Safety Assessment of *Melaleuca alternifolia* (Tea Tree)-Derived Ingredients as Used in Cosmetics

Status:	Draft Report for Panel Review
Release Date:	November 13, 2020
Panel Meeting Date:	December 7-8, 2020

The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; David E. Cohen, M.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; Lisa A. Peterson, Ph.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The Cosmetic Ingredient Review Executive Director is Bart Heldreth, Ph.D. This safety assessment was prepared by Monice M. Fiume, Senior Director, CIR.

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Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Monice M. Fiume MMF
Senior Director
Date: November 13, 2020
Subject: Safety Assessment of *Melaleuca alternifolia* (Tea Tree)-Derived Ingredients as Used in Cosmetics

Enclosed is the Draft Report of the Safety Assessment of *Melaleuca alternifolia* (Tea Tree)-Derived Ingredients as Used in Cosmetics. (It is identified in this report package as *melalt122020rep*.) This is the first time the Panel is seeing the safety assessment on these 8 ingredients. The Scientific Literature Review was announced on August 4, 2020. You will notice that all abbreviations are defined at the front of the document, rather than in the text of the report. Please provide comment as to whether you prefer the comments presented up front (as in this document) or in the text itself (as we have normally done).

The following unpublished data were received either from the Council or as a direct submission to CIR, and are included in the report:

1. Personal Care Products Council. 2019. Concentration of use by FDA product category: *Melaleuca alternifolia* (tea tree)-derived ingredients. Unpublished data submitted by the Personal Care Products Council on April 11, 2019.
2. Anonymous. 2020. Safety data sheet: Tea Tree (*Melaleuca alternifolia*) leaf oil. Submitted by the Australian Tea Tree Industry Association, Ltd on September 28, 2020.
3. Product Investigations Inc. 2016. Report: PII No. 35747: Determination of the irritating and sensitizing propensities of MT#2700253 (10% Melaleuca Alternifolia (Tea Tree) Leaf Oil in Caprylic/Capric Triglyceride) on human skin. Unpublished data submitted by Personal Care Products Council on March 2, 2016.

A concentration of use survey was originally conducted in 2015 (*melalt122020data_4*). Although these data are not included in the safety assessment, they are included with this submission to provide the Panel with information regarding changes in use over the last few years. VCRP data has increased significantly for the Leaf Oil (more than doubling, from 336 uses in 2015 to 724 uses in 2020), but the number of categories for which concentrations of use were reported for the *Melaleuca alternifolia*-derived ingredients, as well as the maximum reported concentration of use for the Leaf Oil, decreased notably. (For example, the maximum concentration of use for the Leaf Oil decreased from 15% (in face and neck products) in 2015 to 0.63% (in cuticle softeners) in 2019.)

Comments on the SLR that were received from the Council (*melalt122020pcpc*) were addressed, and are included. Also received were several sets of comments/emails from the Australian Tea Tree Industry Association (*melalt122020ATTIA_1 through melalt122020ATTIA_4*). Many of these comments focused on the difference in standards for, and the alteration of, tea tree oil, as well as the use of oxidized oil in irritation and sensitization testing. Often, the comments were submitted with attached documents; with the exception of published journal article, these attachments are also included with the comment set. These comments were addressed, when appropriate. However, please consider these comments to determine whether any additional information should be added to the report.

The following are also included as a part of this report package:

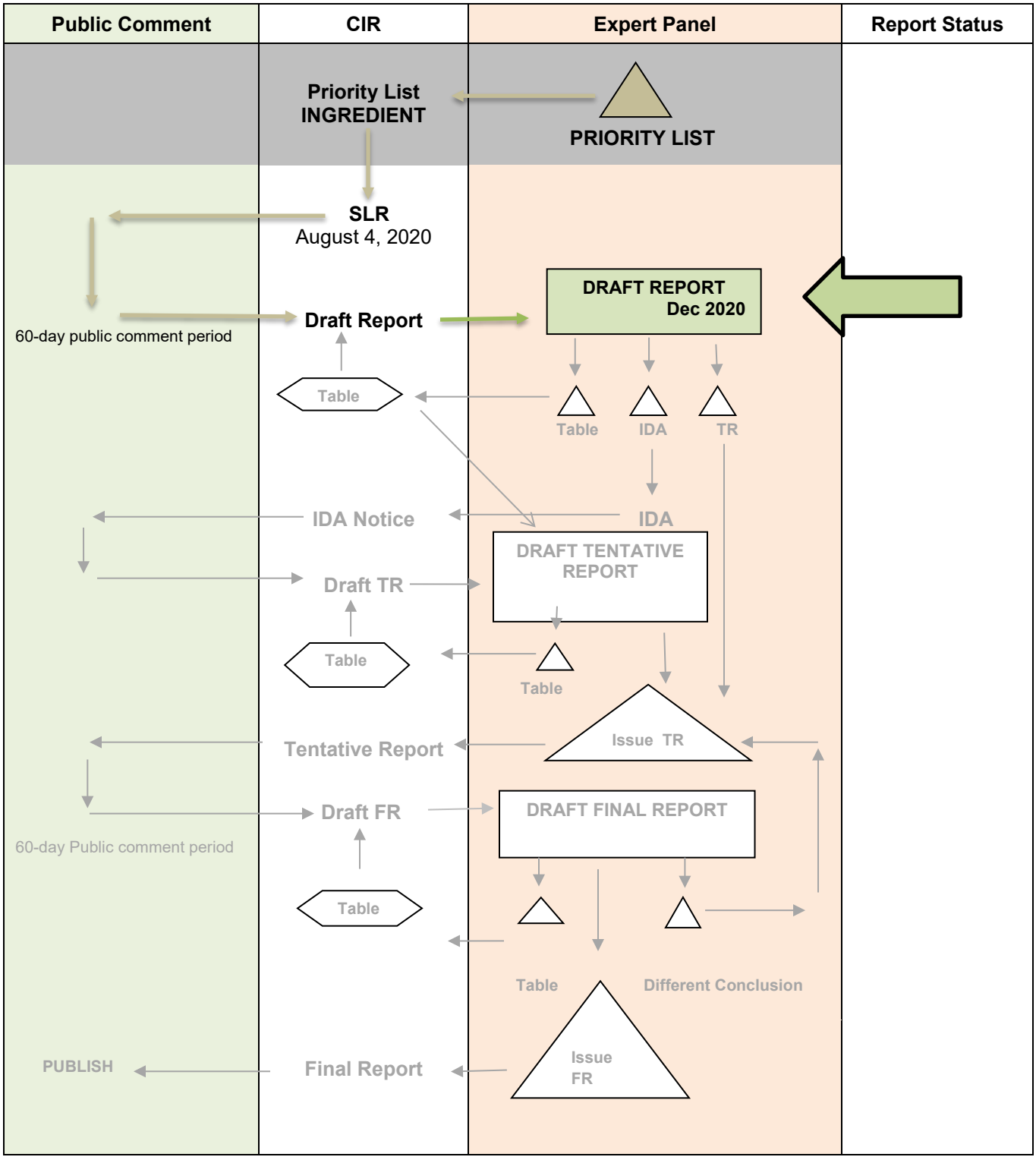
melalt122020flow: report flowchart
melalt122020hist: report history
melalt122020prof: data profile
melalt122020strat: search strategy
melalt122020FDA: 2020 VCRP data

After reviewing these documents, if the available data are deemed sufficient to make a determination of safety, the Panel should issue a Tentative Report with a safe as used, safe with qualifications, or unsafe conclusion, and Discussion items should be identified. If the available data are insufficient, the Panel should issue an Insufficient Data Announcement (IDA), specifying the data needs therein.

SAFETY ASSESSMENT FLOW CHART

INGREDIENT/FAMILY *Melaleuca alternifolia (Tea Tree)*-derived ingredients

MEETING December 2020



CIR Report History: *Melaleuca alternifolia* (Tea Tree)-Derived Ingredients

SLR: August 4, 2020
The following data were received prior to announcing the SLR:

1. Personal Care Products Council. 2016. Concentration of use by FDA product category: *Melaleuca alternifolia* (tea tree)-derived ingredients. (Survey conducted in 2015.) Unpublished data submitted by the Personal Care Products Council on February 8, 2016. [These data were not included in the SLR because updated survey data were provided in 2019.]
2. Personal Care Products Council. 2019. Concentration of use by FDA product category: *Melaleuca alternifolia* (tea tree)-derived ingredients. Unpublished data submitted by the Personal Care Products Council on April 11, 2019.
3. Product Investigations Inc. 2016. Report: PII No. 35747: Determination of the irritating and sensitizing propensities of MT#2700253 (10% Melaleuca Alternifolia (Tea Tree) Leaf Oil in Caprylic/Capric Triglyceride) on human skin. Unpublished data submitted by Personal Care Products Council on March 2, 2016.

Several sets of comments/emails (with attachments) were received from the Australian Tea Tree Industry Association (ATTIA) during the preparation of the SLR.

Draft Report: December 7-8, 2020
The following unpublished data were received as a direct submission to CIR prior to review of the Draft Report:

1. Anonymous. 2020. Safety data sheet: Tea Tree (*Melaleuca alternifolia*) leaf oil. Submitted by the Australian Tea Tree Industry Association, Ltd on October 13, 2020

Several sets of comments/emails (with attachments) were received from the ATTIA in response to the SLR. Comments were also received from the Council.

Melaleuca alternifolia (Tea Tree)-Derived Ingredients * – Dec 7-8, 2020 – Writer, Monice																																		
					Toxico-kinetics		Acute Tox			Repeated Dose Tox			DART		Genotox		Carci		Anti-Carci		Endocrine Activity		Dermal Irritation			Dermal Sensitization				Ocular Irritation		Clinical Studies	
	Reported Use	GRAS	Method of Mfg	Constituents/ Impurities	Dermal Penetration	ADME	Dermal	Oral	Inhalation	Dermal	Oral	Inhalation	Dermal	Oral	In Vitro	In Vivo	Dermal	Oral	In Vitro	Animal	Estrogenic Effects	Anti-Androgenic Effects	In Vitro	Animal	Human	In Vitro	Animal	Human	Phototoxicity	In Vitro	Animal	Retrospective/ Multicenter	Case Reports
Melaleuca Alternifolia (Tea Tree) Extract	X																																	
Melaleuca Alternifolia (Tea Tree) Flower/Leaf/Stem Extract	X																																	
Melaleuca Alternifolia (Tea Tree) Flower/Leaf/Stem Oil																																		
Melaleuca Alternifolia (Tea Tree) Leaf	X																																	
Melaleuca Alternifolia (Tea Tree) Leaf Extract	X																																	
Melaleuca Alternifolia (Tea Tree) Leaf Oil	X			X			X	X								X								X	X			X					
Melaleuca Alternifolia (Tea Tree) Leaf Powder	X			X																													
Melaleuca Alternifolia (Tea Tree) Leaf Water	X		X																															
tea tree oil			X	X	X	X	X	X	X	X	X			X	X				X	X	X	X		X	X		X	X	X	X	X	X	X
tea tree oil (oxidized)																											X					X	X	
tea tree powder																															X			

* "X" indicates that data were available in a category for the ingredient

Melaleuca Alternifolia (Tea Tree)-Derived Ingredients

	CAS #	InfoBase	PubMed	SciFinder	ChemID	NTIS	FDA	ECHA	IUCLID/ SIDS/OECD	WHO/ JEFCA	EU	NICNAS	FEMA	Web
tea tree oil - general							X							
Melaleuca Alternifolia (Tea Tree) Leaf Oil	68647-73-4 8022-72-8	SCCS RIFM TRN	737 hits 80 useful 1/26/16 11/29/18: 393 hits/ 17 selected	297 hits	---	---	X	yesr	---	X	no R SCCP 2008	---	GRAS	yes
Melaleuca Alternifolia (Tea Tree) Flower/ Leaf/Stem Extract	84238-27-7 85085-48-9	---						X	---		no R			
Melaleuca Alternifolia (Tea Tree) Extract	85085-48-9													
Melaleuca Alternifolia (Tea Tree) Flower/Leaf/Stem Oil	85085-48-9	---						X			SCCP 2008			
Melaleuca Alternifolia (Tea Tree) Leaf	85085-48-9	---						X	---		no R			
Melaleuca Alternifolia (Tea Tree) Leaf Extract	85085-48-9	---						X	---		no R			
Melaleuca Alternifolia (Tea Tree) Leaf Powder	85085-48-9	---						preR	---		no R			
Melaleuca Alternifolia (Tea Tree) Leaf Water	85085-48-9	---						---	---		no R			

PubMed Search Strategy

updated, 5/17/2019: (((((((84238-27-7[EC/RN Number]) OR 85085-48-9[EC/RN Number]) OR 68647-73-4[EC/RN Number]) OR 8022-72-8) OR Melaleuca) OR "Melaleuca alternifolia") OR "tea tree") AND ("2015"[Date - Publication] : "3000"[Date - Publication]) – 329 hits/15 selected (alert created)

(((68647-73-4[EC/RN Number]) OR 8022-72-8[EC/RN Number]) OR 85085-48-9[EC/RN Number]) OR (Melaleuca AND alternifolia) OR (tea AND tree) – 737 hits/80 selected (1/26/16; alert created)

((Melaleuca AND Alternifolia) OR (Tea AND Tree)) AND (Flower AND Leaf AND Stem AND Oil) – no hits; (2/1/19; alert created)

Updated 11/29/18): (((((68647-73-4[EC/RN Number]) OR 8022-72-8) OR 85085-48-9[EC/RN Number]) OR (Melaleuca AND alternifolia)) OR ((tea AND tree)) AND ("2015"[Date - Publication] : "3000"[Date - Publication]) – 393 hits/ 17 selected

[***weekly updates received from PubMed***]

FDA

https://www.govinfo.gov/content/pkg/FR-2019-04-12/pdf/2019-06791.pdf Safety and Effectiveness of Consumer Antiseptic Rubs; Topical Antimicrobial Drug Products for Over-the-Counter Human Use (4/12/2019 Federal Register)

http://www.fda.gov/

June 23, 2016 Pharmacy Compounding Advisory Committee Mtg; accessed 1/13/17 as tea tree oil

: http://www.fda.gov/downloads/advisorycommittees/committeesmeetingmaterials/drugs/pharmacycompoundingadvisorycommittee/ucm509958.pdf

associated briefing document

http://www.fda.gov/downloads/advisorycommittees/committeesmeetingmaterials/drugs/pharmacycompoundingadvisorycommittee/ucm505041.pdf

Other Reference Searches:
The Merck Index
USP Pharmacopeia
Food Chemicals Codex

Searched for documents via:
http://www.teatree.org.au/search_abstracts.php
http://www.rirdc.gov.au/publications

LINKS

Search Engines

- Pubmed (- http://www.ncbi.nlm.nih.gov/pubmed)

appropriate qualifiers are used as necessary
search results are reviewed to identify relevant documents

Pertinent Websites

- wINCI - http://webdictionary.personalcarecouncil.org
- FDA databases http://www.ecfr.gov/cgi-bin/ECFR?page=browse
- FDA search databases: http://www.fda.gov/ForIndustry/FDABasicsforIndustry/ucm234631.htm;,
- EAFUS: http://www.accessdata.fda.gov/scripts/fcn/fcnnavigation.cfm?rpt=eafuslisting&displayall=true
- GRAS listing: http://www.fda.gov/food/ingredientspackaginglabeling/gras/default.htm
- SCOGS database: http://www.fda.gov/food/ingredientspackaginglabeling/gras/scogs/ucm2006852.htm
- Indirect Food Additives: http://www.accessdata.fda.gov/scripts/fdcc/?set=IndirectAdditives
- Drug Approvals and Database: http://www.fda.gov/Drugs/InformationOnDrugs/default.htm
- http://www.fda.gov/downloads/AboutFDA/CentersOffices/CDER/UCM135688.pdf
- FDA Orange Book: https://www.fda.gov/Drugs/InformationOnDrugs/ucm129662.htm
- OTC ingredient list: https://www.fda.gov/downloads/aboutfda/centersoffices/officeofmedicalproductsandtobacco/cder/ucm135688.pdf
- (inactive ingredients approved for drugs: http://www.accessdata.fda.gov/scripts/cder/iig/
- ChemPortal: https://www.echemportal.org/echemportal/index.action
- NIOSH (National Institute for Occupational Safety and Health) - http://www.cdc.gov/niosh/
- NTIS (National Technical Information Service) - http://www.ntis.gov/
- NTP (National Toxicology Program) - http://ntp.niehs.nih.gov/
- Office of Dietary Supplements https://ods.od.nih.gov/
- FEMA (Flavor & Extract Manufacturers Association) - http://www.femaflavor.org/search/apachesolr_search/
- EU CosIng database: http://ec.europa.eu/growth/tools-databases/cosing/
- ECHA (European Chemicals Agency – REACH dossiers) – http://echa.europa.eu/information-on-chemicals;jsessionid=A978100B4E4CC39C78C93A851EB3E3C7.live1
- ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals) - http://www.ecetoc.org
- European Medicines Agency (EMA) - http://www.ema.europa.eu/ema/
- OECD SIDS (Organisation for Economic Co-operation and Development Screening Info Data Sets)- http://www.oecd.org/env/ehs/risk-assessment/publishedassessments.htm
- SCCS (Scientific Committee for Consumer Safety) opinions: http://ec.europa.eu/health/scientific_committees/consumer_safety/opinions/index_en.htm
- NICNAS (Australian National Industrial Chemical Notification and Assessment Scheme)- https://www.nicnas.gov.au/

- International Programme on Chemical Safety http://www.inchem.org/
- FAO (Food and Agriculture Organization of the United Nations) - http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/
- WHO (World Health Organization) technical reports - http://www.who.int/biologicals/technical_report_series/en/

- www.google.com - a general Google search should be performed for additional background information, to identify references that are available, and for other general information

