.475–1.482
Optical rotation:	+5° to +15°

3.1.9. Stability**1. Twelve Month In-Use Stability Trial of Tea Tree Oil**

A 12 month in-use stability trial with undiluted Tea Tree Oil has been completed. Two batches of Tea Tree Oil were stored in duplicate (total 4 samples) in 100 mL amber glass bottles with screw neck and fitted with child resistant polypropylene caps at 22°C in a cabinet away from heat sources and light. On a weekly basis the bottles were opened to allow exposure to the atmosphere. On a monthly basis a 5.61 mL sample was taken from each bottle and analysed for composition by gas chromatography (GC) and for Peroxide Value (PV). During sampling the bottles were left open and in contact with the atmosphere and exposed to light within the laboratory for 1 minute. The bottles were then re-capped and returned to the storage cabinet. The composition of the oil remained relatively unchanged for the first 6 months. After this time there was a slight trend toward an increase in p-cymene levels and a slight downward trend in α -terpinene levels. However,

the levels of p-cymene were still less than 6.7% after 12 months of trial. Similarly, the PV was 5 milli-equiv O₂ or less for the first 6 months and did not exceed 8.6 milli-equiv O₂ by the end of the trial. This indicated that there was no appreciable oxidation/degradation of the oil for at least 12 months under these conditions. No detectable levels of 1,2,4-trihydroxymenthane were present in the oil samples at any of the sampling times.

Ref.: 5

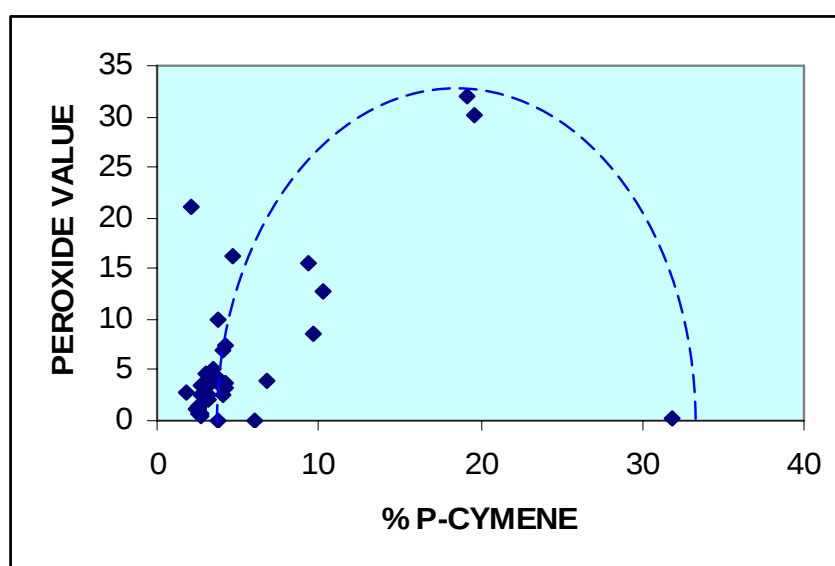
2. Survey of Composition and Peroxide Values of Tea Tree Oil Samples

Three selected Tea Tree Oils samples were chosen as suitable un-oxidised, partially oxidised and oxidised samples, respectively. These three samples were examined in more detail by GC-MS analysis to identify all components present at more than 0.1% and 81 out of at least 88 constituents were identified (see appendix). The samples were found to display mean p-cymene percentages of 2.5 (un-oxidised), 10.5 (partially oxidised) and 19.4 (oxidised) which corresponded with mean peroxide values of 1.1, 11.7 and 30.5 milliequivalents of active oxygen per kg.

A survey of several laboratories that routinely perform peroxide value determinations revealed results from 139 tea tree oil samples, 77 of which had also been analysed for p-cymene by gas chromatography. In general, with increasing peroxide values, the p-cymene concentration also increased. Although a correlation between peroxide value and p-cymene content was apparent, there seem to be anomalies. In an extremely oxidised oil, the peroxide value reduced almost to zero even though the p-cymene percentage exceeded 30%. Conversely one sample showed a high peroxide value and low p-cymene content. The former arise where the peroxides are oxidised further to form more stable alcohols such as 1,2,4-trihydroxymenthane and the latter where a process includes heat and/or rapid air movement or simply the inadequate stirring of large batches.

For selected oils peroxide values and their corresponding p-cymene concentrations are shown in a scatter plot in Figure 4.

Figure 4: Plot of Peroxide Value with respect to p-Cymene content in 40 Tea Tree Oil samples.



Although limited information on peroxide value and p-cymene content with respect to age was available (56 samples), the data suggests that well sealed containers prevent Tea Tree Oil oxidation and degradation for at least 3-4 years.

The determination of peroxide in Tea Tree Oil is not essential because of the presence of inbuilt antioxidants (α -terpinene, γ -terpinene and terpinolene) which oxidise to p-cymene. Hence measurement of p-cymene percentage (which increases with oxidation) is a good measure of the oxidative degradation of Tea Tree Oil. It was also experimentally shown that the p-cymene concentration increases proportionally to the content of the oxidation product 1,2,4-trihydroxymenthane, a suspected skin sensitiser.

Overall, it was concluded that with the difficulty in measuring and identifying the degradation products due to their instability and poor documentation, p-cymene concentration seems a better determinant for oxidation than any other method. Sufficient well accepted standards exist for this to be an easy analysis.

Ref.: 6, 7

3. Stability of Tea Tree Oil in Formulated Products

Analysis of Tea Tree Oil in formulated products was performed by solvent extraction followed by GC-FID of the resultant solution using standard procedures. The stability of the products was monitored using the p-cymene content of the Tea Tree Oil in these products. The composition of the formulations was not indicated. Only single values were provided.

Table 2: Formulation stability studies, selected values

Formulation	Code	Storage	P-Cymene Initial value (%)	P-Cymene Terminal value (%)
White cream	#2923	18 months, 30 °C	1.72	9.02
White cream	#3174	18 months, 30 °C	3.09	6.25
Blemish gel	#11J1	60 months, 22 °C	-	3.25
Blemish gel	#11J1	18 months, 40 °C	-	3.53
Blemish gel	#1131	12 months, 40 °C	-	3.70
Blemish gel	#1131	60 months, 22 °C	-	3.25
Blemish gel	#13AK	48 months, 22 °C	-	3.49
Blemish gel	#13AK	18 months, 40 °C	-	4.02
Solution	#7959	36 months, 22 °C	-	3.42
Solution	#7959	18 months, 40 °C	-	4.08
Solution	#9612	36 months, 22 °C	2.93	3.98
Solution	#9612	18 months, 40 °C	2.93	3.53

Generally (with one exception, #2923), the p-cymene content increased with storage time, but remained below the upper limit, specified in the ISO Standard (8%). The rates of degradation of the oil varied with the medium containing the oil. The degradation in the cream was faster than seen in a gel and a solution. The Tea Tree Oil constituents in the gel were stable over a period of 5 years, in the solution 3 years and in the cream 1½ years in this study.

Ref.: 7, 8

The Australian Tea Tree Industry Association (ATTIA) developed a Code of Practice and a Guidance Document to ensure a common standard of quality management starting on the farms for processing and the supply chain. The measures include control of harvesting, distillation, handling and batching. The use of stainless steel storage vessels for long term storage (> 1 week), storage in the dark and use of nitrogen or argon gas in order to slow down oxidation is recommended. Furthermore, inspection, a quarantine system and recording/documentation is implemented.

Ref.: 9, 10

Comment of the SCCP

Based on the information given, the SCCP is of the opinion that on the basis of the ATTIA Code of Practice and the Guidance document a safe processing and storing of Tea Tree Oil can be achieved which can be controlled by measuring p-cymene content.

3.2. Function and uses

According to the applicant, the annual production of Tea Tree Oil in Australia is estimated to be 400 tonnes per year. The distribution of this oil is 40% USA, 50% Europe and 10% other. Therefore, the volume of Tea Tree Oil imported into Europe is estimated to be 200 tonnes per year.

Tea Tree Oil is used in the following types of cosmetic products. The typical concentration is also provided.

- Skin care – moisturisers (1.25%), body lotion (1.25%)
- Hair care – shampoo and conditioners (2.0%)
- Nail care (20%)
- Oral hygiene – mouth wash (0.2%)
- Personal hygiene - face cleansing wash (0.7%), hand wash (0.7%), soap (2%), foot spray (2%), foot powder (1%)
- Shaving products (2%)
- Post-waxing treatments (1.25%)

No cosmetic function was provided by the applicant.

Tea Tree Oil is considered a universal remedy for acne, eczema, skin infections like herpes, wounds, warts, burns, insect bites and nail mycosis. Other indications mentioned are colds, sore throat and gingival infections, haemorrhoids and vaginal infections. According to a recent review on the use of plants in cosmetics, Tea Tree Oil is widely employed in skin care for the treatment of sores, blisters, spots, rashes, warts, burns and acne. Its antimicrobial activity is well known.

Ref.: 2, 11, 12, 13, 14, 15, 16

Tea Tree Oil is not currently subject to any constraint for the use in cosmetic products. It is sold undiluted and highly concentrated to the public. Furthermore, the oil is used as ingredient of cosmetics, e.g. skin and body care products, toothpaste, mouthwash and in bath oils as well as in products for aromatherapy. A monograph on Tea Tree Oil as an active ingredient being used in cosmetic products was prepared in 2001 by the Norwegian delegation to the Council of Europe Committee of experts on cosmetic products. The following cosmetic usage was evaluated: up to 0.5% in toothpaste and mouth washes, up to 2% in creams for chapped skin, hands and nails and in deodorants, up to 3% in bath preparations, shampoos and special detergents. The Swedish MPA has registered three Tea Tree Oil containing products as "natural medicinal products".