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ABBREVIATIONS

ACC	allergic contact cheilitis
ACD	atopic contact dermatitis
ADR	adriamicin-resistant
ANDA	abbreviated new drug application
AR	androgen receptor
ATTIA	Australian Tea Tree Industry Association
BCOP	bovine corneal opacity and permeability
BrdU	5-bromo-2'-deoxy-uridine
BSA	bovine serum albumin
C1orf116	chromosome 1 open reading frame 116
CAP	compound auditory nerve action potential
CGC/FID	capillary gas chromatography with a flame-ionization detector
CIR	Cosmetic Ingredient Review
COLIPA	European Cosmetic Toiletry and Perfumery Association
Council	Personal Care Products Council
cpm	counts per minute
CTSD	cathepsin D
CYP4F8	cytochrome P450 family 4 subfamily F member 8
DART	developmental and reproductive toxicity
DHT	dihydrotestosterone
Dictionary	*International Cosmetic Ingredient Dictionary and Handbook*
DKG	German Contact Dermatitis Research Group
DMBA	9,10-dimethyl-1,2-benzanthracene
DMEM	Dulbecco's modified Eagle's medium
DMSO	dimethyl sulfoxide
DTH	delayed type hypersensitivity
E2	17β-estradiol
EC	European Commission
EC3	estimated concentration of a substance expected to produce a stimulation index of 3
EC_{50}	concentration for 50% of maximal effect
ECHA	European Chemicals Agency
ELISA	enzyme-linked immunosorbent assay
EMA	European Medicines Agency
ER	estrogen receptor
ERE	estrogen response element
ESCD	European Society of Contact Dermatitis
EU	European Union
FCA	Freund's complete adjuvant
FDA	Food and Drug Administration
FEMA	Flavor and Extract Manufacturer's Association
GC/MS	gas chromatography/mass spectrometry
GPMT	guinea pig maximization test
GRAS	generally recognized as safe
GREB1	growth regulation by estrogen in breast cancer 1
GSD	geometric standard deviation
HaCaT	normal human keratinocytes
HCA	α-hexylcinnamaldehyde
HET-CAM	hen's egg test on the chorioallantoic membrane
HMPC	Committee on Herbal Medicinal Products
HPA	hypothalamic-pituitary-adrenal
HRIPT	human repeated insult patch test
HSE	heat-separated epidermis
HS-SPME	headspace solid-phase microextraction
IC_{50}	concentration eliciting 50% inhibition
ICDRG	International Contact Dermatitis Research Group
IgA	immunoglobulin A
IGFBP3	insulin like growth factor binding protein 3
GSFISO	International Organization for Standardization
K_p	permeability coefficient
LBD	ligand-binding domain
LC/MS/MS	liquid chromatography/tandem mass spectrometry
LC/UV	liquid chromatography with ultraviolet detection
LD	lethal dose
LLNA	local lymph node assay
LOD	limit of detection
MCF-7	Michigan Cancer Foundation-7
MED	minimal erythema dose
MHE	multiple headspace extraction
MMAD	mass median aerodynamic diameter
MMTV	mouse mammary-tumor virus
MOS	margin of safety
MPO	myeloperoxidase
mRNA	messenger RNA
MTS	[(3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2H-tetrazolium)
MTT	3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide
MYC	a proto-oncogene
NACDG	North American Contract Dermatitis Group
NCE	normochromatic erythrocytes
NDA	new drug application
NLT	not less than
NMT	not more than
NOAEL	no-observable-adverse-effect-level
NR	not reported/none reported
NR	nuclear receptor (Table 15)
NS	not specified
NSWPIC	New South Wales Poisons Information Centre
NZW	New Zealand white
OECD	Organisation for Economic Co-operation and Development
OTC	over-the-counter
P_{app}	apparent permeability constant
Panel	Expert Panel for Cosmetic Ingredient Safety
PBMC	peripheral blood mononuclear cells
PBS	phosphate-buffered saline
PCE	polychromatic erythrocytes
PCR	polymerase chain reaction
PEG	polyethylene glycol
pet	petrolatum
PGR	progesterone receptor
PI	propidium iodide
PUVA	psoralen and long-wave ultraviolet radiation
RPE	relative proliferative effect
RPMI	Roswell Park Memorial Institute
SCCP	Scientific Committee on Consumer Products
SCE	stratum corneum and epidermis
SEC14L2	SEC14-like lipid binding 2
SED	systemic exposure dose
SGOT	serum glutamine-oxaloacetic transaminase
SGPT	serum glutamic-pyruvic transaminase
SI	stimulation index
SLS	sodium lauryl sulfate
SPF	specific pathogen-free
SPIN	Significance-Prevalence Index Numbers
SRC	steroid receptor coactivator
TG	test guideline
TNCB	2,4,6-trinitrochlorobenzene
TNF	tumor necrosis factor
UGT2B28	UDP glucuronosyltransferase family 2 member B28
UK	United Kingdom
US	United States
UV	ultraviolet
UVB	mid-wavelength irradiation
V79 cells	Chinese hamster lung fibroblasts
VCRP	Voluntary Cosmetic Registration Program
WHO	World Health Organization
WT	wild type

INTRODUCTION

This assessment reviews the safety of the following 8 *Melaleuca alternifolia* (tea tree)-derived ingredients as used in cosmetic formulations:

Melaleuca Alternifolia (Tea Tree) Extract
Melaleuca Alternifolia (Tea Tree) Flower/Leaf/Stem Extract
Melaleuca Alternifolia (Tea Tree) Flower/Leaf/Stem Oil
Melaleuca Alternifolia (Tea Tree) Leaf
Melaleuca Alternifolia (Tea Tree) Leaf Extract
Melaleuca Alternifolia (Tea Tree) Leaf Oil
Melaleuca Alternifolia (Tea Tree) Leaf Powder
Melaleuca Alternifolia (Tea Tree) Leaf Water

According to the web-based *Dictionary*, 5 of these ingredients are reported to function in cosmetics as skin-conditioning agents (Table 1).[1] Other reported functions include abrasive, antioxidant, fragrance ingredient, flavoring ingredient, anti-acne agent, antifungal agent, and antimicrobial agent. It should be noted that use as an anti-acne agent is not considered a cosmetic function in the US, and therefore, use as such does not fall under the purview of the Expert Panel for Cosmetic Ingredient Safety (Panel).

Melaleuca alternifolia contains over 100 constituents, some of which have the potential to cause adverse effects. For example, 1,8-cineole (also known as eucalyptol[2]) can be an allergen,[3] and terpinolene, α-terpinene, α-phellandrene, limonene, ascaridole (a product of tea tree oil oxidation), and 1,2,4-trihydroxymenthane (a product that might be found in aged tea tree oil) are sensitizers.[4,5] In this assessment, the Panel is evaluating the potential toxicity of each of the *Melaleuca alternifolia* (tea tree)-derived ingredients as a whole, complex substance. Naturally-occurring combinations rarely demonstrate the same biological activity as the individual, separated components;[6] potential toxicity is a functional response to exposure of a mixture of different chemical compounds.[7]

This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. Published data are identified by conducting an exhaustive search of the world's literature. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that the Panel typically evaluates, is provided on the CIR website (https://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; https://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data are provided by the cosmetics industry, as well as by other interested parties.

Some of the data included in this safety assessment were obtained from reviews (such as those issued by the EC SCCP,[8] ECHA,[9] and EMA[3,10,11]). These data summaries are available on the respective websites, and when deemed appropriate, information from the summaries has been included in this report.

The cosmetic ingredient names, according to the *Dictionary*, are written as listed above, without italics and without abbreviations. When referring to the plant from which these ingredients are derived, the standard scientific practice of using italics will be followed (i.e., *Melaleuca alternifolia*). Often in the published literature, the general name "tea tree" is used, especially, tea tree oil. If it is not known whether the substance being discussed is equivalent to the cosmetic ingredient, the test substance will be identified by the name used in the publication that is being cited; it is possible that the oil may be obtained from more than one species of *Melaleuca*, or from parts other than the leaves. However, if it is known that the substance is a cosmetic ingredient, the *Dictionary* nomenclature (e.g., Melaleuca Alternifolia (Tea Tree) Leaf Oil) will be used.

CHEMISTRY

Definition and Plant Identification

According to the *Dictionary*, the most recent definition of Melaleuca Alternifolia (Tea Tree) Extract is the extract of the whole sapling, *Melaleuca alternifolia*; in the past, this ingredient was defined as the extract of the whole tree (Table 1).[1] Each of the other *Melaleuca alternifolia* (tea tree)-derived ingredients is named based on the plant part(s) from which they are obtained. Several of these ingredients have the generic CAS No. 85085-48-9; however, Melaleuca Alternifolia (Tea Tree) Leaf Oil has CAS Nos. (68647-73-4; 8022-72-8) that are specific to that ingredient.

The *Melaleuca* genus belongs to the Myrtaceae family, within the Myrtales order.[12] *Melaleuca alternifolia* occurs in riparian zones of freshwater and swamps. It is a commercially-grown plant that is indigenous to Australia,[13] and plants with the genetic make-up necessary to produce the oil are native to northern New South Wales.[14] However, *Melaleuca alternifolia* has been introduced and cultivated in China, Indonesia, Kenya, Madagascar, Malaysia, South Africa, Tanzania, Thailand, the US, and Zimbabwe.[15,16]

Melaleuca alternifolia is a tall shrub or small tree that typically grows up to 7 m high, with a bushy crown and papery bark.[17] The total biomass (above-ground growth) of the tea tree can be subdivided into three components: leaves, fines stems, and main stems.[18] The fine stems are defined as stems of less than 2.5 mm in diameter, and they carry virtually all the leaves; the leaves and fine stems, together, are referred to as twigs. The main stems make up the remainder. The hairless leaves are scattered to whorled, and are 10 - 35 mm long by about 1 mm wide.[17] The leaves, which have prominent oil glands and are rich in aromatic oil, are borne on a petiole (leaf stalk) that is approximately 1 mm long. Tea tree oil is only

found in the leaves; it is stored in the subepidermal glands that are adjacent to the epidermis, and the glands are equally distributed on both sides of the leaf.[18] The oil glands first appear in immature leaves, and the number per leaf increases as the leaf expands, reaching a maximum just prior to the leaf fully expanding.

The inflorescences are many-flowered spikes, 3 - 5 cm long, with axes bearing short hairs.[17] The white flowers are solitary, each within a bract, and have petals 2 - 3 mm long. There are 30 - 60 stamens per bundle and the style is 3 - 4 mm long. The fruit is cup-shaped and 2 - 3 mm in diameter, with a hole 1.5 - 2.5 mm in diameter that enables release and dispersal of the seeds by wind. Fruits are usually sparsely spaced along the branches.

Chemical Properties

Tea tree oil is a volatile essential oil.[19] The log P_{ow} of Melaleuca Alternifolia (Tea Tree) Leaf Oil is 3.4 – 5.5.[20] Available properties data for *Melaleuca alternifolia* (tea tree) oil are provided in Table 2.

Stability

In a 12-mo study designed to replicate normal consumer use conditions, there was no appreciable oxidation or degradation of tea tree oil.[14,21] No significant change was observed in the level of terpinen-4-ol was reported. A downward trend in α-terpinene and γ-terpinene, and a similar upward trend in *p*-cymene, was observed, and the peroxide levels increased. The amber glass bottles of tea tree oil were regularly opened, exposed to air and light for short periods of time, and a small amount of oil was removed; when not in use, the bottles were stored away from heat and light.

Method of Manufacture

The majority of the methods below are general to the processing of *Melaleuca alternifolia* (tea tree)-derived ingredients, and it is unknown if they apply to cosmetic ingredient manufacturing. In some cases, the definition of the ingredients, as given in the *Dictionary*, provides insight as to the method of manufacture.[1]

Melaleuca Alternifolia (Tea Tree) Leaf Water

Melaleuca Alternifolia (Tea Tree) Leaf Water is an aqueous solution of the steam distillates obtained from the leaves of *Melaleuca alternifolia*.[1]

Tea Tree Oil

Tea tree oil is defined by ISO standard 4730:2017 as the essential oil obtained by steam of the leaves and terminal branchlets of *Melaleuca alternifolia* (Maiden et Betche) Cheel or of *Melaleuca linariifolia* Sm.;[22] steam distillation is required to conform to ISO standards.[23] Tea tree oil also can be prepared by hydrodistillation in a laboratory, usually with a Clevenger-type apparatus.[4]

More than 80% of the world's tea tree oil is produced in Australia.[14] Minor quantities come from China, South Africa and Vietnam. Tea tree oil produced in, and exported from, Australia conforms to the ISO standard (personal communication, T. Larkman, Aug 31, 2020).

A researcher extracted tea tree oil from the leaf, twig (< 0.3 cm in diameter), and branch (0.3 – 0.7 cm in diameter) of *Melaleuca alternifolia* using a Clevenger-type apparatus.[24] After 7 h, the yield of tea tree oil was 2.02% from the leaves, 0.59% from twigs, and 0.01% from branches.

Another possible method for obtaining tea tree oil is solvent extraction.[23] It was reported that solvent extraction methods, including ethanol extraction, have been found to avoid the loss of certain terpenes that occurs during steam distillation, use less leaf material, and are quicker than steam distillation. Total leaf oil content can range from 0.5 – 3%, but yield via "traditional design water distillation" is 1%.[25] A study compared recovery from tea tree leaves by ethanol extraction (3 d) and steam distillation (2 – 6 h) using both dry and fresh leaves from a low- and a high-oil concentration trees.[26] Ethanol extraction gave 48 and 77 mg of oil/g of leaf for the low- and high-oil concentration trees, respectively; with steam distillation, 42 and 63 mg of oil/g of leaf were obtained after 2 h, and 42 and 66 mg of oil/g of leaf were obtained after 6 h for the same low- and high-oil concentration trees, respectively. Absolute amounts of monoterpenoids and sesquiterpenoids extracted with ethanol were higher than those recovered from the 2-h, and most of the 6-h, steam distillations. As a percent of total oil, the oil obtained by steam distillation for 2 h had a higher percentage of total monoterpenoids. Oil yield is considered to be more affected by environmental conditions than oil composition, and has been shown to fluctuate diurnally, seasonally and in response to environmental conditions, particularly moisture levels.[23] However, in the study described above, no significant difference in the quantity or quality of oil extracted from fresh (approximately 50% dry matter) and air-dried leaves (approximately 90% dry matter) sampled from either low- or high-oil concentration trees was found.[26]

Composition/Impurities

There are several varieties, or chemotypes, of *Melaleuca alternifolia*, and each produces oil with a distinct chemical composition.[27] (Chemotypes often occur where a geographical or geological difference influences diversification of biosynthetic pathways, and may result from diverging evolutionary pathways, or from environmental cues, such as soil type or altitude.[6]) Six chemotypes have been described for *Melaleuca alternifolia*, and include a terpinen-4-ol chemotype, a terpinolene chemotype, and four 1,8-cineole chemotypes (Table 3).[23] The terpinen-4-ol chemotype is typically used in commercial tea tree oil production.

Melaleuca Alternifolia (Tea Tree) Leaf Oil

Tea tree oil typically contains approximately 100 constituents;[28] however, one publication reported that over 220 constituents have been identified in tea tree oil samples, and the concentration of these constituents present in the oil can vary widely depending on the sample.[4] Eight constituents (i.e., terpinen-4-ol, α-terpinene, γ-terpinene, 1,8-cineole, terpinolene, *p*-cymene, α-pinene, and α-terpineol) typically comprise up to 90% of the oil,[28] and the 3 constituents reported to be present in the greatest amounts are terpinen-4-ol (up to 48%), γ-terpinene, (up to 28%), and 1,8-cineole (up to 15%).[22] Another notable constituent is limonene (up to 4%). The main constituents of tea tree oil have molecular weights ranging from 134 g/mol (*p*-cymene) to 222 g/mol (globulol and viridiflorol).[8,29,30] The log P of the main constituents ranges from 2.73 (α-terpineol) to 6.64 (δ-cadinene).

Tea tree oil is reported to be composed mainly of monoterpene and sesquiterpene hydrocarbons and their associated alcohols.[27] For one sample, GC/MS analysis determined that oxygenated monoterpenes constituted 51% of the oil, monoterpene hydrocarbons constituted 47%, and the remaining 2% of the oil was composed of sesquiterpene hydrocarbons.[31] Another study reported that GC-MS analysis of ethanolic extracts of mature leaf material of *Melaleuca alternifolia* revealed the presence of 47 compounds, comprising 20 monoterpenes and 27 sesquiterpenes.[32]

According to the ISO standard for tea tree oil, high quality tea tree oil should have an enantiomeric distribution for terpinen-4-ol that is (*R*)(+) 67% - 71% and (*S*)(-) 29% - 33%.[33] The commercial standard for the composition of tea tree oil that conforms to ISO 4730:2017 is identified in Table 4.[22] WHO specifications and *European Pharmacopoeia* specifications also are provided in Table 4.[3] Many of the specifications listed in the *European Pharmacopoeia* are similar to those specified in ISO standard; two notable differences are that the *European Pharmacopoeia* allows a higher maximum of limonene (4% vs. 1.5%) and *p*-cymene (12% vs. 8%) in tea tree oil. (However, for cosmetics, according to EC Regulation No. 344/2013, the presence of limonene in a cosmetic product must be indicated in the list of ingredients when its concentration exceeds 0.001% in leave-on products and 0.01% in rinse-off products; also, the peroxide value must less than 20 mmol, with this limit applied to the substance and not to the finished cosmetic product.[34]) Also, the ISO standard allows only two species, *Melaleuca alternifolia* and *Melaleuca linariifolia*, to be used for the production of tea tree oil, while the *European Pharmacopoeia* monograph also includes *Melaleuca dissitiflora* and other species of *Melaleuca* as sources of tea tree oil.[10,16]

Constituent profiles of tea tree oil from several sources are presented in Table 5.[13,24,28,35-37] Table 6 includes the percentage of constituents, identified using GC/MS, in 97 commercial tea tree oil samples from Australia, Vietnam, and China that were analyzed between 1998 and 2013.[4]

The composition of tea tree oil varies due to environmental factors, method of manufacture, the age of the oil, and whether oxidation occurred. For example, the climate, the time of year, the leaf maceration, the biomass used (i.e., wild or cultivated trees, leaves only, or leaves and branchlets), the age of the leaves, the mode of production (e.g., commercial steam distillation or laboratory hydrodistillation), and the duration of distillation can greatly affect he natural content of the individual constituents of tea tree oil.[4,8,18,28,38] Incomplete distillation results in enhanced terpinen-4-ol levels and lower levels of sesquiterpenoids. The composition of *Melaleuca alternifolia* collected at different times during distillation is provided in Table 7. Levels of α- and γ-terpinene, terpinolene, and α-pinene are almost doubled, and the amount of terpinen-4-ol halved, with distillation for 30 - 90 min as compared to that for 0 - 30 min.

The age of the oil can also affect the composition. Using GC/MS to analyze new and aged tea tree oil, one study found the concentrations of α-terpinene were 10 - 11% in newly purchased oil, 5% in a 10-yr-old oil, and 8% in an oil that was more than 10-yr old.[39] Using LC/UV and LC/MS/MS methods, several oxidation products of α-terpinene were identified in the samples (i.e., *p*-cymene, 1,2-epoxide, diol, and (*E*)-3-isopropyl-6-oxohept-2-enal); the amounts present were not determined, and the possibility that these products originated from another compound present in tea tree oil could not be excluded. A comparison of the monoterpenoid concentrations of *Melaleuca alternifolia* present in aged oils, with various rates of deterioration, is provided in Table 8.[28]

The composition of tea tree oil changes in the presence of atmospheric oxygen, exposure to light, and at higher temperatures, and the relative rate of deterioration plays a role in the changes in concentrations of the components.[8,28] The levels of α-terpinene, γ-terpinene and terpinolene decrease with oxidation, particularly with rapid deterioration, and these substances oxidize, leading to an increased level of, *p*-cymene. Ascaridole and 1,2,4-trihydroxymenthane have been identified as oxidation products; *p*-cymene concentrations are reported to increase proportionally with 1,2,4-trihydroxymenthane.[40] However, one researcher examined 26 samples of tea tree oil and found that the presence of 1,2,4-trihydroxymenthane was rare; when 1,2,4-trihydroxymenthane was found, the oil was extremely old and degraded, and the concentration present was < 5%.[3] [8,28] The composition of tea tree oil at various stages of oxidation is presented in Table 9.[41]

Oxidation processes also lead to the formation of peroxides, endoperoxides, and epoxides.[8,28] As tea tree oil undergoes oxidation, peroxide values increase from zero to "unacceptable" levels in the early stages of oxidative degradation.[40] Once the rate of degradation of the peroxides exceeds the rate of their formation, the peroxide values return to zero in highly degraded aged oil. In a study using GC-MS, it was reported that unoxidized, partially oxidized, and oxidized tea tree oil had *p*-cymene concentrations of 2.5, 10.5, and 19.4%, respectively, and peroxide values of 1.1, 11,7, and 30.5 µeq O_2, respectively.[8]

Methyleugenol is reported as a minor constituent of Melaleuca Alternifolia (Tea Tree) Leaf Oil.[8] Analysis of 128 samples, using GC/MS methods with selected ion monitoring, reported that levels of methyleugenol ranged from 0.01 - 0.06% (mean, 0.02%) for commercial distillations.[42] Longer distillation times can result in slightly higher amounts; however, amounts did not exceed 0.07% for exhaustive laboratory distillations. In the EU, according to the opinion SCCNFP/0373/00 on methyleugenol in fragrances, the highest concentration in the finished products must not exceed 0.01% in fine fragrance, 0.004% in *eau de toilette*, 0.002% in a fragrance cream, 0.0002% in other leave-on products and in oral hygiene products, and 0.001% in rinse-off products.[29] In Norway, purity requirements for tea tree oil state that levels of methyleugenol should not exceed 200 ppm (0.02%) as a minor constituent of tea tree oil, and the content should be indicated in the ingredient list.[30]

According to one supplier, product specifications for tea tree oil stipulate heavy metal limits of ≤ 3 ppm arsenic, ≤ 1 ppm cadmium, ≤ 1 ppm mercury, and ≤ 10 ppm lead.[43] A certificate of analysis states that the presence of these heavy metals was < 1.0 ppm.[44] Heavy metal impurities are expected to be low because steam distillation does not concentrate these impurities.[45]

The recommended maximum pesticides residue limits for aldrin and dieldrin in tea tree oil, according to the WHO, are NMT 0.05 mg/kg.[13] Possible adulterants of tea tree oil include camphor, eucalyptus, cajuput, broadleaf paperbark, Masson pine, maritime pine, and Chir pine.[15] The adulterating materials may not be the essential oil of these species, but materials enriched in terpenes obtained from the waste stream after rectification of camphor, eucalyptus, and pine essential oils.

Melaleuca Alternifolia (Tea Tree) Leaf Powder

Melaleuca Alternifolia (Tea Tree) Powder is reported to contain 3% tea tree oil.[46]

USE

Cosmetic

The safety of the cosmetic ingredients addressed in this assessment is evaluated based on data received from the US FDA and the cosmetics industry on the expected use of this ingredient in cosmetics. Use frequencies of individual ingredients in cosmetics are collected from manufacturers and reported by cosmetic product category in the VCRP database. Use concentration data are submitted by the cosmetic industry in response to a survey, conducted by the Council, of maximum reported use concentrations by product category.

Collectively, the frequency and concentration of use data indicate that 7 of the 8 ingredients included in this safety assessment are used in cosmetic formulations; however, although all 7 in-use ingredients are listed in the VCRP in 2020,[47] concentration of use data collected in 2019 only reported use for 3 ingredients.[48] According to 2020 VCRP data and 2019 Council survey data, Melaleuca Alternifolia (Tea Tree) Leaf Oil has the greatest frequency and concentration of use; it is reported to be used in 724 cosmetic formulations at a maximum leave-on concentration of 0.63% in cuticle softeners (Table 10). The highest concentration reported for use in a leave-on product that result in dermal contact is 0.5% Melaleuca Alternifolia (Tea Tree) Leaf Oil in aerosol deodorants. Melaleuca Alternifolia (Tea Tree) Flower/Leaf/Stem Oil is not reported to be in use.

Melaleuca Alternifolia (Tea Tree) Leaf Oil is reported to be used in products applied near the eye (concentration of use not reported) and in products that can result in incidental ingestion (e.g., at up to 0.02% in lipstick). Several of the *Melaleuca alternifolia* (tea tree)-derived ingredients are used in formulations that come into contact with mucous membranes (e.g., 0.3% Melaleuca Alternifolia (Tea Tree) Leaf Oil in bath soaps and detergents). Additionally, Melaleuca Alternifolia (Tea Tree) Leaf Oil and Melaleuca Alternifolia (Tea Tree) Extract are reported to be used in baby products; concentration of use data were not reported for this category.

Some of the *Melaleuca alternifolia* (tea tree)-derived ingredients are used in cosmetic sprays and could possibly be inhaled; for example, Melaleuca Alternifolia (Tea Tree) Leaf Oil is reported to be used at up to 0.5% in aerosol deodorant formulations.[48] In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters > 10 µm, with propellant sprays yielding a greater fraction of droplets/particles < 10 µm compared with pump sprays.[49,50] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and thoracic regions of the respiratory tract and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.[51,52] There is some evidence indicating that deodorant spray products can release substantially larger fractions of particulates having aerodynamic equivalent diameters in the range considered to be respirable.[51] However, the information is not sufficient to determine whether significantly greater lung exposures result from the use of deodorant sprays, compared to other cosmetic sprays. According to VCRP data, Melaleuca Alternifolia (Tea Tree) Leaf Oil and Melaleuca Alternifolia (Tea Tree) Leaf Water are reported to be used in face powders. Conservative estimates of inhalation exposures to respirable particles during the use of loose powder cosmetic products are 400-fold to 1000-fold less than protective regulatory and guidance limits for inert airborne respirable particles in the workplace.[53-55]

In 2002, COLIPA stated "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.[8] When formulating Tea Tree Oil in a cosmetic product, companies should consider that the sensitisation potential increases if certain constituents of the oil become oxi-

dised. To reduce the formation of these oxidation products, manufacturers should consider the use of antioxidants and/or specific packaging to minimise exposure to light."

In Germany, the Federal Institute for Risk Assessment recommends limiting the concentration of tea tree oil in cosmetics to a maximum of 1%; cosmetic products containing tea tree oil should be protected against light and admixed with antioxidants to avoid oxidation of terpenes.[56] Norway allows Melaleuca Alternifolia (Tea Tree) Leaf Oil to be used at a maximum of 0.5% in mouth care products and 2% in all other cosmetics; it must not be used in products meant for children under 12 years of age.[29] In Australia, typical use concentrations of up to 2% are reported in leave-on (including deodorants and foot sprays) and rinse-off products (including soaps).[14] Use in mouthwash at a typical concentration of 0.2% is also indicated.

Non-Cosmetic

Tea tree oil is listed as a GRAS flavoring substance by FEMA.[57,58]

Tea tree oil is reported to have use as an herbal medicine; it has been used for centuries as a traditional medicine to treat cuts and wounds by the aboriginal people of Australia.[25,59] The EMA EU herbal monograph on *Melaleuca alternifolia* (Maiden and Betch) Cheel, *Melaleuca linariifolia* Smith, *Melaleuca dissitiflora* F. Mueller and/or other species of *Melaleuca aetheroleum* describes traditional cutaneous use (liquid or semi-solid form, up to 100%) in treatment of small superficial wounds and insect bites, small boils, and itching and irritation due to tinea pedis (athlete's foot), as well as oromucosal use (liquid form, diluted in water) for symptomatic treatment of minor inflammation of the oral mucosa;[10] the HMPC concluded that, on the basis of its long-standing use, tea tree oil preparations can be used for these uses.[3,11]

According to the WHO, clinical data supports use of tea tree oil in topical applications for symptomatic treatment of common skin disorders (such as acne, tinea pedis, bromidrosis, furunculosis, and onychomycosis), and of vaginitis due to *Trichomonas vaginalis* or *Candida albicans*, cystitis, or cervicitis.[13] Tea tree oil is reported to have antimicrobial activity. In traditional medicine, it is used as an antiseptic and disinfectant in the treatment of wounds. Additionally, tea tree oil is reported to have antibacterial, anti-viral, anti-inflammatory activity, analgesic, anti-tumoral, insecticidal, and acaricidal activities.[4,14]

The US FDA issued a final action in April 2019 (effective April 13, 2020) for tea tree oil, establishing that its use in non-prescription (OTC) consumer antiseptic products intended for use without water (i.e., antiseptic rubs or consumer rubs) is not eligible for evaluation under the OTC Drug Review for use in consumer antiseptic rubs.[60] Drug products containing these ineligible active ingredients will require approval under an NDA or ANDA prior to marketing.

Additionally, in a 2016 review, the FDA Pharmacy Compounding Advisory Committee did not recommend Melaleuca Alternifolia (Tea Tree) Leaf Oil for inclusion on the list of bulk drug substances that can be used in pharmacy compounding for topical use in the treatment of nail fungus under Section 503A of the Federal Food, Drug, and Cosmetic Act.[45] The final compounded topical formulations being considered were at strengths of 5 - 10%. The Committee considered that although products containing the oil have been commercially available since at least 1982 for use as topical formulations for a wide variety of skin, ocular, oral, and vaginal conditions, the oil may cause local reactions, and a lack of evidence of efficacy in the treatment of onychomycosis and a lack of information on the past use of tea tree oil in pharmacy compounding was cited.

Tea tree oil is reportedly active as an antioxidant.[61] Depending on the testing used, tea tree oil was reported to be a stronger antioxidant than α-lipoic acid, vitamin C, and vitamin E.

TOXICOKINETICS

Dermal Penetration/Absorption

The EMA monograph on *Melaleuca* species stated that because tea tree oil is a semi-volatile substance, the majority of an applied dose would be expected to evaporate from the skin surface before it could be absorbed into the skin.[3] In a study in which tea tree oil 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.[40]

In Vitro

The dermal penetration potential of tea tree oil was estimated in numerous in vitro studies (using both pig ear skin[62,63] and human skin[30,64-67]), and the activities of the components were generally used as markers (Table 11). Because the components are present at different concentrations in the oil, and based on chemical characteristics, these would not be expected to have equal absorption rates.[7] Specifically, the oxygenated terpenes penetrated the skin in much greater amounts than did the hydrocarbons. For example, using a finite dosing regimen for 27 h without occlusion, application of a 5% tea tree oil in an oil/water emulsion to pig ear skin mounted in a static Franz cell resulted in permeation rates (and percent permeation) of 49.1 µg/cm^2 (49.7%) for terpinen-4-ol (aka 4-terpineol); 8.90 µg/cm^2 (53.5%) for α-terpineol, and 3.85 µg/cm^2 (12.4%) for 1,8-cineole; meanwhile, permeation rates could not be measured for α- and β-pinene and α- and γ-terpinene, because very little of these components penetrated.[62] All markers were retained to some extent by the whole skin.

It was also demonstrated that the formulation vehicle affects absorption.[63] Again using pig ear skin, mounted in vertical Franz cell that were sealed to prevent evaporation, and varying amounts of tea tree oil formulated using a cream (2.5 – 10%),

an ointment (5 – 30%), and a hydrophilic gel (5%), the fastest permeation rate was with the 5% tea tree oil gel, followed by the 30% ointment. Additionally, the effect of excipients used as penetration enhancers on the penetration of pure tea tree oil was investigated.[67] Oleic acid enhanced the penetration of tea tree oil (as determined by using terpinen-4-ol as a marker); the amount permeated increased from 0.56 mg/cm^2 pure tea tree oil to 6.06 mg/cm^2 with oleic acid used as an excipient, and lag time decreased from 59 min to 12 min, respectively. Other excipients also had an effect, but to a lesser extent.

Volatility of tea tree oil upon application was also investigated. In the study using pig ear skin in which the donor chamber was not covered, substantial amounts of markers were released into the atmosphere; the highest percentage of oxygenated compounds (i.e., 1,8-cineole, 4-terpineol, α-terpineol) was released into the headspace within the first hour, with approximately 90% of 1,8-cineole and 40 - 45% of 4-terpineol and α-terpineol released.[62] For the hydrocarbons (i.e., α- and β-pinene and α- and γ-terpinene), release into the headspace was constant over the 27-h test period. The vehicle also affected the amount of each component released; for example, in a study using sealed diffusion cells, 52% of the α-terpineol was released from a 5% gel, but only 0.8% was released from a 5% ointment.[63] In a finite dosing study with human skin samples under open test conditions in horizontal Franz cells, the potential total absorption of undiluted tea tree oil (using terpinen-4-ol, 1,8-cineole, and α-terpineol as markers) was determined to be 2.0 – 4.1%; at 20% in ethanol, potential total absorption was determined to be 1.1 – 1.9%.[30] When the donor chamber was partially occluded, potential total absorption of undiluted tea tree oil was 7.1%.

As demonstrated, a difference in bioavailability of the components exists. Therefore, when using in vitro data related to topical use of tea tree oil, the bioavailability, and more specifically, the absorption profile of the individual constituents of the oil, should be considered for in vitro-to-in vivo extrapolation.[68]

Effect on Skin Integrity

Tea Tree Oil

The effect of tea tree oil on skin integrity was determined using full-thickness human breast skin or abdominal skin samples (0.5 – 1.1 mm; 3 - 4 donors) mounted in static diffusion cells.[69] The skin samples were exposed for 24 h to solutions of 0, 0.1, 1.0, or 5.0% tea tree oil (50 µl/cm^2) in an aqueous solution containing 1% Tween, 0.9% saline, and tritiated water, and to tritiated water, using infinite dosing conditions. The median diffusion area was 2.12 cm^2/cell, and donor and receptor cells were covered with wax film to avoid evaporation. Prior to the study, the epidermal site was exposed to ambient laboratory conditions and the dermis exposed to an aqueous solution of 0.9% saline and 1% Tween for 18 h. The maximal flux of tritiated water was significantly reduced with 1.0% tea tree oil, but not at the other two concentrations. At 5%, there was some evidence of damage to the barrier integrity, in that the maximal flux the water increased to was 121% of the controls; however, the increase was not statistically significant.

Comparable results were found in a similar study with concentrations of 1 and 5% tea tree oil (48-h exposure) using full-thickness human breast skin or abdominal skin samples (avg thickness, 0.87 mm) mounted in static diffusion cells.[70] Again, 1% tea tree oil (same vehicle as above) did not affect barrier conditions, but there was an increase in the K_p value for tritiated water with 5% tea tree oil. The researchers stated that this demonstrated that the barrier integrity is affected at this concentration of tea tree oil. However, although the effect on the barrier integrity was statistically significant with 5% tea tree oil in the donor phase, the mean K_p value was still considerably below the cut-off level (35 µm/h) used for assessment of barrier function in percutaneous penetration studies.

Penetration Enhancement

Tea Tree Oil

The effect of tea tree oil on permeation of ketoprofen was examined using excised porcine skin mounted in Franz diffusion cells; degassed PBS was placed in the receptor chamber.[71] The skin samples were pre-treated with 500 µl of tea tree oil or deionized water (negative control) for 1 h. After removal of the pre-treatment solution, 500 µl of ketoprofen in PEG-400 was added to the cell, and the donor chamber was occluded with wax film; the receptor phase was sampled at various intervals for 48 h. The flux of ketoprofen was ~ 7.5 times greater with tea tree oil, as compared to the negative control (38.4 vs 5.19 µg/cm^2/h, respectively), the K_p of ketoprofen increased from 2.1 x 10^{-4} cm/h with deionized water to 15.5 x 10^{-4} cm/h with tea tree oil, and the percentage of ketoprofen that was delivered across the skin in 24 h increased from 0.50% to 3.11% with tea tree oil.

Full-thickness samples from human breast or abdominal skin were used to examine the effect of up to 5% tea tree oil on the dermal absorption of methiocarb and benzoic acid (solubilities of 0.03 and 3.0 g/l, respectively).[70] Using static diffusion cells, with a median diffusion area of 2.12 cm^2/cell, 50 µl/cm^2 of the test substance was applied for 48 h using an infinite dosing regimen. Donor and receptor cells were covered with wax film to limit evaporation. Tea tree oil reduced the maximal flux, thereby reducing the overall amount of benzoic acid and methiocarb entering the receptor chamber.

Absorption, Distribution, Metabolism, and Excretion

Tea Tree Oil

In a study using rats, the pharmacokinetics of tea tree oil was examined.[9] The oral, dermal, and inhalation absorption rates were 70%, 3%, and 100%, respectively. Details were not provided.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

The acute toxicity studies summarized below are presented in Table 12.

In rabbits, following a single 24-h occlusive patch of tea tree oil that was applied to clipped intact or abraded abdominal skin, the LD_{50} was > 5 g/kg; 2 of 10 animals dosed with 5 g/kg died, and mottled livers and stomach and intestinal abnormalities were reported in 3 other animals.[72] In another study, tea tree oil had a dermal LD_{50} > 2 g/kg in rabbits.[8,9] Dermal applications of "very high concentrations" of tea tree oil have been reported to cause tea tree oil toxicosis in dogs and cats.[73,74]

In studies in which Swiss mice were given a single dose of up to 2 g/kg Melaleuca Alternifolia (Tea Tree) Leaf Oil by gavage, animals dosed with 2 g/kg had a wobbly gait, prostration, and labored breathing.[8] In male Wistar rats given a single dose of 1.2 - 5 g/kg Melaleuca Alternifolia (Tea Tree) Leaf Oil by gavage, the LD_{50} was calculated to be 1.9 g/kg bw.[72] For tea tree oil, the LD_{50} was > 2 g/kg (in PEG 400) in female mice[9] and calculated as 2.3 g/kg bw and ~1.7 g/kg bw (in peanut oil) in SPF and non-SPF Sprague-Dawley rats, respectively.[9]

In an acute inhalation study in which groups of 5 male and 5 female Wistar rats were exposed nose-only to tea tree oil for 4 h, the LC_{50} was calculated as 4.78 mg/l for males and females combined, as 5.23 mg/l for males only, and as 4.29 mg/l for females only.[9] No abnormal behavior or signs of toxicity were observed during or after dosing when groups of 10 Sprague-Dawley rats were exposed for 1 h to 50 or 100 mg/l of a test substance that contained 0.3% w/w tea tree oil and 1.8% ethanol in carbon dioxide.[8]

Short-Term Toxicity Studies

Dermal

Tea Tree Oil

Tea tree oil (2%; 50 µl) was applied to the shaved backs of 3 Wistar rats daily for 28 d.[24] (Additional details, including whether or not collars were used or if the test site was covered, were not provided.) SGOT and SGPT levels were measured on days 0, 14, and 28 using blood samples taken from the tail vein. Repeated dermal applications of tea tree oil did not result in any significant changes in SGOT or SGPT levels.