Ref.: 17

Tea Tree Oil does not have marketing authorisation as a pharmaceutical product since a positive clinical effect has not yet been proven according to valid criteria for clinical trials on the efficacy of pharmaceutical products. It can, however, be assumed that consumers use Tea Tree Oil externally and internally for therapeutic purposes.

The European Cosmetic Toiletry and Perfumery Association (COLIPA) in 2002 published the following recommendation:

"COLIPA recommends that Tea Tree Oil should not be used in cosmetic products in a way that results in a concentration greater than 1% oil being applied to the body. When formulating Tea Tree Oil in a cosmetic product, companies should consider that the sensitisation potential increases if certain constituents of the oil become oxidised. To reduce the formation of these oxidation products, manufacturers should consider the use of antioxidants and/or specific packaging to minimise exposure to light."

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Taken from SCCP/0843/04

Guideline:	/
Species/strain:	Sprague Dawley rats
Group size:	5 males + 5 females
Test substance:	Tea Tree Oil in peanut oil
Batch:	88/375
Purity:	/
Dose:	0.83, 0.92, 1 mL/kg bw, once by gavage (SPF animals) 0.34, 0.43, 0.53, 0.56 0.6 mL/kg bw, once by gavage (non SPF animals)
GLP:	/

Results SPF rats

Animals exhibited lack of tonus in the forelimbs. The LD₅₀ was calculated to be 2.6 mL (= 2300 mg) per kg bw.

Results Non-SPF rats

The animals showed similar symptoms. The LD₅₀ was calculated to be 1.9 mL (=1700 mg) per kg bw.

Ref.: 18

In the applicant's dossier a non-GLP study on acute oral toxicity in rats (Sprague Dawley; SPF rats and non-SPF rats) is reported. The study was not submitted to the SCCP. The test substance was Oil of *Melaleuca alternifolia* (Terpinene-4-ol content 41.5% (w/w); 1,8-cineole content 3.8% (w/w)). No analytical data are available for characterization of the substance. According to the dossier the following results were obtained:

LD₅₀ = 2.61 mL/kg (in SPF rats) equivalent to 2300 mg/kg bw
LD₅₀ = 1.9 mL/kg (in non-SPF rats) equivalent to 1682 mg/kg bw

Ref.: 19

Taken from SCCP/0843/04

Reports on human poisoning

There are a few case reports of intoxication caused by Tea Tree Oil in humans.

A 4-year-old boy ingested a small quantity of Tea Tree Oil and became ataxic and progressed to unresponsiveness. But 24 h after admission the child had recovered.

Ref.: 20

A 17-month-old male child developed ataxia and drowsiness following ingestion of less than 10 mL Tea Tree Oil.

Ref.: 21

A 23-month-old boy became confused and was unable to walk 30 minutes after ingesting less than 10 mL of a commercial product containing 100% melaleuca oil. 5h following ingestion the child was asymptomatic.

Ref.: 22

A man aged 60 swallowed about half a teaspoonful of Tea Tree Oil and had a dramatic rash accompanied by leukocytosis.

Ref.: 23

One person lapsed into a coma for 12 hours after ingesting half a cup of pure Tea Tree Oil and suffered disturbances of consciousness for another 36 hours.

Ref.: 24

Pursuant to § 16 Chemicals Act the predecessor of the German Federal Institute for Risk Assessment (BfR), the former Federal Institute for Health Protection of Consumers and Veterinary Medicine (BgVV) received a total of seven intoxication notifications involving Tea Tree Oil between 1996 and 2002. In two infants symptoms of nausea, tiredness and vomiting appeared following the oral intake of Tea Tree Oil; another infant did not develop any symptoms. One adult suffered nausea, stomach pain, loss of appetite and eructation after taking Tea Tree Oil capsules. In three other cases allergic reactions were observed after dermal application.

Ref.: 25

3.3.1.2. Acute dermal toxicity

Taken from SCCP/0843/04

Guideline:	OECD 402
Species/strain:	Albino rabbits (NZ whites)
Group size:	5 males + 5 females
Test substance:	Tea Tree Oil
Batch:	88/375
Purity:	/
Dose:	2000 mg/kg bw, once for 24 h
GLP:	not in compliance

Results

The animals were observed for 14 days. With the exception of slight diarrhoea (1 animal) the animals exhibited no signs of toxicity.

Ref.: 26

In rabbits the dermal LD₅₀ was >5000 mg/kg bw (2/10 deaths).

Ref.: 27

Cases of Tea Tree Oil toxicosis have been reported in dogs and cats following dermal application for therapeutic reasons. Typical signs of neurotoxicity were observed (depression, weakness, incoordination, ataxia, muscle tremors etc.).

Ref.: 28, 29

3.3.1.3. Acute inhalation toxicity

Acute inhalation toxicity in rats

Guideline:	/
Species/strain:	Specific Pathogen Free Sprague Dawley (albino) rats, initial body weights ranged from 120 to 172 grams.
Group size:	5 males + 5 females
Test substance:	Bactigas (0.3% (w/w) Tea Tree Oil (active ingredient) and 1.8% ethanol in carbon dioxide
Purity:	/
Exposure dose:	50, 100 mg/L for one hour
GLP:	not in compliance

The test sample Bactigas consisted of 0.3% (w/w) Tea Tree Oil (active ingredient) and 1.8% ethanol in carbon dioxide. Bactigas is a product used in Australia to sanitize ducted heating and cooling systems. Animals were exposed to Bactigas for a period of one hour under dynamic airflow in a 100 litre acrylic inhalation chamber to a generated level of approximately 50 mg/L of air. A device was used to dispense 3.5 g of gas over 40 seconds at a time. Attached to this small cylinder was an electronic meter which was activated manually. This pulse triggered the device to deliver the sample into the chamber through a hole near the top of the chamber for 40 seconds every 2.1 minutes for a total of 60 minutes. The test material was disseminated in the inhalation chamber by a 17 litre/minute airflow passing through the chamber.

Results

During exposure to the test sample none of the animals showed any abnormal signs. When the animals were returned to boxes after 1 hour of exposure they exhibited normal behaviour. All the animals displayed normal behaviour and appearance during the 14-day holding period. They exhibited a normal weight gain throughout the experimental period. None of the animals' (treated or control) organs showed any abnormalities when subjected to gross pathology.

Histopathology revealed cases of mild interstitial pneumonia in the lung, tubular mineralisation in the kidney and non-supportive pericholangitis which were not considered substance related.

Ref.: 30, 31, 32

Comment of the SCCP

No vehicle-treated control was included. Only 1 control animal was evaluated histopathologically.

3.3.2 Irritation and corrosivity

3.3.2.1. Skin irritation

Guideline	/
Species/strain:	Albino rabbits (NZ whites)
Group size:	6
Test substance:	Tea Tree Oil
Batch:	88/375
Purity:	/
Dose:	0.5 mL
GLP:	not in compliance

The Draize irritation index was found to be 5.0, indicating a severe irritant.

Ref.: 33

A skin irritation test in rabbits was conducted with 25% Tea Tree Oil in paraffin oil and the solution was repeatedly applied over 30 days to the shaved rabbit skin. Minor initial irritations declined; however, skin changes were found microscopically. In the patch test under semioclusive conditions according to OECD 404, 12.5% and 25% Tea Tree Oil was not irritating, while 50% was minimally and 75% Tea Tree Oil was slightly irritating in rabbits; undiluted Tea Tree Oil in the patch test triggered irritations within 24 hours. For Tea Tree Oil the Draize Index for skin irritation in rabbits was determined at 5.0.

Ref.: cited in 34 and 35

Human studies

Tea Tree Oil has been investigated for skin irritancy using an occlusive patch test on 25 human subjects for 21 days and compared with 1,8-cineole in concentrations of 0%, 3.8%, 8%, 12%, 16%, 19.9%, 24% and 28.1% in soft white paraffin. 8 Tea Tree Oil preparations containing 1,8-cineole concentrations similar to the 1,8-cineole groups (from 1.5% to 28.8%) and the 1,8-cineole-treated groups did not show skin irritation. 3 of 28 panellists exhibited an allergic response. They were further tested (see 3.3.3.).

Ref.: 36

In a Danish dermatology clinic, from 2001-2002 (study 1) and in 2003 (study 2) 217 and 160 consecutive patients were patch tested with the European standard series and in addition with 10% Tea Tree Oil in petrolatum (study 1) and commercial lotions containing 5% Tea Tree Oil (study 1 and 2). In the 1st study 44 out of 217 subjects tested (20.3%) showed irritancy from a lotion containing 5% Tea Tree Oil. In the 2nd study 3.1% (5/160) irritant reactions were seen.

Ref.: 37

Various concentrations of Tea Tree Oil (5, 25 and 100%) in different vehicles were applied under occlusive patch testing to the skin of healthy human volunteers (n=311) using a protocol based on the Draize human sensitisation test. The mean irritancy score was 0 for 5% and 0.25 for 100% Tea Tree Oil.

Ref.: 38

Ten different samples of undiluted Tea Tree Oil applied under occlusive conditions for 48 hours to 219 subjects. The prevalence of marked irritancy to 100% tea tree oil ranged from 2.4 to 4.3% (without or with the indistinguishable reactions). Any level of irritancy (mild and marked) ranged from 7.2 to 10.1%.

Ref.: 39

Comment of the SCCP on skin irritation

From the these study it is concluded that neat Tee Tree Oil as well as formulations containing 5% Tea Tree Oil can exhibit skin irritancy.