Oral

Tea Tree Oil

Groups of 5 male and 5 female Sprague-Dawley rats were dosed for 28 d with tea tree oil in corn oil by gavage at doses of 0, 5, 15, and 45 mg/kg/d, in accordance with OECD TG 407.[9] No mortality was observed, and no test-article related clinical signs of toxicity were reported. Additionally, there were not changes in functional observation battery, motor activity body weight, body weight gain, food consumption, or food efficiency during the study. There were no test-article related gross or microscopic findings reported, and absolute and relative organ weights were similar to controls. The NOAEL was determined to be 45 mg/kg/d for both male and female rats.

Subchronic and Chronic Toxicity

Subchronic and chronic toxicity studies on the *Melaleuca alternifolia* (tea tree)-derived ingredients were not found in the published literature, and unpublished data were not submitted.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY

Tea Tree Oil

Groups of 27 mated female Hannover Wistar rats were dosed by gavage with 0, 20, 100, and 250 mg/kg bw/d tea tree oil in PEG 400 on days 5 to 19 of gestation, in a developmental toxicity study performed in accordance with OECD TG 414.[9] The dams were killed on day 20 of gestation. Severe maternal toxicity was observed in dams of the 100 and 250 mg/kg bw/d groups, as evidenced by clinical signs, reduced food consumption, and weight gain reductions of 20% and 45%, respectively, over the gestation period. Seven of the high dose dams died between days 8 and 11 of gestation; there was no mortality in the other test groups. Bilateral enlarged adrenals were observed in all high-dose dams that died during the study and in 6/20 that survived until necropsy; this observation was made in one dam of the mid-dose group. A dose-related decrease in mean fetal weights, related to intrauterine growth retardation, was noted in the mid- and high-dose groups. An increase in the number of late embryonic deaths and post-implantation loss, leading to an overall higher total intrauterine mortality, was observed in the high-dose (but not mid- or low-dose) group; the increase in post-implantation mortality was considered to be secondary to maternal toxicity. There was no statistically significant difference, compared to controls, in the number of visceral malformations in the fetuses of test animals, but there were statistically significant higher numbers of visceral variations reported in the 250 mg/kg bw/d dose group. A statistically significant higher incidence of skeletal malformations unrelated to intrauterine growth retardation was noted in the 250 mg/kg bw/d group, and a statistically significant increase in the number of skeletal variations, secondary to maternal toxicity, was noted in the 100 and 250 mg/kg bw/d groups. The NOAELs for maternal toxicity and for developmental toxicity (secondary to severe maternal toxicity) were 20 mg/kg bw/d tea tree oil.

Effects on Spermatozoa

Animal

The effects of tea tree oil (containing 41.49% terpinen-4-ol, 20.55% γ-terpinene, 9.59% α-terpinene, and 4.42% α-terpineol) on the morpho-functional parameters of porcine spermatozoa were evaluated.[75] Spermatozoa samples (15 x 10^7 spermatozoa in 5 ml of medium) were exposed to 0.2 – 2 mg/ml tea tree oil for 3 h. A concentration-dependent decrease in motility was observed with concentrations of 0.4 mg/ml and greater; the decrease was statistically significant at concentrations ≥ 0.8 mg/ml. Viability of spermatozoa was statistically significant decreased with ≥ 1 mg/ml tea tree oil, and sperm acrosome reaction was statistically significantly increased at concentrations of ≥ 1.4 mg/ml. The effects of terpinen-4-ol alone were also evaluated; a greater concentration of terpinen-4-ol only (relative to the amount in tea tree oil) was needed to have an effect on the morpho-functional parameters.

GENOTOXICITY STUDIES

In vitro, tea tree oil was not mutagenic in an Ames test using *Salmonella typhimurium* and *Escherichia coli* WP2 *uvr* A, with or without metabolic activation,[9,76,77] in chromosomal assays using Chinese hamster V79 cells (≤ 58.6 µg/ml)[9] or human lymphocytes (≤ 365µg/ml),[78] in an in vitro mammalian cell micronucleus assay using human lymphocytes (≤ 365µg/ml), in a mammalian cell transformation assay (120 and 275 µg/ml, without and with metabolic activation, respectively),[9] or in a Comet assay using HaCaT cells(≤ 0.064%).[79] In vivo, Melaleuca Alternifolia (Tea Tree) Leaf Oil was not clastogenic in a mammalian erythrocyte micronucleus test in which mice were dosed orally with up to 1750 mg/kg bw in corn oil.[8] These studies are described in in detail in Table 13.

CARCINOGENICITY STUDIES

Carcinogenicity data on the *Melaleuca alternifolia* (tea tree)-derived ingredients were not found in the published literature, and unpublished data were not submitted.

ANTI-CARCINOGENICITY STUDIES

Tea tree oil exhibited antiproliferative activity against murine AE17 mesothelioma cells and B16 melanoma cells,[80] it impaired the growth of human M14 melanoma cells,[81] and it induced apoptosis in human malignant melanoma (A-375) and squamous cell carcinoma (Hep-2) cells.[82] In human MCF-7 and murine 4T1 breast cancer cells, tea tree oil exhibited an antitumor effect by decreasing cell viability and modulating apoptotic pathways.[83] Tea tree oil also inhibited glioblastoma cell growth in vitro (in human U87MG glioblastoma cells) and in vivo (in a subcutaneous model using nude CD1 mice) at a dose- and time-dependent manner, and the mechanisms were associated with cell cycle arrest, triggering DNA damage and inducing apoptosis and necrosis.[84] The concentration of tea tree oil that elicited 50% inhibition (IC_{50}) in human MDA MB breast cancer cells was 25 µg/ml (48 h).[85] The IC_{50} in several other cancer cell lines ranged from 12.5 µg/ml (24 h) in human HT29 colon cancer cells,[86] to 2800 µg/ml (4 h) in epithelioid carcinomic (HeLa), hepatocellular carcinomic (Hep G2), and human chronic myelogenous leukemia (K-562) cells.[87] In immunocompetent C57BL/6 mice, tea tree oil inhibited the growth of subcutaneous tumors; effectiveness was carrier-dependent.[88] The details of these studies are provided in Table 14.

OTHER RELEVANT STUDIES

Effect on Endocrine Activity

Tea Tree Oil

Studies evaluating the effects of tea tree oil on endocrine activity, summarized below, are described in Table 15.

The effect of tea tree oil on ERα-regulated gene expression was determined in the human MCF-7 breast cancer cell line; ERα target genes showed significant induction when treated with tea tree oil, and the ERE-dependent luciferase activity was stimulated in a dose-dependent manner (maximum activity observed at 0.025%).[89,90] Fulvestrant inhibited transactivation of the 3X-ERE-TATA-luciferase reporter, indicating that the activity observed is ER-dependent. In an E-screen assay using MCF-7 BUS cells, tea tree oil (without E2) induced a weak, but significant, dose-dependent estrogenic response at concentrations ranging from 0.00075% - 0.025%, with a maximal response (corresponding to 34% of the maximal E2 response) induced by a concentration of 0.0125% tea tree oil; when tested in the presence of E2, concentrations of < 0.025% tea tree oil reduced the RPE by 10%.[68] Terpinen-4-ol, α-terpineol, and 1,8-cineole, as well as an 8:1:1 mixture of these constituents, did not induce a significant estrogenic response at concentrations of ≤ 0.1%. A robotic version of the E-screen cell proliferation assay was performed with MCF-7:WS8 cells to evaluate the estrogenic activity (with ≤ 5 x 10^{-6} g/ml) and the anti-estrogenic activity (with ≤ 6.85 x 10^{-7} g/ml) of an ethanol extract of a hair conditioner product that contained tea tree oil.[91] The formulation did not exhibit estrogenic activity, but it did exhibit anti-estrogenic activity; the normalized anti-estrogenic activity (as relative maximum % of the positive control) was 79%. The effects of tea tree oil were also evaluated with human HepG2 hepatocellular cancer cells (ERα-negative).[89] In a luciferase reporter assay using transfected cells, tea tree oil (≤ 0.025%) produced a maximum of an ~20-fold increase in ERα ERE-mediated promotor activity. In a mammalian two-hybrid binding assay to determine binding activity to the ERα LBD, there was a significant induction of ERα ERE-mediated activity with 0.01% tea tree oil, and tea tree oil demonstrated binding to the LBD of ERα.

The effect of tea tree oil (in the presence and absence of DHT) on androgenic activity was evaluated in MDA-kb2 breast cancer cells transfected with an androgen- and glucocorticoid-inducible MMTV-luciferase reporter plasmid.[90] Tea tree oil did not transactivate the reporter plasmid at any concentration tested (≤ 0.01%), and it inhibited plasmid transactivation by DHT in a concentration-dependent manner; maximum inhibition occurred with 0.005% tea tree oil. Additional experiments in MDA-kb2 cells indicated that the anti-androgenic properties of tea tree oil extended to inhibition of DHT-stimulated expression of androgen-inducible endogenous genes. In another luciferase reporter assay with AR MMTV, increasing concentrations of tea tree oil, co-treated with testosterone, significantly inhibited MMTV-mediated activity at concentrations ≥ 0.0005% (v/v); change in activity, as compared to testosterone, was 36%.[89] The effect of tea tree oil on AR-regulated gene expression was determined in MDA-kb2 cells; tea tree oil, co-treated with testosterone, significantly inhibited the target genes.

In an opinion paper, the SCCP commented that an estrogenic potential of tea tree oil was shown in vitro, but in vivo studies were not available to elucidate the relevance of this finding.[8] The potentially endocrine-active constituents of tea tree oil have not been shown to penetrate the skin; therefore, the (hypothesized) correlation of gynecomastia due to the topical use of tea tree oil, in conjunction with lavender oil, in a 10-yr old male,[90] was considered implausible by the SCCP.

Mucosal Toxicity

Tea Tree Oil

The potential for tea tree oil (0.5 – 500 mg/ml) to induce mucosal damage was examined in porcine uterine mucosa (n = 8) using an Evans Blue permeability assay; the highest concentration of tea tree oil was used as a positive control.[92] Emulsifiers only served as the negative control. Tea tree oil induced a dose-dependent increase in the amount of dye absorbed, and the increase was statistically significant at concentrations of 40 and 500 mg/ml. No damage was observed with 0.2, 0.4, or 20 mg/ml tea tree oil; at 40 mg/ml, moderate damage was induced to the uterine mucosa, with a multifocal detachment of the epithelium.

The same researchers also performed an ex vivo study, filling the uterine horns from 8 female sows with 0.2 or 0.4 mg/ml tea tree oil, and incubating the horns for 1 h. After incubation, each uterine horn was emptied, washed with Dulbecco's PBS, and 3 cm x 3 cm section was examined. At these test concentrations, tea tree oil did not alter the structure of swine uterine mucosa.

Ototoxicity

Tea Tree Oil

The ototoxicity of tea tree oil was examined in guinea pigs by measuring the thresholds of the CAP to tone bursts before and after instillation of the oil into the middle ear.[93] After 30 min, undiluted tea tree oil (n = 5) caused a partial CAP threshold elevation at 20 kHz. With 2% tea tree oil in saline (n = 4), no significant lasting threshold change was observed after the same amount of time. Normal saline (n = 4) was used as a negative control.

Immunologic Effects

Tea Tree Oil

In Vitro

The effect of tea tree oil on neutrophil activation was investigated by measuring the TNF-α-induced adherence reaction of human peripheral neutrophils.[94] Tea tree oil was diluted to concentrations of 0.025 – 0.2% using DMSO and RPMI medium (containing 10% fetal calf serum; complete medium). The suppressing activity of tea tree oil was weak; the concentration of tea tree oil providing 50% inhibition (IC_{50}) of neutrophil adherence was 0.033%. Additionally, tea tree oil did not suppress lipopolysaccharide-induced neutrophil-induced adherence.

Animal

Dermal

Five experiments were performed in which BALB/c mice (3/group) were sensitized on shaved abdominal skin with 100 µl of 5% TNCB in acetone; after 7 d, a contact hypersensitivity response was elicited (challenge phase) by application of 50 µl of 1% TNCB in acetone to shaved dorsal skin. [95] Undiluted tea tree oil (20 µl) was applied topically to the shaved area 30 min before or 2, 4, or 7 h after challenge, and the change in double skinfold thickness was determined at various time points for up to 120 h. Controls included mice that were treated with tea tree oil alone (sensitized 7 d prior, but not challenged with TNCB) and mice that were not sensitized 7 d previously, but were challenged with TNCB.

For the first 7 h post-challenge, swelling was detected in the skin of both sensitized and non-sensitized mice. The change in double skinfold thickness in the non-sensitized mice (irritant response) subsided significantly in the following 17 h, but remained high in the sensitized mice. Undiluted tea tree oil applied 30 min before TNCB application to the non-sensitized mice did not reduce the increase in double skinfold thickness observed in the first 7 h after TNCB exposure. However, a significant reduction in swelling was observed in sensitized mice that received a single topical application of undiluted tea tree oil before or after challenge.

The researchers then investigated the effect of a single topical application (30 µl) of 5% tea tree oil ointment, 10% gel, or control gel at 7 h after challenge. The 5% tea tree oil ointment and the 10% tea tree oil gel significantly suppressed TNCB-induced swelling by 39 and 35%, respectively. The control gel had little effect, and did not cause a significant suppression when compared with the TNCB control.

The researchers also examined whether tea tree oil alleviated swelling induced by UVB irradiation. Shaved skin of BALB/c mice (3/group) was exposed to 2 kJ/m^2 (1 trial) or 8 kJ/m^2 (3 trials) UVB (corresponding to an MED of 1 or 4, respectively) using a bank of FS40 sunlamps (250 – 360 nm; wavelengths < 290 nm were screened out). Undiluted tea tree oil (20 µl) was applied topically to the shaved area at either 30 min before or up to 7 h after UVB exposure, and the change in double skinfold thickness was measured at 24, 48, and 120 h. Control mice were treated with tea tree oil, but not exposed to UVB. A single topical application of undiluted tea tree oil after irradiation did not suppress UVB-induced swelling. Furthermore, swelling was significantly increased when tea tree oil was applied before UVB irradiation (8 kJ/m^2).

The effect of the cutaneous application of tea tree oil on MPO activity was examined using groups of 3 - 4 ICR mice.[96] The mice were injected intradermally with a curdlan suspension (10 mg/ml), followed by application of 0.01 ml tea tree oil to the shaved dorsal skin (immediately, and after 3 h). The animals were killed 6 h after curdlan injection, and skin preparations were obtained. Control mice was received applications of 0.1 ml DMSO. Dermal application of tea tree oil decreased MPO activity significantly, from 100% in controls to approximately 55% in the test group.

Inhalation

In mice exposed to tea tree oil via multiple inhalation sessions, there was an increase in the level of circulating blood immunoglobulins and the blood granulocyte number, plus stimulation of the local graft-versus-host reaction of spleen cells.[97] (Details were not available.)

Male $C_{57}BI_{10}$ x CBA/H (F1) mice (number per group not provided) were exposed to tea tree oil via inhalation, 3x/d (15 min each) for 7 d; the animals were subjected to the vapors by applying 5 drops of the oil to cotton wool, and placing the wool near the cage.[97] A negative control group (no inhalation treatment) and a sham control group (water placed on cotton wool) were used. One day before the termination of dosing, subgroups of mice from each group were injected intraperitoneally with zymosan (to induce peritonitis), PBS, or left untreated. Spleens and peritoneal exudates were collected 24 h after injection. The activity of peritoneal leukocytes in the test group was equivalent to that seen in the negative and sham control groups without inflammation, indicating that tea tree oil had anti-inflammatory action. Additionally, tea tree oil stopped the proliferation of splenocytes in response to T- and B-cell mitogens. The effect of tea tree oil in inflammation was reversed by an opioid receptor antagonist (administered in drinking water). An additional inhalation study reported that the HPA axis mediated the anti-inflammatory effect of tea tree oil administered to the same strain of mice.[98]

Human

Dermal

The effect of tea tree oil on a histamine-induced wheal and flare reaction was examined.[99] Subjects were injected intradermally in each forearm with histamine (50 µl of a 100 µg/ml solution), and after 20 min, undiluted tea tree oil (25 µl) was applied topically at the injection site of one arm (test arm) of 21 subjects. In an additional 6 subjects, paraffin oil (25 µl; oil control) was applied to one arm. The arm not treated with any oil served as a negative control. The flare and wheal responses were measured every 10 min for 1 h; wheal scores were normalized as a percentage of the wheal volume at 20 min due to inter- and intraindividual variability. There was no difference in the mean flare area between the control and test arms in the tea tree oil group. However, the mean wheal volume was statistically significantly decreased as of 10 min after tea tree oil application; at 10 min after application, the mean wheal volume was 92% of that measured prior to application, as opposed to 163% at the same time on the control arm. At 20, 30, and 40 min after oil application, the wheal volume decreased to 83, 62, and 43% of that prior to oil application, respectively, on the test arm; on the control arm, the wheal volumes were 175, 130, and 113%, respectively, at the same times. Liquid paraffin had no effect on wheal or flare response. There was no significant difference in itch (subjective scoring), with or without either oil.

A similar study was conducted in 18 subjects, in which undiluted tea tree oil was applied to the injection site at both 10 and 20 min after histamine injection.[100] In this study, tea tree oil significantly reduced both the flare and the wheal response.

Cytotoxicity

Tea Tree Oil

Emulsions of tea tree oil in culture medium containing 10% fetal calf serum were cytotoxic to adherent PBMCs; toxicity ranged from 9% (not significant), with 0.004% tea tree oil, to 69% (significant), with 0.016% tea tree oil.[101] In an MTT assay evaluating the cytotoxic effects of tea tree oil on HaCaT cells following a 24-h exposure to 0.00 – 0.25% w/v, the IC_{50} was determined to be 0.066%.

IRRITATION AND SENSITIZATION

Dermal irritation and sensitization studies summarized below are described in Table 16.

Irritant effects were reported in rabbits after a single 4-h semi-occlusive application[102] and after a single 24-h occlusive application[72,103] of undiluted Melaleuca Alternifolia (Tea Tree) Leaf Oil. Tea tree oil was reported to cause irritation in animals, in a concentration-dependent manner; in rats, application of 5% tea tree oil produced very slight erythema, and 10% produced well-define erythema.[24] In rabbits, tea tree oil was a severe irritant when applied undiluted to intact and abraded skin for 72 h,[8,9] and concentrations of up to 75% were, at most, slightly irritating.[8] In 22 human subjects, a 48-h occlusive patch with 1% Melaleuca Alternifolia (Tea Tree) Leaf Oil in pet. produced no irritation.[103,104] In a clinical 3-wk occlusive patch test, slight irritation was reported with concentrations of up to 10% tea tree oil in sorbolene cream (5 patches/wk, duration not stated; 28 subjects).[18] Two dermal irritation studies were performed with 25% tea tree oil; in one study, no irritation was reported (details were not provided).[18] In the other study, which was a 3-wk occlusive patch test in 28 subjects, no irritation was reported with 25% tea tree oil in soft white paraffin; however, an allergic response (erythema with marked edema and itching) was observed in 3 subjects.[105-107] In a 48-h patch test with undiluted tea tree oil in 219 subjects, the prevalence of marked irritancy was 2.4 - 4.3%, and the prevalence of any irritancy (mild to marked) was 7.2 - 10.1%.[8,14]

In the LLNA, tea tree oil was predicted to be a weak or moderate sensitizer at a concentration up to 50%,[3,8,9] and a moderate sensitizer when tested undiluted.[8,9] In guinea pig studies, tea tree oil was not sensitizing (30% at challenge)[3,9] or had a low sensitizing capacity (tested "pure");[108] however, one study indicated that tea tree oil was possibly a weak sensitizer, with 30% tea tree oil producing positive reactions in 3/10 animals at challenge.[3,109] In guinea pig studies in which "pure" tea tree oil was used at induction and oxidized tea tree oil was used at challenge, an increase in mean response was observed when compared to challenge with "pure" oil.[108] In clinical studies, Melaleuca Alternifolia (Tea Tree) Leaf Oil at 1% in pet. (22 subjects; maximization test)[103,104] and 10% in caprylic/ capric triglycerides (102 subjects; modified HRIPT),[110] was not a sensitizer. In a Draize sensitization study with 5%, 25%, or 100% tea tree oil in various excipients, 3 of 309 subjects (0.97%) developed skin reactions suggestive of active sensitization during the induction period; only 1 of the 3 subjects returned for challenge, and the reaction was confirmed in that subject.[111] Because different samples of tea tree oil were tested simultaneously, it was not possible to determine which specific concentration was responsible for inducing sensitization in this subject at challenge; no other subjects had reactions at challenge. The three subjects (out of an initial 28 subjects) that developed reactions in the irritation study with 25% tea tree oil in soft white paraffin, described previously, had positive reactions when challenged 2 wk after the initial study; testing was also performed using components of tea tree oil, and all 3 sensitized subjects reacted positively to the sesquiterpenoid fractions and sesquiterpene hydrocarbons.[105-107]

Phototoxicity

Animal

Tea Tree Oil

A single application of undiluted tea tree oil was applied to the backs (20 µl/5 cm^2) of 12 Skh hairless mice.[103,112] Thirty min after application, the skin was treated with a combination of PUVA irradiation or broad light spectrum (UV to infrared), Xenon lamps. The test sites were examined at 4, 24, 48, 72, and 96 h, and tea tree oil was not phototoxic in hairless mice; however, some irritation was observed. (Additional details were not provided.)

Cross Allergenicity

Melaleuca alternifolia is contraindicated in cases of known allergy to plants of the *Myrtaceae* family.[13] Tea tree oil can cross react with colophony.[29]

OCULAR IRRITATION

In Vitro

Tea Tree Oil

In a HET-CAM, undiluted tea tree oil and water-soluble tea tree oil had mean irritation indices of 16.1 and 14.7, respectively, and both were classified as a severe irritant.[8] In a surfactant, the control (10% surfactant, 0% tea tree oil), 10% tea tree oil in 10% surfactant, and 25% tea tree oil in 5% surfactant were classified as severe irritants, with mean irritation indices of 10.3, 12.1, and 9.8, respectively. However, 5% tea tree oil in 8% surfactant was classified as a slight irritant, with a mean irritation index of 4.5.

A BCOP test was performed in accordance with OECD TG 437 to evaluate the irritation potential of undiluted tea tree oil.[9] Tea tree oil had an in vitro irritancy score of 2.2, and was considered not to be an ocular corrosive or severe irritant. (The negative and positive controls had in vitro irritancy scores of 2.3 and 44.5, respectively.)

Tea Tree Powder

Tea tree powder and tea tree ground leaf were classified as non-irritants in the HET-CAM assay.[8] Both test substances had a mean irritation index of 0.0.

Animal

Tea Tree Oil

One-tenth ml of 1% or 5% tea tree oil in liquid paraffin was instilled into the conjunctival sac of Japanese white rabbits (3/group).[8] Conjunctival discharge was observed for up to 6 h following instillation of 1% tea tree oil, and conjunctival redness and discharge were observed for up to 24 h following instillation of 5% tea tree oil. Both test concentrations were classified as minimally irritating to rabbit eyes.

Undiluted tea tree oil (0.1 ml) was instilled into the conjunctival sac of the right eye of two NZW rabbits.[9] The eyes, which were not rinsed, were examined at 1, 24, 48, and 72 h after instillation. The contralateral eye served as the untreated control. In both animals, conjunctival irritation was moderate at 1 h, minimal at 24 and 48 h, and resolved at 72 h. Tea tree oil produced a maximum group mean score of 9.0, and was classified as a mild ocular irritant.

CLINICAL STUDIES

Retrospective and Multicenter Studies

Oxidized tea tree oil (5% in pet.) has been part of the NACDG screening series since 2003.[113] Tea tree oil (5% pet, oxidized) was added to the British Society for Cutaneous Allergy facial allergy series in 2019; allergens that had a positive patch test rate > 0.3% were included.[114] Retrospective and multicenter studies are summarized below and described in Table 17.

From 2000 to 2007, the Mayo Clinic tested 869 patients with 5% tea tree oil (oxidized); a positive response was found in 18 patients (2.1%).[115] In screening by the NACDG, when tested at 5% (oxidized, in pet.) in dermatology patients over 2-yr time frames, frequencies of positive reactions ranged from 0.9% (2003 - 2004; 2011 - 2012) to 1.4% (2005 - 2006; 2007 - 2008).[113,116-120] The NACDG measured the positivity ratio (percentage of weak reactions among the sum of all positive reactions) and reaction index (number of positive reactions minus questionable and irritant reactions/sum of all 3) for test results obtained between 2003 - 2006; testing with oxidized tea tree oil had a positivity ratio of 54.5% and a reaction index of 0.73, indicating that 5% tea tree oil (oxidized, in pet.) was an "acceptable" patch test preparation.[121] The NACDG also examined the frequency of positive patch test reactions with oxidized tea tree oil as compared to fragrance markers; in 2003, only 1 of the 5/1603 patients that reacted to oxidized tea tree oil also reacted to the fragrance makers fragrance mix and *Myroxilon pereirae*.[122] During the 2009 - 2014 time frame, 63 of the 123/13,398 patients that reacted to oxidized tea tree oil did not react to any of the fragrance mixes that were tested.[123] Testing at the Northwestern Medicine patch-testing clinic found no difference in positive results between patients with or without atopic dermatitis.[124]

Cross-sectional studies were performed by the NACDG. In a subgroup of 835 patients with moisturizer-associated positive reactions (from a parent group of 2193 patients; 2001 - 2004), 1.2% had positive reactions to oxidized tea tree oil.[125] In subgroups of patients (2003 - 2004) with hand-only reaction, the percent of positive reactions to oxidized tea tree oil was slightly greater in patients with a final diagnosis code of allergic contact dermatitis only (0.4%), as opposed to those whose diagnosis included allergic contact dermatitis (0.2%).[126] Three of 60 patients (5%) with lip ACC (2001 - 2004) had positive reactions to oxidized tea tree oil.[127] Cross-sectional NACDG studies also evaluated the sensitization rates in pediatric and older patients. In 2003 - 2007, 0.4% of pediatric patients (4/1007) that were ≤ 18 yr old had positive reactions to oxidized tea tree oil; during the same time frame, 0.3% of adults (35/11,649) aged 19 – 64 yr old and 0.3% of older patients (8/2409) aged ≥ 65 yr old reacted positively.[128] It was reported that from 2001 - 2004, 14.3% of children aged 0 – 5 yr, and 1.1% of children aged 0 - 18 yr, had a positive reaction to oxidized tea tree oil (total number of patients tested not stated).[129] However, from 2005 - 2012, no pediatric patients (0/40) aged 0 - 5 yr, and 0.3% of patients (n = 876) aged 0 – 18 yr, reacted to the oxidized oil.[130]

Testing was also performed in Europe. In Denmark, 44/217 subjects (September 2001 - January 2002) had weak irritant reactions to a commercial lotion that contained 5% tea tree oil, and 1 subject had a ++ reaction to the lotion and 10% tea tree oil in pet.;[131] in June – August 2003, 5/160 subjects had irritant reactions to lotions containing 5% tea tree oil.[131] In Sweden (prior to 2004), 2.7% of 1075 patients tested had a positive reaction to 5% tea tree oil in alcohol.[132] In Germany, testing with 5% tea tree oil (standardized) in diethyl phthalate produced positive results in 1.1% of the 3375 patients tested (1999 - 2000),[4,8,133] and testing at 5% (oxidized) in pet. (1998 - 2003) produced positive results in 0.9%-1.0% of the patients tested.[134] Testing performed in the Netherlands (2012 - 2013) reported positive results in 0.9% (2/221) of patients patch-tested with 5% tea tree oil (oxidized) in pet.[135] However, when this group and an additional 29 patients from a different study were patch-tested with the 5% oxidized tea tree oil and up to 5% ascaridole (a possible contaminant in aged tea tree oil), 6 of 30 patients that had positive reactions to any concentration of ascaridole also tested positive with tea tree oil; in the 220 patients that did not react to any concentration of ascaridole, none reacted to tea tree oil. In Belgium, 11 of 105 patients (10.5%) had positive reactions to 1 and 5% oxidized tea tree oil in pet.; these patients were a sub-group of 15,980 patients that were tested (1990 - 2016) and identified as being allergic to herbal medicines and/or botanical ingredients.[136] Additional studies performed in Belgium (2000 - 2010) with fragrance and non-fragrance allergens reported positive reactions in skin care products containing tea tree oil, but not in the other cosmetic product categories.[137,138] In testing in Italy with 19 patients that had positive reactions to a botanical integrative series, 2 reacted to 5% tea tree oil in pet.[139] In a Swiss clinic (1997), positive reactions were reported in 0.6% of 1216 patients tested with 5 – 100% tea tree oil in arachis oil,[8,140] and in Spain (prior to

2015), 0.4% of patients had positive reactions to testing with 5% tea tree oil in pet.[141] In the UK (1996 - 1997), 7 of 29 patients thought to have a cosmetic dermatitis had positive patch test reactions to tea tree oil, applied neat,[142] and in 2001, 2.4% of 550 patients tested with neat, oxidized tea tree oil had positive reactions.[4] Between 2008 and 2016, positive reactions from testing with 5% tea tree oil in pet. ranged from 0.1 – 0.29% in the UK,[143,144] and in 2016 - 2017, 0.45% of 4224 patients in the UK and Ireland that were patch-tested with 5% tea tree oil (oxidized) in pet. had positive reactions.[114]

In Australia, positive reaction rates generally appear to be higher than those reported in the US or Europe. The Skin and Cancer Foundation reported a positive reaction rate of 1.8% (41/2320 patients) with 5 and 10% tea tree oil (oxidized);[145] however, the same group reported that from 2001 - 2010, the positive reaction rates with 5% (oxidized) and 10% tea tree oil were 3.5% (794 subjects) and 2.5% (5087 subjects), respectively.[146] Additionally, positive reaction rates of up to 4.8% have been reported with 10% tea tree oil.[145]

Provocative Testing

Tea Tree Oil

Eight subjects confirmed to previously be sensitized to tea tree oil were tested using occlusive patches to determine their allergic reaction threshold.[3,14] Reaction threshold concentrations varied among the subjects, from 0.5% in one subject to a doubtful reaction at 10% in another subject. For the remaining subjects, a 1-3 response was produced in one subject with 1%, in 3 subjects with 2%, and in 2 subjects with 5% tea tree oil. Eleven individual components of tea tree oil were also tested; *p*-cymene, terpinolene, α-terpinene, and γ-terpinene produced reactions in the sensitized subjects. The study authors commented that they were concerned that the oil samples may have become oxidized during the study.

Forty-three patients with the primary complaint of vulval pruritus were patch-tested with a battery of allergens, including tea tree oil (undiluted) and common OTC topical vulval treatments.[147] Of 21 patients that reported using 4 or more topical treatments, 5 of these patients had a positive reaction to tea tree oil. However, tea tree oil was not considered clinically relevant because it was not reported by the patients as being used directly on the vulva to alleviate pruritus.

Cross-Reactivity

Studies noting cross-reactivity with tea tree oil, summarized below, are described in Table 18.

Cross-reactivity with tea tree oil was indicated in some retrospective and multi-center studies. With testing of up to 100% tea tree oil in arachis oil, 2 of the 7 patients that had positive reactions to tea tree oil also exhibited a type IV hypersensitivity towards fragrance mix or colophony; the researchers stated study there was a possibility of an allergic group reaction caused by contamination of the colophony with the volatile fractions of turpentine.[8,140] In one study in which 36/3375 patients reacted to 5% tea tree oil in diethyl phthalate, 14 of those 36 also had positive patch test reactions to turpentine.[133] However, in another study, no correlation was reported between positive reactions to tea tree oil and to colophony.[132] In 45 patients that had positive patch tests to compound tincture of benzoin, 9 of the 45 also had positive reactions to tea tree oil.[148] In several case reports of reactions to tea tree oil (described later in this report), reactions were also noted with eucalyptol,[38] colophony,[149,150] and ascaridole.[151]

Case Reports

Tea Tree Oil

Numerous case reports of reaction to tea tree oil are available in the published literature; in 2005, tea tree oil was the most common botanical reported to cause allergic contact dermatitis.[4] A sampling of dermal case reports describing reactions from use of treatment of dermatitis and/or psoriasis,[38,108,109,141,151-153] other direct skin applications,[108,149-151,154-161] and from use of hand wash or shampoos[108,162,163] is presented in Table 19. Patients with sensitivity to tea tree oil (dermal and/or oral) were also reported to have reactions to constituents or degradation products of tea tree oil.[164] Positive reactions were also reported in a patient with hand eczema following inhalation of tea tree oil vapors.[165]

Oral ingestion can be poisonous; serious symptoms, such as confusion and ataxia, can occur.[59] In 2011, the National Capital Poison Center received nearly twice as many calls about tea tree oil than any other named essential oil, including cinnamon oil, clove oil, and eucalyptus oil.[166] In Australia, a retrospective study of essential oil exposure was conducted by analyzing calls to the NSWPIC during July 2014 – June 2018; NSWPIC takes about half of all calls to poisons information centers in Australia.[167] Tea tree oil was involved in 17% of the reported poisonings.

RISK ASSESSMENT

In a 2008 opinion on tea tree oil, the SCCP concluded that an MOS had not been calculated, and the safety of tea tree oil could not be assessed.[8] The following factors led to this conclusion: tea tree oil is a sensitizer, and sensitization may be enhanced by irritancy; neat tea tree oil and some formulations of 5% or more can induce skin and eye irritation; tea tree oil is prone to oxidation when exposed to air and heat, yielding epoxides and further oxidation products which are considered to contribute to the skin sensitizing potential; and, percutaneous absorption of some constituents of tea tee oil may occur following topical application of the oil and oil-containing products leading to a considerable systemic exposure, but the magnitude of systemic exposure to tea tree oil was uncertain due to a lack of adequate dermal absorption studies.

Daily exposure of tea tree oil was calculated for the various product types, using a rate of percutaneous absorption of 3%, and was adjusted for the skin retention factor according to SCCP Notes of Guidance (version not specified).[8] 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. SED estimates between 0.0017 mg/kg/d (2% tea tree oil in a hand soap) and 3.33 mg/kg/d (undiluted tea tree oil) were obtained. The SEDs that were calculated for various formulations containing tea tree oil are presented in Table 20.

Another source reported SEDs for several product types using an assumption of 100% dermal absorption.[29] MOS were then calculated; an NOAEL of 117 mg/kg bw/d (for renal effects, derived based on repeated dose systemic toxicity of tea tree oil constituents) was chosen for illustrative purposes. Assuming complete absorption as % of applied dose, SED values for different product types ranged from 0.030 mg/kg bw/d (2.0% tea tree oil in a shampoo) to 1.54 mg/kg/d (1.25% tea tree oil in a body lotion), and MOS values ranged from 76 (body lotion) to 3900 (shampoo). Based on an aggregate exposure (shampoo + deodorant stick + foot powder + body lotion + hand wash soap + neat tea tree oil (nails)), the SED was calculated as 2.22 mg/kg bw/d, and the overall MOS was 53. The SED and MOS values for several types of cosmetic formulations are presented in Table 21.

SUMMARY

Five of the 8 *Melaleuca alternifolia* (tea tree)-derived ingredient included in this assessment are reported to function in cosmetics as skin-conditioning agents. Other reported cosmetic functions include abrasive, antioxidant, fragrance ingredient, flavoring ingredient, antifungal agent, and antimicrobial agent.

Often, in the published literature, the general name "tea tree" is used, especially, tea tree oil; however, it is not known whether the substance being discussed is equivalent to the cosmetic ingredient. Some constituents of *Melaleuca alternifolia* have the potential to cause adverse effects. For example, 1,8-cineole (also known as eucalyptol) can be an allergen, and terpinolene, α-terpinene, α-phellandrene, and limonene, ascaridole (a product of tea tree oil oxidation), and 1,2,4-trihydroxymenthane (a product that might be found in aged tea tree oil) are sensitizers. However, the Panel evaluates each ingredient as a whole, complex substance, and not the safety of the individual components.

Melaleuca Alternifolia (Tea Tree) Leaf Water is an aqueous solution of the steam distillates obtained from the leaves of *Melaleuca alternifolia*. Tea tree oil is the essential oil obtained by steam distillation of the leaves and terminal branchlets of *Melaleuca alternifolia* (or of *Melaleuca linariifolia*); it also can be prepared by hydrodistillation, or by solvent extraction.