3.3.2.2. Mucous membrane irritation

Hen's egg test on the chorio-allantoic membrane (HET-CAM assay)

Guideline:	/
Species/strain:	fertilised fresh chicken eggs (white leghorn)
Group size:	substance treated 6, controls 2
Test substance:	Tea Tree Oil several oral products (see Table)
Batch:	6081 and 871
Purity:	pharmaceutical grade

Dose: 0.1 g / egg, controls: 300 µL/egg
 GLP: In compliance

Results

Substance	Mean irritation index	Evaluation
Negative control (0.9% NaCl solution)	0.0	non-irritant
Tea Tree Oil batch no. 6081	16.1	severe
Tea Tree powder	0.0	non-irritant
Tea Tree ground leaf	0.0	non-irritant
water-soluble Tea Tree Oil	14.7	severe
Placebo (0% Tea Tree Oil) 10% surfactant	10.3	severe
25% Tea Tree Oil 5% surfactant	9.8	severe
5% Tea Tree Oil 8% surfactant	4.5	slight
10% Tea Tree Oil 10% surfactant	12.1	severe
Positive control (0.1 N NaOH)	19.3	severe
Positive control (1% SDS)	11.3	severe

Neat Tea Tree Oil and 25 and 10% solutions in surfactant as well as 10% surfactant are severe irritants in the assay while 5% TTO is only slightly irritant. Tea Tree powder and ground leaf are non-irritant.

Ref.: 40

Comment

The description of the analysed substances is poor. The identity of the used surfactant is not indicated and the reasoning of the used dilutions with different surfactant concentrations is not clear. Historical control data on the range of response to positive control agents are included. The HET-CAM assay has been extensively used and is showing promise as a potential alternative assay for eye irritation. However, it has not yet been validated.

Primary eye irritation of TTO was studied in the rabbit (female, Japanese White) under GLP conditions. Two groups of three rabbits were given a single ocular dose (0.1 mL) of TTO (1% or 5% in liquid paraffin). After instillation of the test substance, no abnormal signs in the clinical conditions were observed among the rabbits. Ocular responses using Draize's criteria demonstrated a conjunctival discharge lasting for up to six hours following instillation of 1% TTO and conjunctival redness and discharge for up to 24 hours following instillation of 5% TTO. In both groups, the maximal response was observed after one hour. Based on these observations, the author concludes, that both TTO solutions can be classified as "minimally irritating".

Ref.: cited in 35

Comment of the SCCP on eye irritation

No definite conclusions regarding mucous membrane irritation of Tea Tree Oil containing formulations can be drawn from the data provided.

3.3.3. Skin sensitisation

Local Lymph Node Assay, study 1

Guideline: OECD 429
 Species/strain: Female mice; strain CBA/J
 Group size: 5 animals per group
 Test substance: Tea Tree Oil
 Batch: #MC4061

Purity:	ISO 4730
Exposure:	3 days
Dose:	5, 25 and 50% of Tea Tree Oil diluted in PEG 400
GLP:	in compliance

Three groups of five animals per dose group were treated by topical application of three concentrations (5%, 25% and 50%) of the test substance, a negative control group was treated with PEG 400 used as vehicle, and a positive control was included (25% alpha-hexylcinnamaldehyde, HCA).

Ear thickness measurements were performed prior to application on Day 1, after 48 h and prior to the third application at Day 3 and lastly on Day 6 before sacrifice to screen for ear swelling as a consequence of skin irritation. Five days following the initial application the mice were injected with 5-bromo-2'-deoxy-uridine (BrdU) and auricular lymph nodes were then isolated at sacrifice. The Stimulation Index (SI) was measured as percentage of proliferating BrdU+ lymph node cells in fixed cell preparations relative to the vehicle control. In addition, the B:T cell ratio in the lymph node preparations was measured by immunotyping.

Results

None of the concentration tested resulted in dermal irritating response (no changes in ear thickness). The SI measured after treatment with Tea Tree Oil indicated a sensitizing response at 25% and 50% of the test article (SI: 2.1 at 5%, 7.7 at 25%, and 7.9 at 50%). The sensitizing effect of Tea Tree Oil application was supported by the immunotyping experiments (increase of B cells in Tea Tree Oil treated groups compared to control > 25%; increase of B:T cell ratio in Tea Tree Oil treated groups compared to control > 25%).

Conclusion

Tea Tree Oil was shown to be a skin sensitiser in the Local Lymph Node Assay. The EC3 value was calculated to be 8.3% (moderate sensitiser).

Ref.: 41

Local Lymph Node Assay, study 2

Guideline:	OECD 429
Species/strain:	Female mice; strain CBA/CaHdsRcc (SPF)
Group size:	5 animals per group
Test substance:	Tea Tree Oil
Batch:	#RP-05-346
Purity:	ISO 4730
Exposure:	3 days
Dose:	2, 20 and 100% of Tea Tree Oil diluted in PEG 300
GLP:	in compliance

Three groups of five animals per dose group were treated by topical application to the dorsum of each ear lobe (left and right) of three concentrations (2%, 20% and 100%) of the test substance; a negative control group was treated with PEG 300 used as vehicle. No positive control was included.

Results

The SI measured after treatment with Tea Tree Oil were 2.4 at 2%, 6.9 at 20%, and 16 at 100%).

Conclusion

Tea Tree Oil was shown to be a skin sensitiser in the Local Lymph Node Assay. The EC3 value was calculated to be 4.4% (moderate sensitiser).

Ref.: 42

Local Lymph Node Assay, study 3

Guideline: OECD 429
Species/strain: Female mice; strain CBA/CaHdsRcc (SPF)
Group size: 5 animals per group
Test substance: Tea Tree Oil
Batch: #1219
Purity: ISO 4730
Exposure: 3 days
Dose: 2, 20 and 100% of Tea Tree Oil diluted in PEG 300
GLP: in compliance

Three groups of five animals per dose group were treated by topical application to the dorsum of each ear lobe (left and right) of three concentrations (2%, 20% and 100%) of the test substance; a negative control group was treated with PEG 300 used as vehicle. No positive control was included.

Results

The SI, measured after treatment with Tea Tree Oil, were 1.8 at 2%, 2.8 at 20%, and 6.5 at 100%).

Conclusion

Tea Tree Oil was shown to be a skin sensitiser in the Local Lymph Node Assay. The EC3 value was calculated to be 24.3% (moderate sensitiser).

Ref.: 43

Local Lymph Node Assay, study 4

Guideline: OECD 429
Species/strain: Female mice; strain CBA/CaHdsRcc (SPF)
Group size: 5 animals per group
Test substance: Tea Tree Oil
Batch: #1219
Purity: ISO 4730
Exposure: 3 days
Dose: 2, 20 and 100% of Tea Tree Oil diluted in PEG 300
GLP: in compliance

Three groups of five animals per dose group were treated by topical application to the dorsum of each ear lobe (left and right) of three concentrations (2%, 20% and 100%) of the test substance; a negative control group was treated with PEG 300 used as vehicle. No positive control was included.

Results

The SI, measured after treatment with Tea Tree Oil, were 1.6 at 2%, 2.8 at 20%, and 5.7 at 100%).

Conclusion

Tea Tree Oil was shown to be a skin sensitiser in the Local Lymph Node Assay. The EC3 value was calculated to be 25.5% (moderate sensitiser).

Ref.: 44

Comment on LLNA

PEG is not a recommended vehicle of LLNA. All four studies showed that ISO 4730 quality Tea Tree Oil itself and in PEG solution is a moderate sensitizer.

Human studies

Sensitisation tests (HRIPT)

A study was performed based on the skin sensitisation method of Draize 1965. 6 Tea Tree Oil products were investigated which consisted of 100% Tea Tree Oil and 25% and 5% Tea Tree Oil in cream, ointment or gel formulation. Cream base was used as negative control. 151 adult male and female panellists were selected. They gave their consent in conformance with the Declaration of Helsinki. On day 1, 100 µl of the respective product was placed in Finn chambers onto the upper arm or the back. After 48 h the chambers were removed and the skin was assessed. If needed, the volunteers returned 48 h later for a further assessment. Skin reaction was assessed on a 5-graded scale. The test products were applied to the skin 9 times over a 3 week period and any response for irritancy was recorded (induction). After a 2 week rest phase the products were applied on a new site (challenge). 2 days later and - if necessary - again after 4 days the skin reaction was assessed. Any doubtful results were repeated 2 weeks later.

Results

Irritancy:

148 of 151 panellists were evaluated. The average daily score for irritancy was 0.1922 for the neat Tea Tree Oil. The other samples showed scores from 0.0000 to 0.0060.

Sensitization:

150 of 151 panellists were evaluated. 3 out of 150 (2%) became sensitized to Tea Tree Oil. In a second follow-up trial the number of panellists was increased to a joint number of 306 (irritancy) and 308 (sensitization). The second study confirmed the results of the first one but no details were presented. Since different samples of Tea Tree Oil were tested simultaneously on subjects it was not possible to determine which specific concentrations were responsible for inducing sensitisation.

Ref.: 45

Various concentrations of Tea Tree Oil (5, 25 and 100%) in different vehicles were applied under occlusive patch testing to the skin of healthy human volunteers (n=311) using a protocol based on the Draize human sensitisation test. 3 subjects developed grade 3 skin reactions suggestive of an allergic reaction. Since different samples of Tea Tree Oil were tested simultaneously on subjects it was not possible to determine which concentration was responsible for inducing sensitisation.

Ref.: 38

Comment

It seems possible that these 2 reports relate to the same study, however, this could not be verified.

The SCCP considers HRIPT as unethical.

Clinical Patch testing

3 of 28 panellists of a skin irritancy study exhibited an allergic response to Tea Tree Oil. They were further tested for allergic responses with major constituents of Tea Tree Oil. 3 positive reactions were seen against a sesquiterpenoid hydrocarbon fraction and 1 against α-terpinene

Ref.: 36

1216 patients were patch tested in a Swiss dermatologic clinic. The tested concentrations were 5, 10, 50 and 100% in Arachis oil. 7 cases showed an allergic contact dermatitis due to Tea Tree Oil while 2 of them also exhibited a type IV hypersensitivity towards fragrance-mix or colophony. The effective concentrations were not given in the publication.