Six chemotypes have been described for *Melaleuca alternifolia*; the terpinen-4-ol chemotype is typically used in commercial tea tree oil production. Tea tree oil is reported to contain approximately 100 constituents, with 8 constituents (i.e., terpinen-4-ol, α-terpinene, γ-terpinene, 1,8-cineole, terpinolene, *p*-cymene, α-pinene, and α-terpineol) typically comprising up to 90% of the oil. Commercial standards for tea tree oil that conform to an ISO specification are indicated. The natural content of the individual constituents of tea tree oil varies considerably depending on the climate, the time of year, the leaf maceration, the biomass used, the age of the leaves, the mode of production, and the duration of distillation. The composition can change as the oil ages, especially when exposed to air, light, and/or high temperatures. Methyleugenol is reported as a minor constituent of Melaleuca Alternifolia (Tea Tree) Leaf Oil.

According to 2020 US FDA VCRP data and Council survey results, 7 of the 8 ingredients included in this safety assessment are currently used in cosmetic formulations. Melaleuca Alternifolia (Tea Tree) Leaf Oil has the greatest frequency and concentration of use; it is reported to be used in 724 cosmetic formulations at a maximum leave-on concentration of 0.63% in cuticle softeners. The highest concentration reported for use in a leave-on product that result in dermal contact is 0.5% Melaleuca Alternifolia (Tea Tree) Leaf Oil, in aerosol deodorants. Collectively, the *Melaleuca alternifolia* (tea tree)-derived ingredients are reported to be used in products applied near the eye, in products that can result in incidental ingestion, in formulations that come into contact with mucous membranes, and in baby products. Additionally, some of these ingredients are used in spray and powder formulations.

Tea tree oil is listed as a GRAS flavoring substance by FEMA. It is reported to have antimicrobial and antioxidant activity, and has been used as a traditional herbal medicine for centuries. The EMA HMPC concluded that, on the basis of its long-standing use, tea tree oil preparations are approved for a variety of traditional uses. However, the US FDA issued a final action for tea tree oil, establishing that its use in non-prescription OTC consumer antiseptic products intended for use without water is not eligible for evaluation under the OTC Drug Review for use in consumer antiseptic rubs. Additionally, the FDA Pharmacy Compounding Advisory Committee did not recommend Melaleuca Alternifolia (Tea Tree) Leaf Oil for inclusion on the list of bulk drug substances that can be used in pharmacy compounding for topical use in the treatment of nail fungus.

In rats, the oral, dermal, and inhalation absorption rates of tea tree oil were 70, 3, and 100%, respectively. Because tea tree oil is a semi-volatile substance, the majority of an applied dose would be expected to evaporate from the skin surface before it could be absorbed into the skin. In in vitro studies that used the individual components as markers for penetration, it was demonstrated that the components have distinctly different absorption rates. Additionally, formulation vehicle affects absorption, as does excipients that are used as penetration enhancers.

Tea tree oil increased the percentage of ketoprofen that was delivered across excised porcine skin. However, using human skin samples, it reduced the overall amount of benzoic acid and methiocarb entering the receptor chamber of a static diffusion cell.

In an acute dermal toxicity tests in rabbits, the LD_{50} of tea tree oil was > 5 g/kg. Dermal applications of "very high concentrations" of tea tree oil have been reported to cause tea tree oil toxicosis in dogs and cats. In an acute oral study, Swiss mice that were given a single dose of 2 g/kg Melaleuca Alternifolia (Tea Tree) Leaf Oil by gavage exhibited a wobbly gait, prostration, and labored breathing. In male Wistar rats dosed once with ≤ 5 g/kg Melaleuca Alternifolia (Tea Tree) Leaf Oil by gavage, the LD_{50} was calculated to be 1.9 g/kg bw. For tea tree oil, the LD_{50} was > 2 g/kg (in PEG 400) in female mice, and calculated as 22.3 g/kg bw and ~1.7 g/kg bw (in peanut oil) in SPF and non-SPF Sprague-Dawley rats, respectively.

In an acute inhalation study in which groups of 5 male and 5 female Wistar rats were exposed nose-only to tea tree oil for 4 h, the LC_{50} was calculated as 4.78 mg/l for males and females combined, as 5.23 mg/l for males only, and as 4.29 mg/l for females only. No abnormal behavior or signs of toxicity were observed during or after dosing when groups of 10 Sprague-Dawley rats were exposed for 1 h to 50 or 100 mg/l of a test substance that contained 0.3% w/w tea tree oil and 1.8% ethanol in carbon dioxide.

Repeated dermal applications of 2% tea tree oil to the shaved back of rats for 28 d did not result in any significant changes in SGOT or SGPT levels. In a 28-d gavage study (OECD TG 407) with doses of up to 45 mg/kg/d tea tree oil in corn oil, the NOAEL was determined to be 45 mg/kg/d for both male and female rats.

A developmental toxicity study was performed in accordance with OECD TG 414, in which gravid female rats were dosed by gavage with up to 250 mg/kg bw/d tea tree oil in PEG 400 on days 5 to 19 of gestation. The NOAELs for maternal toxicity and for developmental toxicity (secondary to severe maternal toxicity) were 20 mg/kg bw/d tea tree oil. An increase in the number of late embryonic deaths and post-implantation loss, leading to an overall higher total intrauterine mortality, was observed in the high-dose group; the increase in post-implantation mortality was considered to be secondary to maternal toxicity. A statistically significant higher incidence of skeletal malformations unrelated to intrauterine growth retardation was noted in the high-dose group, and a statistically significant increase in the number of skeletal variations secondary to maternal toxicity was noted in the 100 and 250 mg/kg bw/d groups.

The effects of tea tree oil on the morpho-functional parameters of porcine spermatozoa were evaluated.by exposing spermatozoa samples to ≤ 2 mg/ml tea tree oil for 3 h. Viability of spermatozoa was statistically significant decreased with ≥ 1 mg/ml tea tree oil, and a concentration-dependent decrease in motility was observed with concentrations of 0.4 ml and greater.

Tea tree oil did not demonstrate genotoxic activity. In vitro, tea tree oil was not mutagenic in an Ames test using *S. typhimurium* and *E. coli* WP2 *uvr* A, with or without metabolic activation, in chromosomal assays using Chinese hamster V79 cells (≤ 58.6 µg/ml) or human lymphocytes (≤ 365µg/ml), in an in vitro mammalian cell micronucleus assay using human lymphocytes (≤ 365µg/ml), in a mammalian cell transformation assay (120 and 275 µg/ml, without and with metabolic activation, respectively), or in a Comet assay using HaCaT cells (≤ 0.064%). In vivo, Melaleuca Alternifolia (Tea Tree) Leaf Oil was not clastogenic in a mammalian erythrocyte micronucleus test in which mice were dosed orally with up to 1750 mg/kg bw in corn oil.

Carcinogenicity studies were not identified in the published literature. However, numerous studies investigating ant-carcinogenic potential of tea tree oil were found. Tea tree oil exhibited antiproliferative activity against murine AE17 mesothelioma cells and B16 melanoma cells, it impaired the growth of human M14 melanoma cells, and it induced apoptosis in human malignant melanoma (A-375) and squamous cell carcinoma (Hep-2) cells. In human MCF-7 and murine 4T1 breast cancer cells, tea tree oil exhibited an anti-tumor effect by decreasing cell viability and modulating apoptotic pathways. Tea tree oil also inhibited glioblastoma cell growth in vitro (in human U87MG glioblastoma cells) and in vivo (in a subcutaneous model using nude CD1 mice) in a dose- and time-dependent manner, and the mechanisms were associated with cell cycle arrest, triggering DNA damage and inducing apoptosis and necrosis. The IC_{50} of tea tree oil in human MDA MB breast cancer cells was 25 µg/ml (48 h). The IC_{50} in several other cancer cell lines ranged from 12.5 µg/ml (24 h) in human HT29 colon cancer cells, to 2800 µg/ml (4 h) in epithelioid carcinomic (HeLa), hepatocellular carcinomic (Hep G2), and human chronic myelogenous leukemia (K-562) cells. In immunocompetent C57BL/6 mice, tea tree oil inhibited the growth of subcutaneous tumors; effectiveness was carrier-dependent.

Human MCF-7 breast cancer cells were used to examine the effect of tea tree oil on ERα-regulated gene expression; ERα target genes showed significant induction when treated with tea tree oil, and the ERE-dependent luciferase activity was stimulated in a dose-dependent manner (maximum activity observed at 0.025%). Fulvestrant inhibited transactivation of the 3X-ERE-TATA-luciferase reporter, indicating that the activity observed is ER-dependent. In an E-screen assay using MCF-7 BUS cells, tea tree oil (≤ 0.1%; without E2) induced a weak, but significant, dose-dependent estrogenic response at concentrations ranging from 0.00075% - 0.025%, with a maximal response (corresponding to 34% of the maximal E2 response) induced by a concentration of 0.0125% tea tree oil; when tested in the presence of E2, concentrations of < 0.025% tea tree oil reduced the RPE effect by 10%. A robotic version of the E-screen cell proliferation assay was performed with MCF-7:WS8 cells to evaluate the estrogenic activity (with ≤ 5×10^{-6} g/ml) and the anti-estrogenic activity (with ≤ 6.85×10^{-7}

g/ml) of an ethanol extract of a hair conditioner product that contained tea tree oil. The formulation did not exhibit estrogenic activity, but it did exhibit anti-estrogenic activity; the normalized anti-estrogenic activity (as relative maximum % of the positive control) was 79%. Human HepG2 hepatocellular cancer cells were also used to examine estrogenic effects. In a luciferase reporter assay using transfected cells, tea tree oil (≤ 0.025%) produced a maximum of an ~20-fold increase in ERα ERE-mediated promotor activity, and in a mammalian two-hybrid binding assay to determine binding activity to the ERα LBD, there was a significant induction of ERα ERE-mediated activity with 0.01% tea tree oil, and tea tree oil demonstrated binding to the LBD of ERα.

The androgenic activity of tea tree oil was evaluated in MDA-kb2 breast cancer cells (in the presence and absence of DHT). In cells transfected with an MMTV-luciferase reporter plasmid, tea tree oil did not transactivate the reporter plasmid at any concentration tested (≤ 0.01%), and it inhibited plasmid transactivation by DHT in a concentration-dependent manner; maximum inhibition occurred with 0.005% tea tree oil. Additional experiments indicated that the anti-androgenic properties of tea tree oil extended to inhibition of DHT-stimulated expression of androgen-inducible endogenous genes. In another luciferase reporter assay AR MMTV, increasing concentrations of tea tree oil, co-treated with testosterone, significantly inhibited MMTV-mediated activity at concentrations ≥ 0.0005% (v/v); change in activity, as compared to testosterone, was 36%. In a study examining the effect of tea tree oil on AR-regulated gene expression, tea tree oil, co-treated with testosterone, significantly inhibited the target genes.

The potential for tea tree oil to induce mucosal damage was examined in porcine uterine mucosa; no damage was observed with up to 20 mg/ml tea tree oil, but at 40 mg/ml, moderate damage was induced to the uterine mucosa, with a multifocal detachment of the epithelium. In an ex vivo study using uterine horns from female sows, tea tree oil (≤ 0.4 mg/ml) did not alter the structure of the uterine mucosa.

Immunological effects of tea tree oil were examined in vitro, in mice (via dermal route and inhalation), and in humans (dermal application). In vitro, tea tree oil had a weak effect on suppression of neutrophil activation; the IC_{50} of neutrophil adherence was 0.033%.

In dermal studies using mice, undiluted tea tree oil (applied before or after challenge) reduced swelling induced by TNCB in sensitized, but not in non-sensitized, mice. In examining whether the oil had an effect on swelling associated with UVB irradiation, a single topical application of undiluted tea tree oil after irradiation did not suppress swelling in mice; additionally, swelling was significantly increased when tea tree oil was applied before UVB irradiation. Cutaneous application of tea tree oil to mice decreased MPO activity, from 100% in controls to approximately 55% in the treated group. In mice exposed to tea tree oil via inhalation, there was an increase in the level of circulating blood immunoglobulins and the blood granulocyte number. Additionally, in mice exposed to tea tree oil vapors, and then induced with peritonitis, peritoneal leukocyte activity in the test group was equivalent to that seen in control groups without inflammation, indicating that tea tree oil had anti-inflammatory action.

In one study using human subjects, undiluted tea tree oil did not have an effect on the mean flare area induced by histamine when the oil was applied 20 min after histamine injection; however, the mean wheal volume was statistically significantly decreased. In another study, in which undiluted tea tree oil was applied to the injection site at both 10 and 20 min after histamine injection, a significant reduction in both the flare and the wheal response was observed.

Emulsions of tea tree oil in in culture medium containing 10% fetal calf serum were cytotoxic to adherent PBMCs. Significant toxicity was reported at a concentration of 0.016%.

Irritant effects were reported in rabbits after a single 4-h semi-occlusive application and after a single 24-h occlusive application of undiluted Melaleuca Alternifolia (Tea Tree) Leaf Oil. Tea tree oil was reported to cause irritation in animals, in a concentration-dependent manner; in rats, application of 5% tea tree oil produced very slight erythema, and 10% produced well-define erythema. In rabbits, tea tree oil was a severe irritant when applied undiluted to intact and abraded skin for 72 h, and concentrations of up to 75% were, at most, slightly irritating. In 22 human subjects, a 48-h occlusive patch with 1% Melaleuca Alternifolia (Tea Tree) Leaf Oil in pet. produced no irritation. In a clinical 3-wk occlusive patch test, slight irritation was reported with concentrations of up to 10% tea tree oil in sorbolene cream (5 patches/wk, duration not stated; 28 subjects). Two dermal irritation studies were performed with 25% tea tree oil; in one study, no irritation was reported. In the other study, which was a 3-wk occlusive patch test in 28 subjects, no irritation was reported with 25% tea tree oil in soft white paraffin; however, an allergic response (erythema with marked edema and itching) was observed in 3 subjects. In a 48-h patch test with undiluted tea tree oil in 219 subjects, the prevalence of marked irritancy was 2.4 - 4.3%, and the prevalence of any irritancy (mild to marked) was 7.2 - 10.1%.

In the LLNA, tea tree oil was predicted to be a weak or moderate sensitizer at a concentration up to 50%, and a moderate sensitizer when tested undiluted. In guinea pig studies, tea tree oil was not sensitizing (30% at challenge) or had a low sensitizing capacity (tested “pure”); however, one study indicated that tea tree oil was possibly a weak sensitizer, with 30% tea tree oil producing positive reactions in 3/10 animals at challenge. In guinea pig studies in which “pure” tea tree oil was used at induction and oxidized tea tree oil was used at challenge, an increase in mean response was observed when compared to challenge with “pure” oil. In clinical studies, Melaleuca Alternifolia (Tea Tree) Leaf Oil at 1% in pet. (22 subjects; maximization test) and 10% in caprylic/capric triglycerides (102 subjects; modified HRIPT), was not a sensitizer.

In a Draize sensitization study with 5, 25, or 100% tea tree oil in various excipients, 3 of 309 subjects (0.97%) developed skin reactions suggestive of active sensitization during the induction period; only 1 of the 3 subjects returned for challenge, and the reaction was confirmed in that subject. Because different samples of tea tree oil were tested simultaneously, it was not possible to determine which specific concentration was responsible for inducing sensitization in this subject at challenge; no other subjects had reactions at challenge. Three of an initial 28 subjects that developed reactions in the irritation study with 25% tea tree oil in soft white paraffin, had positive reactions when challenged 2 wk after the initial study; testing was also performed using components of tea tree oil, and all 3 sensitized subjects reacted positively to the sesquiterpenoid fractions and sesquiterpene hydrocarbons. *Melaleuca alternifolia* is contraindicated in cases of known allergy to plants of the *Myrtaceae* family. Tea tree oil can cross react with colophony.

A single application of undiluted tea tree oil was not phototoxic in hairless mice. However, some irritation was observed.

Tea tree powder and tea tree ground leaf were classified as non-irritants in the HET-CAM assay. Undiluted tea tree oil and water-soluble tea tree oil were both classified as a severe irritant in the HET-CAM assay; however, tea tree oil was classified as not to be an ocular corrosive or severe irritant in a BCOP test. Additionally, using rabbits, tea tree oil was classified as minimally irritating to rabbit eyes when tested at a concentration of up to 5%, and undiluted tea tree oil was considered a mild ocular irritant.

Oxidized tea tree oil (5% in pet.) has been part of the NACDG screening series since 2003, and it was added to the British Society for Cutaneous Allergy facial allergy series in 2019. From 2000 to 2007, the Mayo Clinic tested 869 patients with 5% tea tree oil (oxidized0; the positive response rate was 2.1%. In screening by the NACDG, when tested at 5% (oxidized) in pet. in dermatology patients over 2-yr time frames, frequencies of positive reactions ranged from 0.9% to 1.4%. The NACDG also examined the frequency of positive patch test reactions with tea tree oil as compared to fragrance markers; in 2003, only 1 of the 5/1603 patients that reacted to oxidized tea tree oil also reacted to the fragrance makers fragrance mix and *Myroxilon pereirae*. During the 2009 - 2014 timeframe, 63 of the 123/13,398 patients (51%) that reacted to oxidized tea tree oil did not react to any of the fragrance mixes that were tested. Testing at the Northwestern Medicine patch-testing clinic found no difference in positive results between patients with or without atopic dermatitis.

Cross-sectional studies were also performed by the NACDG examining the effects of oxidized tea tree oil, based on symptoms or age. In patients with moisturizer-associated positive reactions (2001 - 2004), 1.2% had positive reactions to oxidized tea tree oil. In subgroups of patients (2003 - 2004) with hand-only reactions, the percent of positive reactions to oxidized tea tree oil was slightly greater in patients with a final diagnosis code of allergic contact dermatitis only (0.4%), as opposed to those whose diagnosis included allergic contact dermatitis (0.2%) among the diagnoses. In 60 patients with lip ACC (2001 - 2004), 3 (5%) had positive reactions to oxidized tea tree oil. In 2003 - 2007, 0.4% of pediatric patients that were ≤ 18 yr had positive reactions to oxidized tea tree oil; during the same time frame, 0.3% of adults aged 19 – 64 yr and 0.3% of older patients aged ≥ 65 yr reacted positively. It was reported that from 2001 - 2004, 14.3% of children aged 0 – 5 yr, and 1.1% of children aged 0 – 18 yr, had a positive reaction to oxidized tea tree oil; however, from 2005 - 2012, no pediatric patients (0/40) aged 0 – 5 yr, and 0.3% of patients aged 0 – 18 yr, reacted to the oxidized oil.

Testing was also performed in Europe. Frequencies of positive reactions varied greatly, especially when examining reactions in subgroups of patients. In Denmark, 20% of subjects (September 2001 - January 2002) had weak irritant reactions to a commercial lotion that contained 5% tea tree oil, and 1 subject had a ++ reaction to the lotion and 10% tea tree oil in pet.; in June – August 2003, 3.1% of subjects had irritant reactions to lotions containing 5% tea tree oil. In Sweden (prior to 2004), 2.7% of patients tested had a positive reaction to 5% tea tree oil in alcohol.[132] In Germany, testing with 5% tea tree oil (standardized) in diethyl phthalate produced positive results in 1.1% of the patients tested (1999 - 2000), and testing at 5% (oxidized) in pet. (1998 - 2003) produced positive results in 0.9% - 1.0% of the patients tested. Testing performed in the Netherlands (2012 - 2013) reported positive results in 0.9% of patients patch-tested with 5% tea tree oil (oxidized, in pet). However, when this group and an additional 29 patients from a different study were patch-tested with the 5% oxidized tea tree oil and up to 5% ascaridole (a possible contaminant in aged tea tree oil), 6 of 30 patients (20%) that had positive reactions to any concentration of ascaridole also tested positive with tea tree oil; in the 220 patients that did not react to any concentration of ascaridole, none reacted to tea tree oil. In Belgium, 10.5% of patients had positive reactions to 1 and 5% oxidized tea tree oil in pet.; these patients were a sub-group of 15,980 patients that were tested (1990 - 2016) and identified as being allergic to herbal medicines and/or botanical ingredients. Additional studies performed in Belgium (2000 - 2010) with fragrance and non-fragrance allergens reported positive reactions in skin care products containing tea tree oil, but not in the other cosmetic product categories. In testing in Italy with 19 patients that had positive reactions to a botanical integrative series, 2 (10.5%) reacted to 5% tea tree oil in pet. In a Swiss clinic (1997), positive reactions were reported in 0.6% of patients tested with 5 – 100% tea tree oil in arachis oil, and in Spain (prior to 2015), 0.4% of patients had positive reactions to testing with 5% tea tree oil in pet. In the UK (1996 - 1997), 7 of 29 patients (24%) thought to have a cosmetic dermatitis had positive patch test reactions to tea tree oil, applied neat, and in 2001, 2.4% of 550 patients tested with neat, oxidized tea tree oil had positive reactions. Between 2008 and 2016, positive reactions from testing with 5% tea tree oil in pet. ranged from 0.1 – 0.29% in the UK, and in 2016 - 2017, 0.45% of 4224 patients in the UK and Ireland that were patch-tested with 5% tea tree oil (oxidized) in pet. had positive reactions.

In Australia, positive reaction rates generally appear to be higher than those reported in the US or Europe when patch-testing general populations of patients. The Skin and Cancer Foundation reported a positive reaction rate of 1.8% with 5 and 10% tea tree oil (oxidized); however, the same group reported that from 2001 - 2010, the positive reaction rates with 5% and 10% tea tree oil were 3.5% and 2.5%, respectively. Additionally, positive reaction rates of up to 4.8% have been reported with 10% tea tree oil.

Cross-reactivity with tea tree oil was indicated in some retrospective and multi-center studies. With testing of up to 100% tea tree oil in arachis oil, 2 of the 7 patients that had positive reactions to tea tree oil also exhibited a type IV hypersensitivity towards fragrance mix or colophony; the researchers stated study there was a possibility of an allergic group reaction caused by contamination of the colophony with the volatile fractions of turpentine. In one study in which 36/3375 patients reacted to 5% tea tree oil in diethyl phthalate, 14 of those 36 also had positive patch test reactions to turpentine. However, in another study, no correlation was reported between positive reactions to tea tree oil and to colophony. In 45 patients that had positive patch tests to compound tincture of benzoin, 9 of the 45 also had positive reactions to tea tree oil. In several case reports of reactions to tea tree oil, reactions were also noted with eucalyptol, colophony, and ascaridole.

Numerous cases of reaction to tea tree oil have been reported. Adverse reactions were reported with use for treatment of dermatitis and/or psoriasis, other direct skin applications, and from use of hand wash or shampoos. Patients with sensitivity to tea tree oil (dermal and/or oral) were also reported to have reactions to constituents or degradation products of tea tree oil, and positive reactions were reported in a patient with hand eczema following inhalation of tea tree oil vapors. Oral ingestion can be poisonous; serious symptoms, such as confusion and ataxia, can occur.

Daily exposure to tea tree oil was calculated for various product types. Using a rate of percutaneous absorption of 3%, SED estimates between 0.0017 mg/kg/d (2% tea tree oil in a hand soap) and 3.33 mg/kg/d (undiluted tea tree oil) were obtained. When assuming complete absorption as % of applied dose, SED values for different product types ranged from 0.030 mg/kg bw/d (2.0% tea tree oil in a shampoo) to 1.54 mg/kg/d (1.25% tea tree oil in a body lotion). Using 100% absorption and an NOAEL of 117 mg/kg bw/d (for renal effects, derived based on repeated dose systemic toxicity of tea tree oil constituents), and MOS values ranged from 76 (body lotion) to 3900 (shampoo). Based on an aggregate exposure, the SED was calculated as 2.22 mg/kg bw/d, and the overall MOS was 53.

DISCUSSION

To be developed.

CONCLUSION

To be determined.

TABLES

Table 1. Definitions and reported cosmetic functions[1]

Ingredient (CAS No.)	Definition	Cosmetic Function(s)
Melaleuca Alternifolia (Tea Tree) Extract (85085-48-9 [generic])	the extract of the whole sapling, *Melaleuca alternifolia*	skin-conditioning agent -emollient
Melaleuca Alternifolia (Tea Tree) Extract was previously defined as the extract of the whole tree, Melaleuca alternifolia		
Melaleuca Alternifolia (Tea Tree) Flower/Leaf/Stem Extract (84238-27-7; 85085-48-9 [generic])	the extract of the leaves, flowers, and stems of *Melaleuca alternifolia*	skin-conditioning agent - miscellaneous
Melaleuca Alternifolia (Tea Tree) Flower/Leaf/Stem Oil (85085-48-9 [generic])	the volatile oil obtained from the flowers, leaves, and stems of *Melaleuca alternifolia*	flavoring agent; fragrance ingredient; skin-conditioning agent - miscellaneous
Melaleuca Alternifolia (Tea Tree) Leaf	the leaves of *Melaleuca alternifolia*	abrasive; skin-conditioning agent - miscellaneous
Melaleuca Alternifolia (Tea Tree) Leaf Extract (85085-48-9 [generic])	the extract of the leaves of the tea tree, *Melaleuca alternifolia*	skin-conditioning agent - miscellaneous
Melaleuca Alternifolia (Tea Tree) Leaf Oil (68647-73-4; 8022-72-8)	the oil distilled from the leaves of the *Melaleuca alternifolia*	antioxidant; fragrance ingredient
Melaleuca Alternifolia (Tea Tree) Leaf Powder (85085-48-9 [generic])	the powder obtained from the dried, ground leaves of *Melaleuca alternifolia*	abrasive
Melaleuca Alternifolia (Tea Tree) Leaf Water (85085-48-9 [generic])	an aqueous solution of the steam distillates obtained from the leaves of *Melaleuca alternifolia*	antiacne agent; antifungal agent; antimicrobial agent

Table 2. Chemical properties

Property	Description	Reference
	Melaleuca Alternifolia (Tea Tree) Leaf Oil	
physical characteristics	pale yellow to yellow clear mobile liquid with a myristic, characteristic odor	20
solubility		
in water (mg/l at 25°)	insoluble in water	20
	332.1 (estimated)	168
other	1 part miscible with 2 parts ethanol (85% v/v) at 20°C	20
	soluble in alcohol, fixed oil, paraffin oil; insoluble in glycerin	168
	miscible in non-polar solvents	27
freezing point (°C)	-22	20
boiling point (°C)	97 - 220	20
relative density	0.885 – 0.906	20
refractive index (at 20°)	1.475 – 1.482	168
optical rotation	+7° to +12°	20
	+5º to + 15º	168
log P_{ow}	3.4 – 5.5	20
peroxide value (µeq O_2)	< 10 (good quality, fresh oil)	3
	Tea Tree Oil	
physical characteristics	colorless to pale yellow clear, mobile liquid with a "characteristic" odor	22
	colorless to pale yellow liquid, with a myristic odor	13
	colorless to pale yellow, clear mobile liquid that has a "terpeny," coniferous and "minty–camphoraceus" odor	4
	clear colorless liquid with a green/yellow tinge and "antiseptic" odor	9
solubility	insoluble in water; soluble in 2 volumes of 85% ethanol (20ºC)	8
	sparingly soluble in water; miscible with non-polar solvents	
freezing point (°C)	-22	9
boiling point (°C)	97 - 220	9
relative density (at 20ºC)	0.885-0.906	22
	0.89	9
refractive index	1.475 - 1.482	8
	1.465 - 1.495	44
vapor pressure (Pa at 25°C)	2100	8
optical rotation	+ 7° to + 12°	22
log P_{ow} of constituents	2.82 – 6.64	8
$\log_{10} P_{ow}$ of constituents	3.4 - 5.5	9
α-terpineol	3.4	
terpinen-4-ol	3.5	
α-terpinene	5.2	
γ-terpinene	5.3	

Table 3. Composition of the 6 *Melaleuca alternifolia* chemotypes measured by headspace GC[23]

	1,8-cineole	terpinen-4-ol	terpinolene
Type 1 (terpinen-4-ol)	0-17%	22-40%	2-6%
Type 2 (terpinolene)	22-44%	< 3%	41-60%
Type 3 (1,8-cineole)	34-46%	10-14%	16-24%
Type 4 (1,8-cineole)	41-63%	6-14%	0-3%
Type 5 (1,8-cineole)	72-86%	<1%	<1%
Type 6 (1,8-cineole)	65-80%	<1%	6-14%

Table 4. Standards and specifications for tea tree oil

Constituent	ISO 4730:2017 standard (GC)[22]	European Pharmacopoeia[3]	WHO Specifications[13] (Melaleuca Alternifolia (Tea Tree) Leaf Oil)
α-pinene	1-4%	1-6%	NS
sabinene	trace – 3.5%	NMT 3.5%	NLT 3.5%
α- terpinene	6-12%	5-13%	1-6%
limonene	0.5-1.5%	0.5-4%	NS
p-cymene	0.5-8%	0.2-12%	0.5-12%
1,8-cineole	trace (i.e., < 0.01%) – 10%	NMT 15%	NMT 15%
γ- terpinene	14-28%	10-28%	10-28%
terpinolene	1.5-5%	1.5-5%	NS
terpinen-4-ol	35-48%	NLT 30%	NLT 30%
α- -terpineol	2-5%	1.5-8%	1.5-8%
aromadendrene	0.2 – 3%	NMT 7%	NS
ledene (aka viridiflorene)	0.1 – 3%	NS	NS
δ-cadinene	0.2 – 3%	NS	NS
globulol	trace – 1%	NS	NS
viridiflorol	trace – 1%	NS	NS

Table 5. Constituent profiles of tea tree oil

Constituent	WHO (steam distillation)[13]	Supplier Information (GC)[35] (Melaleuca Alternifolia (Tea Tree) Leaf Oil)	Test Samples (steam-distilled; (GC or GC-MS)[28]	Test Sample (GC-MS)[36]	Test Sample (steam-distilled from leaves; GC-MS)[24]	Essential Oil (from leaves)[37]
α-pinene	1-5%	1-6%	2.6%	2.52%	2.0%	2.4%
sabinene	NR	trace – 3.5%	0.2%	0.4%	1.6%	NR
α-terpinene	2.7-13%	5-13%	10.4%	10.2%	9.6%	9.6%
limonene	1-5%	0.5-1.5%	1.0%	0.9%	0.5%	1.1%
p-cymene	1-5%	0.5-8%	2.9%	1.5%	1.5%	2.7%
1,8-cineole	4.5-16.5%	trace-15%	5.1%	2.1%	1.7%	3.1%
γ-terpinene	10-28%	10-28%	23%	21.2%	20.6%	20.1%
terpinolene	1-5%	1.5-5%	3.1%	3.5%	3.0%	3.5%
terpinen-4-ol	29-45%	30-48%	40%	41.5%	47.3%	39.8%
α-terpineol	NR	1.5-8%	2.4%	2.9%	3.0%	2.8%
aromadendrene	NR	trace – 3%	1.5%	1%	< 0.1%	2.1%
ledene	NR	trace – 3%	NR	NR	NR	1.8%
δ-cadinene	NR	trace – 3%	1.3%	1%	NR	1.6%
globulol	NR	trace – 1%	0.2%	0.6%	0.3%	NR
viridiflorol	NR	trace – 1%	0.1%	0.3%	NR	NR

Table 6. Constituents identified by GC/MS in 97 commercial tea tree oil samples from Australia, Vietnam, and China[a] [4]

Constituent	Concentration (%)	Constituent	Concentration (%)
1,8-cineole	0.5 – 18.3	α-eudesmol	0.03 – 0.5
terpinen-4-ol	6.2 – 44.9	α-gurjunene	0.2 – 1.0
terpinolene	0.04 – 45.7[b]	*cis*-3-hexen-1-ol	0.01– 0.07
α-terpinene	2.3 – 11.7	*cis*-3-hexenyl acetate	0 – 0.02
γ-terpinene	3.1 – 23.0	α-humulene	trace – 0.2
α-terpineol	1.9 – 4.2	ledol	0.02 – 0.3
limonene	0.5 – 3.0	linalool	0.06 – 0.8
sabinene	0.03 – 1.3	*p*-menth-2-en-1-ol	0.04 – 0.7
aromadendrene	0.1 – 0.2	methyleugenol	0.01 – 0.4
δ-cadinene	0.1 – 1.9	γ-muurolene	0 – 0.3
globulol	0.02 – 0.6	myrcene	0.2 – 4.1
viridiflorol	0.08 – 0.8	α-phellandrene	0.2 – 0.6
α-pinene	1.8 – 9.2	β-phellandrene	trace – 5.2
p-cymene	0.3 – 19.4	β-pinene	0.3 – 1.7
ledene	0.3 – 2.1	piperitol	0.05 – 0.3
bicyclogermacrene	0 – 1.2	*cis*-sabinene hydrate	trace – 19.4
calamenene	trace – 0.2	*trans*-sabinene hydrate	0.01 – 0.3
camphene	trace – 0.07	spathulenol	trace – 1.1
β-caryophyllene	0.2 – 1.5	α-thujene	0.05 – 1.4
p-cymenene	0.04 – 3.1		

[a] 1 sample from China

[b] the concentration of 45.7% was found in one sample from China only; the median value for all oils was 3.1%

Table 7. Composition of *Melaleuca alternifolia* at different collection times during distillation[28]

Constituent	0-30 min	30-90 min
α-pinene	1.4%	3.5%
sabinene	0.2%	0.1%
α-terpinene	7.8%	14%
p-cymene	1.3%	1.4%
γ-terpinene	15.6%	29.1%
α-terpineol	3.8%	2.1%
terpinolene	2.6%	4.8%
terpinen-4-ol	55.9%[b]	25.1%
aromadendrene	0.3%	1.2%
ledene	0.5%	1.5%
δ-cadinene	0.3%	1.2%
limonene/β-phellandrene/1,8-cineole[a]	5.7%	4.1%
α-thujene[a]	0.6%	1.1%
β-pinene[a]	0.5%	0.9%
myrcene[a]	0.7%	1.3%
α-phellandrene[a]	0.2%	0.4%

[a] not included in the ISO 4730 standard

[b] the values in red text fail to meet the ISO 4730: 2017 standard

Table 8. Monoterpenoid composition comparison of aged oils of Melaleuca alternifolia [28]

age of sample relative deterioration rate	unaged sample	1 yr moderate	2 yr rapid	5 yr rapid	10 yr rapid	10 yr slow
α-pinene	2.6%	2.5%	2%	trace	3.2%	2.2%
sabinene	0.2%	trace	trace	NR	0.1%	NR
α-terpinene	10.4%	6.6%	0.1%	NR	0.2%	5.8%
limonene	1.0%					
p-cymene	2.9%	8.0%	35.3%	21.7%	32%	4.3%
1,8-cineole	5.1%					
γ-terpinene	23%	17.6%	trace	trace	trace	15.9%
terpinolene	3.1%	3.1%	trace	trace	trace	2.7%
terpinen-4-ol	40%	37.3%	23.8%	45.9%	31.5%	41.6%
α-terpineol	2.4%	2.9%	8.2%	9.6%	6.4%	3.7%
limonene/β-phellandrene/1,8-cineole[a]		8%	35.3%	21.7%	32%	4.3%
α-thujene[a]	0.9%	0.8%	0.2%	NR	NR	0.6%
β-pinene[a]	0.3%	0.7%	0.4%	trace	0.3%	0.6%
myrcene[a]	0.5%	0.7%	0.1%	trace	0.2%	0.5%
α-phellandrene[a]	0.3%	0.4%	trace	NR	trace	0.2%
1,2,4-trihydroxymenthane[a]	trace	trace	3.6%	2.5%	4.6%	trace

[a] not included in the ISO 4730 standard

Table 9. Composition of tea tree oil at various stages of oxidation[41]

Component	Un-oxidized Oil	Intermediate Oxidation	Oxidized Oil
α-pinene	2.4%	2.5%	2.6%
sabinene	0.3%	0.2%	NR
α-terpinene	9.1%	5.3%	1.1%
limonene	1.2%	1.2%	1.2%
p-cymene	2.4%	10.2%	19.2%
1,8-cineole	4.5%	4.8%	5.0%
γ-terpinene	19.5%	13.6%	6.9%
terpinolene	3.5%	2.6%	1.5%
terpinen-4-ol	37.7%	36.1%	34.3%
α-terpineol	3.0%	3.1%	3.1%
aromadendrene	1.4%	1.6%	1.9%
ledene	1.0%	1.0%	0.9%
δ-cadinene	1.3%	1.2%	1.2%
globulol	0.4%	0.4%	0.4%
viridiflorol	0.3%	0.3%	0.4%

the values in red text fail to meet the ISO 4730:2017 standard

Table 10. Frequency (2020)[47] and concentration of use (2019)[48] according to duration and type of exposure