Ref.: 46

A case of positive patch testing with 1% Tea Tree Oil solution was reported on a 74-year-old man with a history of blistering dermatitis who had treated warts with Tea Tree Oil as wart paint.

Ref.: 47

An immediate systemic hypersensitivity reaction of a 38-year-old man associated with topical application of Tea Tree Oil was observed. The patient had placed a drop of Tea Tree Oil on his finger and had applied this to psoriatic lesions on his leg.

Ref.: 48

A case of an allergic contact dermatitis to Tea Tree Oil with erythema multiforme-like Id reaction was reported.

Ref.: 49

A further case report of a contact allergy to Tea Tree Oil and cross-sensitization to colophony origins from Norway.

Ref.: 50

In Wales a combined contact allergy to Tea Tree Oil and lavender oil complicating chronic vulvovaginitis was observed.

Ref.: 51

A case of contact dermatitis (face eczema) to Tea Tree Oil was reported which was explained by the use of Tea Tree Oil against pimples. The epicutaneous testing of the patient revealed positive reactions to α -terpinene, terpinolene and ascaridole.

Ref.: 52

7 patients were seen in a dermatology clinic during a 3-year period reactive to a 1 % solution of melaleuca oil. 6 of them also reacted to limonene, 5 to α -terpinene and aromadendrene, 2 to terpinen-4-ol and 1 to p-cymene and α -phellandrene.

Ref.: 53

Contact dermatitis was observed with a 12-year-old boy who had applied Tea Tree Oil on his face to treat a minor skin complaint.

Ref.: 54

The case reports in the literature were summarized and the potentially causative substances were discussed.

Ref.: 34, 55

In a Danish dermatology clinic from 2001-2002 (study 1) and in 2003 (study 2) 217 and 160 consecutive patients were patch tested with the European standard series and in addition with 10% Tea Tree Oil in petrolatum (study 1) and commercial lotions containing 5% Tea Tree Oil (study 1 and 2). In the 1st study only 1 person showed a relevant positive patch test to 5% and 10% Tea Tree Oil. In the 2nd study no allergic but 3.1% (5/160) irritant reactions were seen to 5% Tea Tree Oil.

Ref.: 37

In an Italian study of 725 consecutive patients were patch tested with undiluted, 5%, 1% and 0.1% Tea Tree Oil in petrolatum. While in 5.9% of the patient positive reactions were

observed to undiluted Tea Tree Oil, only 1 patient was positive with the 1% dilution, none with 0.1% Tea Tree Oil.

Ref.: 56

An increase in sensitization to oil of turpentine between 1992 and 1997 was found in a multicenter study on 45,005 patients from the German-Austrian information network of departments of dermatology (IVDK). Oil of turpentine is a mixture of terpenes including α -pinene, carenes and β -pinene. While between 1992 and 1995 the prevalence rate was as low as 0.5% in the years 1996 and 1997 a dramatic increase was observed showing a sensitization rate of 4.8%. It was hypothesized that the increase in the use of Tea Tree Oil may be responsible due to cross-reaction with turpentine.

Ref.: 57

In a parallel guinea-pig and human study, freshly distilled as well as oxidised Tea Tree Oil and some fractions thereof were analysed and compared as to their sensitizing properties. Photooxidation of Tea Tree Oil was demonstrated by an increase of the p-cymene content (2.0% to 11.5%) accompanied by a decrease in the content of α -terpinene (11.2% to 5.0%), γ -terpinene and terpinolene. Simultaneously, the peroxide number increased from < 50 ppm to > 500 ppm.

All 11 Tea Tree Oil-sensitive patients reacted positively to α -terpinene, terpinolene and ascaridol. Experimental sensitization in guinea pigs showed a low sensitizing capacity for fresh Tea Tree Oil while photo-oxidised oil was a 3 times stronger sensitiser. The mono-terpene fraction obtained by fractional distillation showed to be a stronger sensitiser than the sesquiterpene fraction.

Ref.: 58

1,2,4-Trihydroxymenthane was shown to be an oxidation product of Tea Tree Oil formed from terpinen-4-ol. By epicutaneous patch testing of 15 patients sensitive to Tea Tree Oil 11 of them reacted positively to 1,2,4-trihydroxymenthane.

Ref.: 4

3 out of 28 normal healthy volunteers tested strongly positive to patch testing with Tea Tree Oil. All 3 reacted positive to a sesquiterpenoid fraction of the oil, 1 to α -terpinene.

Ref.: 59

In the draft monograph submitted to the Council of Europe by the Norwegian delegation data of the Swedish MPA (Medicinal Products Agency) on adverse effects induced by Tea Tree Oil in Sweden was mentioned. The reports concerned mainly contact dermatitis and eczema (27 out of 33 cases). Only products having a concentration of 2% or more seem to cause skin reactions.

Ref.: 17

In a multicenter study with 11 dermatological departments in Austria and Germany 36 out of 3375 patients (1.1%) reacted positive to a 5% solution of Tea Tree Oil in diethylphthalate. Great regional differences in frequencies were found (0 to 2.3%). 10 of 10 positively tested persons also reacted positive to terpinolene, ascaridol and α -terpinene, 9 of 10 to 1,2,4-trihydroxymenthane. 14 of these 36 persons (38.9%) also showed a positive response to oil of turpentine.

Ref.: 60

In a review on cutaneous effects of Tea Tree Oil, prevalence rates for allergic contact dermatitis reactions were cited. In an Australian study conducted with 219 volunteers 2.9 to 4.8% reacted positively when patch-tested with Tea Tree Oil dilutions. Within the subjects with previous exposure to Tea Tree Oil the rate rose to 4.3 to 7.2%. The same authors report on personal communications related to a study of the North American Contact Dermatitis Group. 0.5% of patients reacted to oxidized Tea Tree Oil (5% in petrolatum) on patch testing.

Ref.: 61

Allergic skin reactions related to oral intake of Tea Tree Oil have also been documented. After initial external treatment of an atopic dermatitis with undiluted Tea Tree Oil a man ingested the oil. This resulted in obvious exacerbation of the dermatitis.

Ref.: 62

A case of Tea Tree Oil dermatitis associated with linear IgA disease was described. The patient had applied Tea Tree Oil to her recently pierced umbilicus.

Ref.: 63

An airborne allergic contact dermatitis was observed following inhalation of the vapours of a hot aqueous solution of Tea Tree Oil.

Ref.: 64

Comment on human studies

Neat Tea Tree Oil is a sensitizer in humans. In a human sensitisation study with 9 applications, 3 of 150 (2%) volunteers became sensitized. Since different samples of Tea Tree Oil were tested simultaneously on subjects in this study it was not possible to determine which specific concentrations were responsible for inducing sensitisation. Such Human Repeated Insult Patch Testing (HRIPT) is considered by SCCP as unethical to conduct.

In a Swiss study (ref. 46), 7 of 1216 (0.6%) patch tested patients reacted positive to Tea Tree Oil. The elicitation concentrations were not indicated.

In a large multicenter study with 11 dermatological departments in Austria and Germany, 36 out of 3375 patients (1.1%) reacted positive to a 5% solution of Tea Tree Oil. The prevalence for allergic contact dermatitis exhibited regional differences.

In an Australian study conducted with 219 volunteers 2.9 to 4.8% reacted positively when patch-tested with Tea Tree Oil dilutions

It is not fully understood which of the constituents is responsible for sensitisation. Terpinolene, α -terpinene, a sesquiterpenoid fraction, limonene and/or oxidative degradation products like ascaridole, 1,2,4-trihydroxymenthane, peroxides and epoxides have been discussed. In one study, oxidised Tea Tree Oil was shown to be 3 times more potent than fresh oil. The sensitising potency may also be influenced by the content of irritants such as p-cymene and 1,8-cineole.

3.3.4. Dermal / percutaneous absorption

Guideline	OECD 428
Test system:	<i>in-vitro</i> human epidermal skin
No. of samples:	three female human skin donors (6 samples per treatment)
Test substance:	Tea Tree Oil (neat) and 20% solution in ethanol
Batch:	RP05-347
Exposure:	24 h in horizontal static diffusion cells (Franz-type glass cells, application area approx. 1.3 cm ²) with 200 μ L receptor phase removal and replacement with fresh solution at 2, 4, 8, 12 and 24 h.
Dose:	10 μ L/cm ² donor phase (pure oil and a 20% solution)
Receptor fluid:	phosphate buffered saline containing 4% bovine serum albumin
GLP:	/

The potential of Tea Tree Oil components to penetrate the epidermal membrane was analyzed in an *in-vitro* human epidermal membrane penetration study. The test substance was applied topically (as the pure oil and as a 20% solution in ethanol; applied amount 10

$\mu\text{L}/\text{cm}^2$) over a 24h period onto the skin in horizontal Franz-type glass cells. In order to consider inter-individual variability, skin samples from three different human skin donors were used.

Solubility of Tea Tree Oil components in the receptor phase were determined to be 4.08 mg/mL for terpinen-4-ol, 6.07 mg/mL for α -terpineol and 3.17 mg/mL for 1,8-cineole. Concentrations in the receptor phase determined in the study were well below 10% of this solubility. Experiments to assess penetration of each of the 15 Tea Tree Oil components (according to ISO 4730) into the receptor phase showed that only terpinen-4-ol, α -terpineol and 1,8-cineole (only under occlusion) could be detected in the receptor phase samples after 12 h or 24 h, respectively.

Results

Approximately 3.7 - 8.0% (138 - 303 $\mu\text{g}/\text{cm}^2$) of the applied amount of terpinen-4-ol and 0.5 - 1.1% (14 - 33 $\mu\text{g}/\text{cm}^2$) of α -terpineol appeared in the receptor phase over the 24 h period from the pure oil. After application of the 20% tea tree oil formulation, 2.2 - 4.0% of the applied amount of terpinen-4-ol (19 - 33 $\mu\text{g}/\text{cm}^2$) and no α -terpineol was detected in the receptor phase.

The epidermal retention of terpinen-4-ol varied between 0.01 - 0.02% (5 - 8 μg) of the applied amount per diffusion cell for the pure oil formulation and <0.05% (<0.5 μg) for the 20% oil formulation. The retention within the epidermis for α -terpineol and other constituents was much higher. It accounted for 21 - 34 μg (application of the pure oil) and 0.7 - 1.6 μg (application of the 20% formulation) of material recovered from the epidermis. The recovery of the Tea Tree Oil components was low (0.5 - 10%). None of the more volatile mono-terpenes were recovered in the surface material and the epidermis at the end of the 24 h application period. This observation was attributed to loss due to evaporation from the skin surface. The assumption is supported by the observation made under occluded conditions, where the occlusion of the chamber by Parafilm resulted in an increase in the total recovery by up to 3-fold. Furthermore, 1,8-cineole was detected in the receptor phase in addition to terpinen-4-ol and α -Terpineol in the occluded cells.

Conclusion

The percutaneous absorption study used an *in vitro* human epidermal skin model. The experimental design (non-occluded chambers) was chosen to mimic the situation under normal 'in use' conditions. Under non-occluded conditions only two of the components of Tea Tree Oil were able to penetrate the entire thickness of the epidermal preparation, α -terpineol and terpinen-4-ol. Following application of pure Tea Tree Oil a total of 2.99% (249.6 $\mu\text{g}/\text{cm}^2$) of components can be found either permeating the skin into receptor fluid (2.59%) or retained within the epidermis (0.40%) in 24 h. The absorption of terpinen-4-ol (sum of receptor and epidermis) was 212.94 $\mu\text{g}/\text{cm}^2$ for neat Tea Tree Oil, for a 20 % ethanolic solution 24.6 $\mu\text{g}/\text{cm}^2$.

Ref.: 65, 66

Further studies on dermal absorption

Hayes *et al.* (1997) found in an experimental study on dermal penetration of different ingredients of Tea Tree Oil that terpinen-4-ol was the first component which penetrates the skin and reach the subcutaneous fat layer within 1h. α -terpineol appears after two hours exposure in the subcutaneous fat layer. As exposure time was increased, more ingredients were detected (1,8-cineole, α -terpinene, p-cymene, α -terpinolene), but all in considerably lower amounts.

Ref.: 67

The potential of α -terpineol to penetrate the entire thickness of the epidermal preparation certainly point to a considerable uptake from Tea Tree Oil. This is remarkable in so far as α -terpineol was shown to cause a slight, but dose related effect in the bacterial reverse mutation assay with *S. typhimurium* tester strain TA102 (Gomez-Carneiro *et al.* 1998).

Ref.: 68

Human skin permeation *in vitro* of terpinen-4-ol was investigated by Reichling et al. (2006) with pure Tea Tree Oil (44.7 Vol% terpinen-4-ol) and 3 preparations (O/W emulsion, white petrolatum, ambiphilic cream) containing 5 % Tea Tree Oil using an infinite dose regimen. The receptor fluid was 50 % ethanol. Derived from the flux the absorption of terpinen-4-ol after 24 h for neat Tea Tree Oil was 6.3 $\mu\text{l}/\text{cm}^2$ (ca. 5.9 mg/cm^2), for the 3 preparations the values were as follows: O/W emulsion 1.5 mg/cm^2 ; white petrolatum 1.2 mg/cm^2 ; ambiphilic cream 0.5 mg/cm^2 . The absorption of terpinen-4-ol from the 5 % preparations was 4 - 12 times lower compared with the neat oil. For neat Tea Tree Oil, the value is about 28 times the value of the Cross study. But in the Cross study a finite dose was used and the receptor fluid was phosphate buffered saline containing 4 % bovine serum albumin.

Ref.: 69

Nielsen and Nielsen (2006) identified only terpinen-4-ol, α -terpineol and eucalyptol (1,8-cineole) as being absorbed through the skin among seven major constituents of Tea Tree Oil tested. The penetration rates for the Tea Tree Oil constituents eventually penetrating the skin were low (penetration coefficient K_p around 20 $\mu\text{m}/\text{h}$ for terpinene-4-ol). At 5% Tea Tree Oil concentration skin barrier integrity was affected as shown by penetration of tritiated water. For methiocarb and benzoic acid the dermal absorption was reduced.

Ref.: 70

Furthermore, the cyclic terpenes, terpinene-4-ol and α -terpineol, were shown to affect skin integrity (Magnusson *et al.* 1997). It was speculated that terpenes open new polar pathways across the *stratum corneum* thereby creating micro-pores in the intercellular lipids through which both ions and polar drugs may pass (Cornwell and Barry 1993).

Ref.: 71, 72

Some components included in Tea Tree Oil might enhance the percutaneous penetration of substances (as shown for terpenes like thymol, menthone and 1,8-cineole; see Doliwa, *et al.* 2001, Vaddi *et al.* 2002, Pillai and Panchagnula 2003, Narishetty and Panchagnula 2004, Amnuakit *et al.* 2005) but seem not to penetrate the skin in considerable amounts.

Ref.: 73, 74, 75, 76, 77

The distribution of terpinen-4-ol in different layers of human skin *in vitro* and in the receptor fluid (phosphate buffer, pH 7.4) following administration of terpinen-4-ol, Tea Tree Oil (neat) and 3 preparations containing 5% terpinen-4-ol was investigated. No terpinen-4-ol was detected in the receptor fluid. 4 h after application of terpinen-4-ol the absorption in the epidermis plus dermis was 780 $\mu\text{g}/\text{cm}^2$. 8 h after application of Tea Tree Oil the absorption of terpinen-4-ol in the epidermis plus dermis was 1500 $\mu\text{g}/\text{cm}^2$. A 4 h dermal application of an oily solution, a hydrogel and a W/O emulsion containing 5% terpinen-4-ol resulted in values of 140, 530, and 100 $\mu\text{g}/\text{cm}^2$ absorption in the epidermis plus dermis, respectively.

Ref.: 78, 79, 80, 81

An oily solution and a hydrogel containing 5% terpinen-4-ol were applied to the skin of human volunteers as infinite dose and in non-occlusive conditions. After 1 h application of oily solution, 20 $\mu\text{g}/\text{cm}^2$ of terpinen-4-ol in *stratum corneum* were measured, and, after hydrogel application, 110 $\mu\text{g}/\text{cm}^2$, respectively. After 2 h the terpinen-4-ol amount in *stratum corneum* decreased to about 5 $\mu\text{g}/\text{cm}^2$ in both cases.

Ref.: 82, 83

Comment of the SCCP on dermal absorption

The comparison of the various studies on percutaneous absorption of terpinen-4-ol from different sources reveals huge differences, as compiled in Table 3.

Table 3: Overview on dermal absorption studies of Tea Tree Oil, values for terpinen-4-ol

Source	Dermal absorption ($\mu\text{g}/\text{cm}^2$)	Study details
A Cross et al. 2008 (Ref. 65, 66)	a) 213 b) 24.6	Human epidermal skin <i>in vitro</i> a) neat Tea Tree Oil b) Tea Tree Oil 20 % ethanolic solution; finite dose (10 mg/cm ²) 24 h exposure
B Reichling et al. 2006 (Ref. 69)	1509	Human epidermal skin <i>in vitro</i> Tea Tree Oil 5 % in an ambiphilic cream Infinite dose conditions, 24 exposure (calculated)
C Cal et al. 2006a,b,c Ref. 78-81	530	Human skin <i>in vitro</i> Tea Tree Oil 5 % in a hydrogel Infinite dose conditions, 4 h exposure
D Cal et al. 2006d, 2007 Ref. 82, 83	110 (Stratum corneum only)	Human skin <i>in vivo</i> Tea Tree Oil 5 % in a hydrogel Infinite dose conditions, 1 h exposure

In the studies with infinite dose conditions (B, C, D) using the same concentration of 5% Tea Tree Oil in water containing formulations, a nearly linear relationship between exposure time and dermal absorption is apparent. In one study (study A) an adequate exposure dose, but no relevant cosmetic formulation was investigated. These studies also demonstrated that the type of formulation has a significant influence on dermal absorption of Tea Tree Oil. Overall, none of the available studies is adequate as a sound basis for assessment of exposure to Tea Tree Oil from cosmetic products.

3.3.5 Repeated dose toxicity

No repeated dose toxicity study with Tea Tree Oil was provided.

In the dossier the repeated dose toxicity studies of some constituents of Tea Tree Oil were discussed. For the constituents with a maximum content of 5% and more they are briefly summarized in the following. The order is according to the maximum content in Tea Tree Oil.

Ref.: 35

Terpinen-4-ol (max. 48%)

Terpinen-4-ol on average constitutes 40% of Tea Tree Oil. Based on the 28-days study on kidney toxicity in rats, the NOAEL for terpinen-4-ol after oral exposure may be estimated to be 400 mg/kg bw/day.

γ -Terpinene (max. 28%)

The available literature on systemic effects of γ -terpinene is not sufficient to reach conclusions on chronic toxicity or estimate a NOAEL. The dermal LD₅₀ value above 5 g/kg indicates low toxicity.

1,8-Cineole (eucalyptol, max. 15%)

1,8-Cineole (eucalyptol) has been studied subchronically in rats and mice: a NOAEL of 300 mg/kg bw/day has been established based on hepatic and renal toxicity.

Ref.: 84

Eucalyptol was evaluated as component of natural sources of flavourings by the Committee of Experts on Flavouring Substances of the Council of Europe (CEFS), resulting in the allocation of a provisional TDI of 0.2 mg/kg bw. This TDI was derived from a minimum lethal dose of 60 mg/kg bw/day for children applying a safety factor of 300 (Council of Europe, 2000).

Ref.: 85

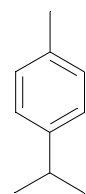
α -Terpinene (max. 13%)

Exposure to α -terpinene (125 or 250 mg/kg bw) for nine consecutive days caused decreased body weight gain in pregnant Wistar rats. No maternal toxicity was observed at 60 mg/kg bw/day, and a NOAEL of 60 mg/kg bw/day for systemic effects following repeated exposure to α -terpinene is suggested.

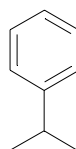
Ref.: 86

***p*-Cymene (max. 8 %)**

Cumene is a chemical similar to cymene: cumene is only lacking the methyl-group residing in the para-position on p-cymene. Studies on the metabolism of cumene and cymene demonstrate considerable similarities and they are expected to have comparable toxicological profiles. A review on the toxicity of cymene and cumene has been submitted to the US EPA. A limited number of relevant repeat-dose studies are available and the inhalation route is often used for cumene. A LOAEL of 769 mg/kg bw/day based on kidney effects in a study with oral exposure was identified. Based on the oral study and using an uncertainty factor of 10, a NOAEL for cumene/p-cymene of 75 mg/kg bw/day was suggested.



p-Cymene



Cumene

Ref.: 87

 α -Terpineol (max. 8%)

10 male and 10 female weanling Osborne-Mendel rats were fed α -terpineol acetate in the diet for 20 weeks at concentrations of 0, 1000, 2500 or 10,000 ppm. These dietary levels were calculated to result in daily intakes of 0, 50, 125 and 500 mg/kg bw/day, respectively. All animals were examined for growth, haematology, and macroscopic changes in the tissues. Microscopic examination was performed on 6 - 8 male and female animals in the high dose and control groups. No statistically significant adverse effects were reported. Based on this study a NOAEL of 500 mg/kg bw/day for α -terpineol can be suggested.

 α -Pinene (max. 6 %)

α - and β -pinene are bicyclic terpene hydrocarbons and the major components of turpentine. With the structurally related bicyclic terpene hydrocarbon camphene a 28-day repeat dose study has been performed according to OECD Guideline 407 in both sexes of Wistar rats. Groups of animals (5/sex/group) were given daily oral doses of 0, 62.5, 250, or 1000 mg/kg bw by gavage for 28 days. Weekly measurement of body weight and food intake revealed no significant differences between test and control animals. Animals of both sexes at the 1000 mg/kg bw/day dose exhibited vacuolization of hepatocytes and increase in liver weights. Male rats also exhibited α 2u-microglobulin-type nephrotoxicity at all dose levels. The repeat-dose study for camphene provides a NOAEL of 250 mg/kg bw/day. It is expected that α -pinene, if subjected to a 28-day study at similar dose levels would exhibit α 2u-microglobulin nephrotoxicity in male rats and would be expected to exhibit a NOAEL of at least 250 mg/kg bw/day.

Ref.: 88

Terpinolene (max. 5%)

No subchronic, chronic, or foeto-toxicity studies were identified for terpinolene, and a NOAEL for systemic toxicity can not be estimated.

Comment of the SCCP

No repeated dose toxicity study with Tea Tree Oil itself was performed. However, data and read-across considerations were provided regarding the systemic toxicity of some constituents or related compounds. There are 8 major constituents of Tea Tree Oil with a maximum content of 5% and more are: terpinen-4-ol (max. 48%), γ -terpinene (max. 28%), 1,8-cineole (eucalyptol, max. 15%), α -terpinene (max. 13%), p-cymene (max. 8%), α -terpineol (max. 8%), α -pinene (max. 6%) and terpinolene (max. 5%). For these constituents, the following NOAELs were established or estimated:

Compound	Max. Content in TTO (%)	Established or Estimated NOAEL (mg/kg bw/day)
Terpinen-4-ol	48	400
1,8-Cineole (eucalyptol)	15	300
α -Terpinene	13	60
Cumene / p-Cymene	8	75
α -Terpineol	8	500
α -Pinene	6	250
Terpinolene	5	no data
γ -Terpinene	28	no data

Derivation of a NOAEL for Tea Tree Oil based on systemic toxicity of its constituents

The direct extrapolation from a NOAEL for a constituent to a NOAEL for Tea Tree Oil might be only acceptable when no other constituent is reported to affect the same target. Three constituents have been reported to affect the kidneys: terpinen-4-ol (content 40%, NOAEL 400 mg/kg bw/day), 1,8-cineole (content 4.5%, NOAEL 300 mg/kg bw/day) and cumene/p-cymene (content 6%, NOAEL 75 mg/kg bw/day).

To estimate a NOAEL for Tea Tree Oil based on the renal toxicity data, information on the estimated constituent-specific NOAEL as well as relative presence in Tea Tree Oil needs to be considered. When available data from terpinen-4-ol, 1,8-cineole, and cumene is used, a NOAEL may be estimated using the formula:

$$(40\% / 400 \text{ mg/kg} + 4.5\% / 300 \text{ mg/kg} + 6\% / 75 \text{ mg/kg}) \times \text{NOAEL} = 100\%$$

This formula gives an estimated NOAEL for Tea Tree Oil of 510 mg/kg bw/day

Lack of data on possible renal effects of the remaining constituents may decrease the NOAEL further. As a possible scenario one could assume that the remaining 49.5% of Tea Tree Oil had a constituent-specific NOAEL equal to cumene. Incorporating this estimate in the calculation of a NOAEL for Tea Tree Oil gives an adjusted additivity formula:

$$(40\% / 400 \text{ mg/kg} + 4.5\% / 300 \text{ mg/kg} + 6\% / 75 \text{ mg/kg} + 49.5\% / 75 \text{ mg/kg}) \times \text{NOAEL} = 100\%$$

Based on the available information on repeated dose toxicity, using these assumptions and following this approach an estimate for a NOAEL for Tea Tree Oil for renal effects would be 117 mg/kg bw/day.

3.3.5.1. Repeated Dose (28 days) oral / dermal / inhalation toxicity

No data submitted

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

No data submitted

3.3.5.3. Chronic (> 12 months) toxicity

No data submitted

3.3.6. Mutagenicity / Genotoxicity3.3.6.1. Mutagenicity / Genotoxicity *in vitro*

The mutagenic potential of Tea Tree Oil (*Melaleuca alternifolia*) was examined using the Ames Test. One of the major components, the monoterpenoid terpinen-4-ol, was also examined to determine if it demonstrated any mutagenic potential. *Salmonella typhimurium* (TA102, TA100 and TA98) was utilised in the Ames test. Commercially available Tea Tree Oils were tested. No mutagenic effect was determined in any of the brands of Tea Tree Oil on any of the strains of *Salmonella* examined with or without metabolic activation. The same negative results were obtained for the terpinen-4-ol component examined. There was a clear evidence of toxicity of Tea Tree Oil on all *Salmonella* strains and also by terpinen-4-ol at higher dose levels. It is suggested that terpinen-4-ol may contribute significantly to the widely reported antibacterial activity of Tea Tree Oil.

Ref.: 89

A genotoxicity study using *S. typhimurium* tester strains TA98, TA100 and TA102 with and without S9-mix performed by the test facility PSC (1989), NSW Australia, reported no effect for a Tea Tree Oil application in a range of 100 up to 1500 µg/plate. It is worth to note that the latter study was performed under non-GLP conditions. No details were provided.

There are a number of data available from testing of Tea Tree Oil or of individual constituents in bacterial test systems (For review see Hammer *et al.* 2006). While Tea Tree Oil and most of its constituents were non-mutagenic, α-terpineol caused a slight but dose-related effect in the bacterial reverse mutation assay using *S. typhimurium* tester strain TA102 with and without S9 mixture (tested dose levels: 0-2500 µg/plate). Other bacterial mutagenicity test systems gave no significant response in this study which points to an induction of base-pair substitutions affecting an A-T base pair by terpineol (Gomes-Carneiro *et al.* 1998). In various tests with mammalian cells, several constituents of Tea Tree Oil had no mutagenic effect.

Ref.: 90, 68

Comment

It should be considered that the antimicrobial activity of Tea Tree Oil certainly reduces the relevance of the results obtained with bacterial test systems. Furthermore, chemical modification of individual constituents during inadequate handling of Tea Tree Oil (e.g. generation of epoxides) might lead to a mutagenic activity of Tea Tree Oil to a yet unknown extent.

3.3.6.2. Mutagenicity / Genotoxicity *in vivo***Mammalian Erythrocyte Micronucleus Test**

Guideline: OECD 474
Species/strain: mouse (SPF ARC(S) Swiss)
Group size: 5 mice/sex/group

Test substance: *Melaleuca alternifolia* (Tea Tree) Leaf Oil (INCI)
 Batch: ATTIA/0501
 Purity: ISO 4730-2004
 Dose level: 0, 1000, 1350 and 1750 mg/kg bw
 Route: oral gavage (application of the test item suspensions in 10.8 mL/kg)
 Vehicle: corn oil (10.61 mL/kg)
 Sacrifice times: 24 h for all dose levels and the vehicle control;
 48 h for test item highest dose, vehicle control and positive control
 GLP: in compliance

The potential of Australian Tea Tree Oil (Batch ATTIA/0501) to induce micronuclei in the bone marrow of mouse was investigated. A preliminary study at oral doses of 2000, 1750, 1500, 1250, 1000 and 500 mg/kg, respectively, was performed in order to identify a suitable dose range. Since, at the highest dose (2000 mg/kg) all mice exhibited wobbly gait, prostration and laboured breathing between 30 min. and 5 h after dosing, 1750 mg/kg was selected as the highest dose level in a subsequent main study. Doses of 1350 and 1000 mg/kg respectively were used as medium and low dose in the micronucleus assay.

In the main study, the test item was administered orally to 4 groups of 10 mice (5M/5F) at concentrations of 17.5%, 13.5% and 10% w/w in corn oil. Three groups were sacrificed at 24 hours and a further high dose group, a vehicle control group (corn oil) and the positive control group (40 mg/kg bw of 9,10-dimethyl-1,2-benzanthracene) at 48 hours after dosing. A total of 2000 PCEs prepared from bone marrow of each animal were counted for the incidence of MPCE. At least 200 polychromatic erythrocytes per animal were counted to determine the proportion of PCE among total erythrocytes.

Results

In all test item treated groups, there was no significant increase in the frequency of micronucleated erythrocytes when compared with negative control groups at 24 and 48 hours sampling times. However, all animals (males and females) treated with 1750 mg/kg bw were characterized by a reduced weight gain at the 24-hour sacrifice time. There was also a statistically significant depression of PCE and PCE + NCE ratio in the high dose test item treated groups in both sexes when compared with the vehicle control groups at 48 hours as an indication of the toxicity of the test item at this dose.

Conclusion

The test item Australian Tea Tree Oil (Batch ATTIA/0501) was considered to be non-clastogenic in the mouse micronucleus test under the conditions of the study.

Ref.: 91

3.3.7. Carcinogenicity

No data submitted

3.3.8. Reproductive toxicity

3.3.8.1. Two generation reproduction toxicity

No data submitted

3.3.8.2. Teratogenicity

There are no teratogenicity data available for Tea Tree Oil. In addition, the available literature on reproductive toxicity of constituents of Tea Tree Oil is limited. Therefore, results from studies on myrcene, linalool, and cumene which are terpenes/terpenoids with some structural and chemical resemblances with the major components of Tea Tree Oil, were included in the dossier (β -myrcene, cumene, coriander oil (72.9% linalool, 22.3%

other terpenoids, balance unknown)). The NOAELs of reproductive toxicity were between 250 and 365 mg/kg bw/d.

Ref.: cited in 35

α -Terpinene was given to female Wistar rats at 30, 60, 125 and 250 mg/kg bw on days six to 15 of pregnancy. The two highest doses were maternally toxic, and the highest dose also caused a reduction in the proportion of pregnant females. Foetuses from rats given 250 mg/kg bw/day had reduced body weights and increased kidney weights. Abnormal ossification of bones and minor skeletal abnormalities were evident in foetuses from females given 60 mg/kg bw/day or more. Thus the NOAEL for embryo-foetotoxicity was set at 30 mg/kg body weight (oral route). The NOAEL of maternal toxicity was 60 mg/kg bw/day. It was argued that α -terpinene is typically present at 9% in tea tree oil and that this NOEL would equate to 333 mg/kg bw/day of Tea Tree Oil.

Ref.: 86

Comment of the SCCP

It is obvious that from the data available a definite NOAEL for reproductive toxicity cannot be assessed. However, from the data from other terpenoids it can be deduced that the use of the NOAEL of α -terpinene (30 mg/kg bw/d) as a representative of Tea Tree Oil is a conservative approach.

3.3.9. Toxicokinetics

No data provided.

3.3.10. Photo-induced toxicity

No data submitted

3.3.11. Human data

/

3.3.12. Special investigations

Gynecomastia in 3 prepubertal boys (4, 7, and 10 years old) was reported and related to the topical use of lavender and/or Tea Tree Oil containing products. It was shown that both essential oils exert oestrogen-like activity *in vitro* by inducing growth in MCF-7 cells.

Ref.: 92

The estrogenic potency of Tea Tree Oil was confirmed with an identical *in vitro* model. The main constituents of Tea Tree Oil which have been demonstrated to penetrate human skin (terpinen-4-ol, α -terpineol and eucalyptol) were analysed separately and as mixture in a ratio penetrating the skin and no oestrogenicity was found. It was argued that the active components of the oil may not be bioavailable.

Ref.: 93

Comment of the SCCP

An estrogenic potential of Tea Tree Oil was shown *in vitro*. No *in vivo* studies are available to elucidate the relevance of this finding for the *in vivo* situation. Since the hormonal active ingredients of Tea Tree Oil were shown not to penetrate the skin, the hypothesized correlation of the finding of 3 cases of gynecomastia to the topical use of Tea Tree Oil is considered implausible.

3.3.13. Safety evaluation (including calculation of the MoS)

Not applicable

3.3.14. Discussion

As no clear cosmetic function was provided by the applicant and several non-cosmetic applications are known, it is necessary to state the cosmetic function of TTO.

Physico-chemical properties

The European Inventory contains 3 Melaleuca-type ingredients (INCI names): Melaleuca alternifolia oil (antimicrobial), Melaleuca cajuputi extract (tonic), Melaleuca leucadendron extract (tonic). Tea Tree Oil is the essential oil obtained by steam distillation of the foliage and terminal branchlets of Melaleuca alternifolia, Melaleuca linariifolia and Melaleuca dissitiflora as well as other species of Melaleuca provided that the oil obtained conforms to the requirements given in the International Standard (ISO 4730-2004). Using GC-MS analysis, all 88 components present at more than 0.1% were separated and 54 were identified.

The major constituents with a maximum content of 5% and more are:

Terpinen-4-ol (max. 48%), γ -terpinene (max. 28%), 1,8-cineole (eucalyptol, max. 15%), α -terpinene (max. 13%), p-cymene (max. 8%), α -terpineol (max. 8%), α -pinene (max. 6%) and terpinolene (max. 5%). 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. The levels of α -terpinene, γ -terpinene and terpinolene decrease whereas the level of p-cymene increases up to tenfold. Oxidation processes lead to the formation of peroxides, endoperoxides (ascaridole) and epoxides. As a further oxidation product 1,2,4-trihydroxymenthane was identified. Measurement of p-cymene percentage (which increases with oxidation) gives a good indication of the oxidative degradation of Tea Tree Oil. It was also shown that the p-cymene concentration increases proportionally to the content of the oxidation product 1,2,4-trihydroxymenthane, a suspected skin sensitiser.

In an EMEA position paper on herbal medicinal products containing the natural potential carcinogen methyleugenol a content of 0.28 to 0.9% methyleugenol in Tea Tree Oil essential oil fractions was stated (Ref. 94). Methyleugenol may be present in lower amounts (below 0.1%) according to Ref. 35. Exposure from Tea Tree Oil to these traces of methyleugenol is considered low when comparing it to the intake from food (max. 0.53 mg/kg bw/d, see Ref. 95). Methyleugenol is not classified as carcinogenic in the EU. Methyl eugenol is classified in the US Annual Report on Carcinogen as "Reasonably anticipated to be a human carcinogen". It is also classified by US State of California EPA as: Chemical known by the State to cause cancer.

Skin / eye irritation, dermal sensitisation

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.

In human studies, diverging results were observed. In some studies, no irritant results were seen with diluted or neat oil, other studies indicated that neat Tea Tree Oil as well as cosmetic formulations containing 5% Tea Tree Oil can exhibit skin irritancy. These differences may be due to the vehicle used.

In a HET-CAM assay, neat Tea Tree Oil as well as 25 and 10% solutions in a surfactant were severe irritants in the assay while 5% was only slightly irritant. Primary eye irritation of Tea Tree Oil was studied in the rabbit under GLP conditions. The author concludes that 1% or 5% Tea Tree Oil solutions can be classified as "minimally irritating".

LLNA studies showed that Tea Tree Oil itself and in PEG solution at ISO 4730 quality is a moderate sensitiser in mice. Neat Tea Tree Oil also is a sensitiser in humans. In a number

of patch test studies, different prevalence rates for allergic contact dermatitis to Tea Tree Oil up to 4.8% of the tested patients were observed.

No data on phototoxicity is provided.

It is not fully understood which of the constituents of Tea Tree Oil is responsible for sensitisation. Terpenoid fraction, limonene and/or oxidative degradation products like ascaridole, 1,2,4-trihydroxymenthane, peroxides and epoxides were discussed. Oxidised Tea Tree Oil is a 3 times more potent sensitiser than fresh oil. The sensitising potency may also be influenced by the content of irritants such as p-cymene and 1,8-cineole.

Oxidative bioactivation of prohaptens to haptens in the skin has been recently subject of interest. In studies with model alkenes it was shown for allylic conjugated dienes that the metabolically formed epoxides are the haptens. This includes α -terpinene, a major constituent of Tea Tree Oil (Ref. 96, 97).

Dermal absorption

The comparison of the various studies on percutaneous absorption of terpinen-4-ol from different sources reveals huge differences in absorption rates. In one study an adequate exposure dose, but no relevant cosmetic formulation was investigated. In studies with infinite dose conditions using the same concentration of 5% in different formulations, a nearly linear relationship between exposure time and dermal absorption is apparent. These studies also demonstrated that the type of formulation has a significant influence on dermal absorption of Tea Tree Oil. None of the available studies on dermal absorption is adequate as a sound basis for assessment of systemic exposure to Tea Tree Oil from cosmetic products.

Mutagenicity/ Carcinogenicity

The mutagenic potential of commercially available Tea Tree Oil and its major component terpinen-4-ol was examined using the Ames test (*Salmonella typhimurium* strains TA102, TA100 and TA98). No mutagenic effect was determined with or without metabolic activation.

A non-GLP genotoxicity study using *S. typhimurium* tester strains TA98, TA100 and TA102 with and without S9 mixture reported no effect for Tea Tree Oil in a range of 100 up to 1500 μ g/plate. α -Terpineol caused a slight but dose-related effect in the bacterial reverse mutation assay using *S. typhimurium* tester strain TA102 with and without S9-mix. In a mouse micronucleus test Tea Tree Oil was considered to be non-clastogenic under the conditions of the study. No data on carcinogenicity are available.

General toxicity

No repeated dose toxicity study with Tea Tree Oil was provided. However, for some of the constituents of Tea Tree Oil and related compounds a range of toxic effects has been reported after repeated exposure to them and could be used to estimate NOAEL values. Based on the available information on renal effects in the repeated dose toxicity studies an estimate based on assumed additivity would result in an NOAEL for Tea Tree Oil of 117 mg/kg bw/d.

No reproductive toxicity study with Tea Tree Oil was provided. In a reproductive toxicity study of α -terpinene the NOAEL of maternal toxicity of α -terpinene was 60 mg/kg bw/d while the NOAEL of embryo-/foetotoxicity was 30 mg/kg bw/d. The latter is based on abnormal ossification of bones and minor skeletal abnormalities. Given the presence of 9% α -terpinene in Tea Tree Oil, this would equal a NOAEL for Tea Tree Oil of 333 mg/kg according to the applicant. However, from the data available a definite NOAEL for reproductive toxicity of Tea Tree Oil cannot be assessed. From data of other terpenoids it can be deduced that the use of the NOAEL for reproductive toxicity of α -terpinene as a representative of Tea Tree Oil (30 mg/kg bw/d) would be a conservative approach.

4. CONCLUSION

The cosmetic function of Tea Tree Oil needs to be indicated, as no clear cosmetic function was given by the applicant and several non-cosmetic applications are known.

When exposed to air and heat, Tea Tree Oil is prone to oxidation, yielding epoxides and further oxidation products which are considered to contribute to the skin sensitising potential of Tea Tree Oil. It is important to consider that certain formulations tend to reduce stability. According to the Code of Practice and the Guidance document introduced by the Australian Tea Tree Oil Association, safe processing and storage may be achieved which can be controlled by the p-cymene content.

Tea Tree Oil is a skin sensitiser. Skin sensitisation may also be enhanced by irritancy. Neat Tea Tree Oil and certain formulations at concentrations of 5% or more can induce skin and eye irritation.

Based on clinical data, the current use levels of TTO are shown to induce contact allergy.

Methyleugenol was reported as a minor constituent of Tea Tree Oil; the content should be indicated. According to the opinion SCCNFP/0373/00 on methyleugenol in fragrances the content in finished leave-on products should not exceed 0.0002% (2 ppm) and in rinse-off products 0.001% (10 ppm).

Following topical application of Tea Tree Oil and Tea Tree Oil containing products, percutaneous absorption of some constituents may occur, leading to a considerable systemic exposure, especially from neat oil, body lotion and foot spray/powder (see appendix). Because of inadequate dermal absorption studies available, the magnitude of systemic exposure to Tea Tree Oil from cosmetic products is uncertain. Only worst case estimations for NOAELs for general systemic and reproductive toxicity can be made. A Margin of Safety has not been calculated and the safety of Tea Tree Oil cannot be assessed.

Should there be reliable data on percutaneous absorption covering relevant concentrations and cosmetic formulations, a reassessment of the safety of Tea Tree Oil is envisaged by the SCCP.

5. MINORITY OPINION

Not applicable

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Appendix

Chemical composition

The chemical composition of the three oils selected for testing for the Tea Tree Oil safety dossier. Area percentages were measured by total ion chromatography on a HP5MS 30m column and are hence not comparable with the flame ionisation detection used for standard quantitation.

Library Search Report

Pk#	RT	Library/ID	050106	Area% 050108	050196
1	7.42	THUJENE, α-	0.85	0.96	0.91
2	7.57	PINENE, α-	0.46	2.44	2.46
3	8.55	SABINENE		0.30	0.15
4	8.61	PINENE, β-	0.66	0.70	0.69
5	8.96	MYRCENE	0.19	0.70	0.45
6	9.23	PHELLANDRENE, α-	0.10	0.45	0.28
8	9.50	TERPINENE, α-	1.15	10.22	5.93
11	9.67	CYMENE-P	19.90	2.89	11.40
12	9.75	PHELLANDRENE-β + LIMONENE	1.39	1.91	1.65
13	9.80	CINEOLE, 1,8-	5.37	5.05	5.20
14	10.15	OCIMENE, TRANS-β		0.02	
15	10.37	TERPINENE-γ	6.90	19.15	13.59
16	10.53	SABINENE HYDRATE, TRANS-		0.12	0.08
17	10.92	TERPINOLENE	1.57	3.80	2.73
19	11.11	SABINENE HYDRATE, CIS-		0.22	0.16
22	11.52	p-MENTH-2-EN-1-OL, TRANS-	0.14	0.29	0.23
25	11.84	p-MENTH-2-EN-1-OL, CIS-	0.16	0.25	0.21
28	12.51	TERPINEN-4-OL	30.06	33.11	31.16
29	12.60	UNIDENTIFIED	0.47	0.19	0.28
30	12.69	TERPINEOL, α-	4.82	3.39	4.08
31	12.77	PIPERITOL, TRANS-	0.21	0.26	0.22
33	12.96	PIPERITOL, CIS-	0.20	0.28	0.24
37	13.90	UNKNOWN m/z 155	0.43		0.22
38	14.16	ASCARIDOLE GLYCOL	0.69		0.34
39	14.37	UNKNOWN m/z 170	0.94		0.45
40	14.50	TERPINOLENE-4,8-DIOL	0.28		0.13
41	14.63	UNKNOWN m/z 170	1.85		0.85
42	14.90	UNKNOWN m/z 170	0.24		0.12
44	15.16	UNKNOWN m/z 170	0.50		0.23
48	15.43	COPAENE, α-	0.32	0.24	0.31
52	15.90	GURJUNENE, α-	0.47	0.45	0.47
54	16.04	CARYOPHYLLENE	0.36	0.55	0.50
58	16.29	AROMADENDRENE	1.60	1.48	1.56
59	16.34	SELINA-5,11-DIENE	0.20	0.18	0.18
60	16.42	CADINA-3,5-DIENE		0.21	0.15
61	16.48	HUMULENE	0.13	0.16	0.15
62	16.58	AROMADENDRENE, ALLO-	0.70	0.61	0.66
63	16.71	7,10-CADINA-1,(6),4-DIENE	0.27	0.57	0.44
64	16.77	MENTHANE-1S,2S,4S-TRIOL	1.64		0.74
66	16.93	GUAIENE, CIS-β-	0.35	0.41	0.52
67	17.00	LEDENE	1.57	1.38	2.15
68	17.02	BICYCLOGERMACRENE		1.22	
71	17.31	CADINENE-δ	2.22	1.85	1.98
72	17.35	ZONARENE		0.39	0.29
73	17.43	CADINA-1,4-DIENE	0.20	0.32	0.27
76	17.81	EPI-GLOBULOL	0.13	0.12	0.14
77	17.91	UNIDENTIFIED	0.17	0.12	0.14
78	18.02	SPATHULENOL	0.18	0.09	
80	18.10	GLOBULOL	0.71	0.46	0.59
81	18.21	VIRIDIFLOROL	0.47	0.41	0.45
82	18.31	ROSIFOLIOL	0.12	0.10	0.11
85	18.54	GUAIA - 5 - EN - 11 - OL	0.18	0.16	0.19
86	18.59	CUBENOL, 1 EPI-	0.30	0.30	0.31
88	18.76	CUBENOL	0.23	0.20	0.22

Ref.: 7

Hypothetical exposure estimation

8.2.1 Exposure estimation using absolute skin penetration

The applicant argued that the *in vitro* percutaneous absorption study showed a 24-hour penetration rate for undiluted Tea Tree Oil of 250 µg/cm² (total components in receptor phase and epidermis). The penetration rate based on terpinen-4-ol was 213 and 25 µg/cm² for undiluted oil and 20 % formulation, respectively. This demonstrates that the rate of penetration of terpinen-4-ol was dependent on the concentration of Tea Tree Oil, but the relationship was not linear since the penetration rate for the 20 % formulation was 1/8th of that for the undiluted oil. This indicates that a linear extrapolation of the penetration of the undiluted oil for lower concentration would result in a conservative overestimation of the absorption for the lower concentrations according to the applicant.

Comment of the SCCP

It is generally well known and was also demonstrated in the published literature of Tea Tree Oil that the vehicle plays an important role in dermal absorption studies. However, no relevant cosmetic formulation was investigated by the applicant.

An extrapolation of the absorption rate based on concentration over several orders of magnitude seems not to be justified. Therefore, a MoS cannot be calculated using this approach.

8.2.2. Exposure estimation using the percentage of applied substance

The rate of absorption of Tea Tree Oil was measured to be about 3% in the percutaneous absorption study submitted in the current dossier (see reference 65 and 66). The daily exposure was calculated for the various product types. This was adjusted for the skin retention factor according to SCCP Notes of Guidance. Where retention factors were not stipulated by the SCCP, a value of 0.01 was used for rinse-off products and a value of 1 was used for leave-on products. The Table contains the exposure estimates for the various product types. Systemic exposure estimates between 0.0017 and 3.33 mg/kg/day were obtained.

Table: Exposure estimates using the absorption as % of applied dose

Use	Concentration (%)	Amount applied (mg)	Retention Factor	Systemic Exposure Dose (mg/kg/day)
100% Tea Tree Oil	100	200	1	3.33
Bath additive	15	10,000	0.01	0.25
Cleansing Face Wash	0.7	5,000	0.01	0.006
Anti-Dandruff Shampoo	2.0	8,000	0.01	0.027
Deodorant stick/roller	2.5	500	1	0.21
Foot Powder	1.0	2,000	1	0.33
Foot Spray	2.0	2,000	1	0.67
Body Lotion	1.25	8,000	1	1.67
Hand Wash	0.7	3,000	0.01	0.0035
Mouthwash	0.2	10,000	0.1	0.033
Hand wash soap solid	2.0	500	0.01	0.0017

Comment of the SCCP

From the table above it can be deduced that following topical application of Tea Tree Oil and Tea Tree Oil containing products percutaneous absorption of some constituents occurs leading to a considerable systemic exposure, especially from neat Tea Tree Oil, body lotion and foot spray/powder.