	# of Uses	*Max Conc of Use (%)*	*# of Uses*	*Max Conc of Use (%)*	*# of Uses*	*Max Conc of Use (%)*
	Melaleuca Alternifolia (Tea Tree) Extract		**Melaleuca Alternifolia (Tea Tree) Flower/Leaf/Stem Extract**		**Melaleuca Alternifolia (Tea Tree) Leaf**	
Totals*	**62**	**NR**	**29**	**0.001-0.01**	**17**	**NR**
Duration of Use						
Leave-On	*48*	*NR*	*18*	*0.01*	*15*	*NR*
Rinse-Off	*13*	*NR*	*11*	*0.001*	*2*	*NR*
Diluted for (Bath) Use	*1*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type						
Eye Area	NR	NR	NR	NR	1	NR
Incidental Ingestion	1	NR	NR	NR	NR	NR
Incidental Inhalation-Spray	19[a]; 17[b]	NR	4[a]; 9[b]	NR	6[a]; 3[b]	NR
Incidental Inhalation-Powder	17[b]; 1[c]	NR	9[b]	NR	3[b]	NR
Dermal Contact	56	NR	20	0.001-0.01	14	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	4	NR	7	NR	1	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR
Nail	1	NR	2	NR	2	NR
Mucous Membrane	8	NR	2	NR	NR	NR
Baby Products	2	NR	NR	NR	NR	NR

	Melaleuca Alternifolia (Tea Tree) Leaf Extract		**Melaleuca Alternifolia (Tea Tree) Leaf Oil**		**Melaleuca Alternifolia (Tea Tree) Leaf Powder**	
Totals*	**17**	**0.0001-0.001**	**724**	**0.003-0.63**	**3**	**NR**
Duration of Use						
Leave-On	*13*	*0.0001*	*418*	*0.003-0.63*	*NR*	*NR*
Rinse-Off	*4*	*0.001*	*285*	*0.0003-0.3*	*3*	*NR*
Diluted for (Bath) Use	*NR*	*NR*	*21*	*NR*	*NR*	*NR*
Exposure Type						
Eye Area	NR	NR	4	NR	NR	NR
Incidental Ingestion	NR	NR	19	0.0003-0.02	NR	NR
Incidental Inhalation-Spray	1[a]; 11[b]	NR	23; 132[a]; 95[b]	0.01-0.3[a]; 0.03[b]	NR	NR
Incidental Inhalation-Powder	11[b]	NR	5; 95[b]; 5[c]	0.03[b]	NR	NR
Dermal Contact	17	0.0001-0.001	557	0.0003-0.5	3	NR
Deodorant (underarm)	NR	NR	27[a]	not spray: 0.2; spray: 0.5	NR	NR
Hair - Non-Coloring	NR	NR	135	0.0072-0.3	NR	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR
Nail	NR	NR	11	0.005-0.63	NR	NR
Mucous Membrane	1	NR	129	0.0003-0.3	1	NR
Baby Products	NR	NR	9	NR	NR	NR

	Melaleuca Alternifolia (Tea Tree) Leaf Water					
Totals*	**7**	**NR**				
Duration of Use						
Leave-On	*7*	*NR*				
Rinse-Off	*NR*	*NR*				
Diluted for (Bath) Use	*NR*	*NR*				
Exposure Type						
Eye Area	NR	NR				
Incidental Ingestion	NR	NR				
Incidental Inhalation-Spray	4[a]; 1[b]	NR				
Incidental Inhalation-Powder	2; 1[b]	NR				
Dermal Contact	7	NR				
Deodorant (underarm)	NR	NR				
Hair - Non-Coloring	NR	NR				
Hair-Coloring	NR	NR				
Nail	NR	NR				
Mucous Membrane	NR	NR				
Baby Products	NR	NR				

*Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.

[a] Includes products that can be sprays, but it is not known whether the reported uses are sprays

[b] Not specified whether this product is a spray or a powder or neither, but it is possible it may be a spray or a powder, so this information is captured for both categories of incidental inhalation

[c] Includes products that can be powders, but it is not known whether the reported uses are powders

Table 11. In vitro dermal penetration studies of tea tree oil using skin samples

Test Article	Concentration	Diffusion Cell	Skin Sample	Receptor Fluid	Procedure	Penetration/Absorption/Other Parameters	Reference
Animal Skin Samples							
tea tree oil	5% o/w emulsion	conventional static Franz cell; modified static Franz cell to monitor volatiles	pig ear skin; 1 mm thickness	PBS, 0.05 M (pH 5.5), containing 0.1% sodium dodecyl sulfate	Distribution of 7 tea tree oil components was measured Finite dosing regimen using 12 mg of formulation; donor compartment was kept open; sampling was carried out up to 27 h; after withdrawal, the same volume of fresh buffer was added; tape-stripping was used to remove stratum corneum; 3 trials were performed Conventional static Franz evaluated both the components that permeated and distributed in ear pig skin layers (area surface, 2.54 cm^2), and the donor compartment was kept open. The static Franz cell was modified to measure the amounts of components vaporized during the tests; a hermetically sealed glass vessel (75ml) connected online to a donor compartment to collect the components released by the formulation. Amount of each marker in the receiving phase was determined by HS-SPME-GC-MS (20 ml vial); the amount of each marker retained by the total skin, and by epidermis and dermis (separated via the cryostat method), were quantified by HS-SPME-GC-MS using the MHE approach	The skin layers contained less than 1% of each tea tree oil marker in total; only oxygenated terpenes significantly permeated across the skin, while hydrocarbons were only absorbed at trace levels. Over 27 h, permeation rates (and percent permeation) were 49.1 µg/cm^2 (49.7%) for 4-terpineol; 8.90 µg/cm^2 (53.5%) for α-terpineol, and 3.85 µg/cm^2 (12.4%) for 1,8-cineole; permeation rates could not be measured for α- and β-pinene and α- and γ-terpinene because very low amounts permeated at each time All markers were retained by the whole skin, and the amounts ranged from 0.031 µg (β-pinene) to 1.3 µg (4-terpineol). The amounts found in the epidermis ranged from 0.012 µg (α-terpineol) to 0.042 µg α-pinene; β-pinene and α-terpinene were below the LOD. The amounts found in the dermis ranged from 0.031µg β-pinene to 1.26 µg 4-terpineol. Almost no components remained in the residual formulation after 27 h. Substantial amounts of markers were released into the atmosphere; the highest percentage of oxy-genated compounds (i.e., 1,8-cineole, 4-terpineol, α-terpineol) was released into the headspace within the first hour, with approximately 90% of 1,8-cineole, and 40-45% of 4-terpineol and α-terpineol, released into the headspace. For the hydrocarbons (i.e., α- and β-pinene, α- and γ-terpinene), release into the headspace was constant over 27 h	[62]
tea tree oil	2.5, 5, and 10% in a cream 5, 15, and 30% in an ointment 5% in a hydrophilic gel	static glass vertical Franz diffusion cell	pig ear skin for permeation tests; 1 mm thickness synthetic cellulose membrane for release studies	PBS, 0.05 M (pH 5.5), containing 0.1% sodium dodecyl sulfate	Eight marker compounds were identified. Infinite dose regimen; donor compartment contained 1 g of the test article, and was sealed with wax film to prevent evaporation Skin surface has a diffusion area of 1.54 cm^2 18 sampling times, over a 50-h period; receptor phase was completely replaced at each sampling time. Receiving phases were analyzed by HS-SPME with GC–MS; experiments were repeated 3 times	The fastest permeation rate was with the 5% gel, followed by the 30% ointment. All markers (α-pinene, α-terpinene, p-cymene, 1,8-cineole, γ-terpinene, α-terpinolene, 4-terpineol, α-terpineol) permeated the skin; the oxygenated monoterpenes (i.e. 1,8-cineole, 4-terpineol, and α-terpineol) preferentially diffused through the skin; hydrocarbons were only present in the skin (as well as the receptor fluid) at trace levels. ***1,8-cineole (33 mg/g (3.3%) of the oil)*** Amount Released (% of the total amount initially present in the formulations) 5% gel: 236 µg/cm^2 (16.7%) 2.5% cream: 72 µg/cm^2 (8.8%) 5% cream: 137 µg/cm^2 (8.4%) 10% cream: 318 µg/cm^2 (7.2%) 5% ointment: 88 µg/cm^2 (4.7%) 15% ointment: 482 µg/cm^2 (7.3%) 30% ointment: 3642 µg/cm^2 (32.2%) Amount Permeated	[63]

Table 11. In vitro dermal penetration studies of tea tree oil using skin samples

Test Article	Concentration	Diffusion Cell	Skin Sample	Receptor Fluid	Procedure	Penetration/Absorption/Other Parameters	Reference
						5% gel: 235 µg/cm^2 (14.5%) 2.5% cream: 74 µg/cm^2 (9.1%) 5% cream: 31 µg/cm^2 (1.9%) 10% cream: 93 µg/cm^2 (2.1%) 5% ointment: 29 µg/cm^2 (1.6%) 15% ointment: 142 µg/cm^2 (2.1%) 30% ointment: 2.1 µg/cm^2 (1.9%) ***4-terpineol (450 mg/g (45%) of the oil)*** <u>Amount Released</u> 5% gel: 5437 µg/cm^2 (43.6%) 2.5% cream: 354 µg/cm^2 (5.0%) 5% cream: 874 µg/cm^2 (6.1%) 10% cream: 1648 µg/cm^2 (4.2%) 5% ointment: 277 µg/cm^2 (1.7%) 15% ointment: 2496 µg/cm^2 (4.3%) 30% ointment: 10,047 µg/cm^2 (10.1%) <u>Amount Permeated</u> 5% gel: 2103 µg/cm^2 (14.7%) 2.5% cream: 182 µg/cm^2 (2.5%) 5% cream: 84 µg/cm^2 (0.6%) 10% cream: 248 µg/cm^2 (0.6%) 5% ointment: 71 µg/cm^2 (0.4%) 15% ointment: 550 µg/cm^2 (0.9%) 30% ointment: 663 µg/cm^2 (0.7%) ***α-terpineol (65 mg/g (6.5%) of the oil)*** <u>Amount Released</u> 5% gel: 941 µg/cm^2 (52.0%) 2.5% cream: 38 µg/cm^2 (3.6%) 5% cream: 102 µg/cm^2 (4.9%) 10% cream: 190 µg/cm^2 (3.3%) 5% ointment: 20 µg/cm^2 (0.8%) 15% ointment: 275 µg/cm^2 (3.2%) 30% ointment: 1120 µg/cm^2 (7.7%) <u>Amount Permeated</u> 5% gel: 312 µg/cm^2 (15.0%) 2.5% cream: 14 µg/cm^2 (1.3%) 5% cream: 6.3 µg/cm^2 (0.3%) 10% cream: 21 µg/cm^2 (0.4%) 5% ointment: 5.2 µg/cm^2 (0.2%) 15% ointment: 46 µg/cm^2 (0.5%) 30% ointment: 2.58 µg/cm^2 (0.4%) Only 4-terpineol and α-terpineol are retained in the skin; the highest retention was observed with the 30% ointment (0.52 µg/cm^2 4-terpineol; 0.41 µg/cm^2 α-terpineol), and the lowest was with the 5% gel (0.09 µg/cm^2 4-terpineol; 0.15 µg/cm^2 α-terpineol)	

Table 11. In vitro dermal penetration studies of tea tree oil using skin samples

Test Article	Concentration	Diffusion Cell	Skin Sample	Receptor Fluid	Procedure	Penetration/Absorption/Other Parameters	Reference
Human Skin Samples							
monolayer patch formulations containing 10.10% (w/w) tea tree oil; terpinen-4-ol content, 42.7%	as prepared	vertical Franz cells	female (n = 1) abdominal skin; SCE	degassed mixture of ethanol/water (50:50 v/v)	Penetration was estimated using terpinen-4-ol as a marker. Six patch formulations were made of a self-adhesive controlled-release matrix containing methacrylic copolymers or a silicone resin; 3 contained 3.2% oleic acid as a skin penetration enhancer. Terpinen-4-ol content/patch ranged from: 265 ± 52 µg/cm^2 to 485 ± 45 µg/cm^2 Diffusion area of the cell was 0.636 cm^2. Upper and lower parts of the cell were sealed with wax film. Samples were taken at various intervals for up to 24 h, and assayed using CGC/FID. Three replicates were used.	A linear profile was observed for all patches, both with and without oleic acid Formulations containing the silicone resin had the highest flux (6.8 ± 1.0 µg/cm^2/h without, and 8.6 ± 0.4 µg/cm^2/h with, oleic acid); greatest permeation of terpinen-4-ol occurred with this patch (184.6 ± 28.0 µg/cm^2 without, and 217.1 ± 28.3 µg/cm^2 with, oleic acid) Avg flux from the 2 methacrylic copolymer patches was 3.7 ± 0.5 and 4.1 ± 1.9 µg/cm^2/h without, and 3.7 ± 1.4 and 6.6 ± 0.4 µg/cm^2/h with, oleic acid, respectively; amts of terpinen-4-ol that penetrated from these patches were 85.8 ± 10.6 and 128.0 ± 2.3 µg/cm^2 without, and 97.7 ± 31.0 and 161.9 ± 9.9 µg/cm^2 with, oleic acid, respectively Total amount of terpinen-4-ol retained in the skin sample ranged from 2.4 to 16.1 µg/cm^2	[64]
tea tree oil	100%	static Franz diffusion cells	Caucasian female abdominal skin; HSE	ethanol/water mixture	All experiments measured terpinen-4-ol. Liberation experiments were performed by placing the test material in the donor compartment, and using an Isopore® membrane; concentration of saturation of terpinen-4-ol was 10.5 µl/ml, and samples were withdrawn at various intervals for up to 18 h. Permeation were determined using an infinite dosing regimen. HSE, which was rehydrated for 1 h prior to use with PBS, was transferred onto a cellulose membrane for handling. Samples were withdrawn at various intervals up to 48 h. GC was used to assay the components in the receptor fluid.	<u>terpinen-4-ol data (447.4 µl/ml in oil)</u> flux through HSE: 0.262 ± 0.019 µl/cm^2/h P_{app}: 1.62 ± 0.12 cm/s x 10^7 permeation: ~ 4.5 µ1/cm^2 (24 h); ~ 11.7 µl/cm^2 (48 h)	[65]
cream	3, 5, and 10%					<u>from 5% cream (contained 22.37 µl/ml terpinen-4-ol)</u> flux through HSE: 0.022 ± 0.001 µl/cm^2/h P_{app}: 2.74 ± 0.06 cm/s x 10^7 permeation: ~ 0.5 µl/cm^2 (24 h); ~ 1 µl/cm^2 (48 h) overall, release rate ranged from 0.184 ± 0.007 (3% cream) to 0.663 ± 0.017 µl/cm^2/h (10% cream)	
ointment (in white pet)	3, 5, and 10%					<u>from 5% ointment (contained 22.37 µl/ml terpinen-4-ol)</u> flux through HSE: 0.051 ± 0.002 µl/cm^2/h P_{app}: 6.36 ± 0.21 cm/s x 10^7 permeation: ~ 1 µl/cm^2 (24 h); ~ 2 µl/cm^2 (48 h) overall, release rate ranged from 0.416 ± 0.010 (3% ointment) to 1.581 ± 0.035 µl/cm^2/h (10% ointment)	
semisolid o/w emulsion	3 and 5% (phase separation occurred at 10%)					<u>from 5% emulsion (contained 22.37 µl/ml terpinen-4-ol)</u> flux through HSE: 0.067 ± 0.001 µl/cm^2/h P_{app}: 8.41 ± 0.15 cm/s x 10^7 permeation: ~ 1.7 µl/cm^2 (24 h); ~ 3 µl/cm^2 (48 h) overall, release rates were 0.565 ± 0.012 (3% emulsion) and 0.659 ± 0.038 µl/cm^2/h (5% emulsion)	

Table 11. In vitro dermal penetration studies of tea tree oil using skin samples

Test Article	Concentration	Diffusion Cell	Skin Sample	Receptor Fluid	Procedure	Penetration/Absorption/Other Parameters	Reference
tea tree oil; contained 37.5% terpinin-4-ol; 4.5% 1,8-cineole; 3.0% α-terpineol	20% in ethanol and 100%	horizontal Franz cells	female abdominal skin; HSE (n = 3 donors; 6 samples/donor)	PBS (pH 7.4) containing 4% BSA	Penetration and skin retention of components of tea tree oil were studied. Exposed skin area was ~ 1.3 cm^2; membranes were hydrated overnight with PBS placed in the receptor chamber. A finite dose of 10 µl/cm^2 (8.9 mg/cm^2) was used to simulate normal "in use" conditions. Samples were taken at various intervals for up to 24 h, and assayed using GC/MS..	Only terpinen-4-ol and α-terpineol were found in the receptor fluid, but some other sesquiterpenes (not specified) were retained in the skin sample. The amounts varied among the 3 donors. ***Undiluted oil*** Penetration: 138.2 – 302.5 µg/cm^2 terpinen-4-ol (3.6 – 8.0% of the applied dose) and 14.2 – 33.0 µg/cm^2 α-terpineol (3.6 –8.4% of the applied dose) was found in the receptor fluid over the 24-h period; total penetration: 1.73 - 3.82% Epidermal retention: 4.1 – 6.6 µg/cm^2 terpinen-4-ol (0.1 – 0.2% of the applied dose) and 16.3 – 25.7 µg/cm^2 α-terpineol + other components; total found in the epidermis: 0.23 – 0.37% Potential total absorption: 2.0 – 4.1% ***20% formulation*** Penetration: 18.6 – 32.9 µg/cm^2 terpinen-4-ol (1.1 – 1.9% of the applied dose) was found in the receptor fluid after 24 h; α-terpineol was not found Epidermal retention: 0.25 – 0.38 µg/cm^2 terpinen-4-ol (< 0.02% of the applied dose) and 0.5 – 1.18 µg/cm^2 α-terpineol + other components; total found in the epidermis: 0.05 – 0.09% Potential total absorption: 1.1 -1.9%	[30]
	100%		n = 1 donor		Effect of partial occlusion was also evaluated by placing a glass slipcover on top of the donor chamber.	Penetration: terpinen-4-ol (289.7µg/cm^2) and α-terpineol (22.8 µg/cm^2) were found in the receptor fluid after 12 h, and terpinen-4-ol (531.4 µg/cm^2), α-terpineol (44.7 µg/cm^2), and 1,8-cineole (19.8 µg/cm^2) were present at 24 htotal penetration of all 3 components after 24 h was 6.8%. (No other components were detected.) Epidermal retention (24 h): 4.3 µg/cm^2 terpinen-4-ol and 23.3 µg/cm^2 α-terpineol + 14 other components (0.27% of total dose) were found in the epidermis; total retained in epidermis: 0.31% Potential total absorption: 7.1%	
tea tree oil; terpinen-4-ol content, 30%	100%	flow-through Teflon® diffusion cells	female cadaver thorax skin	isotonic phosphate buffer	200 mg of oil was applied to the skin sample for 8 h; donor compartment was occluded with wax film. Cells had a diffusion area of 0.65 cm^2. Stratum corneum layers were separated by tape-stripping. Assayed for 4-terpinen-ol using CGC/FID. Four replicates were used.	amounts of terpinen-4-ol found in the skin layers: outer stratum corneum: 711.5 µg/cm^2 middle stratum corneum: 128.3 µg/cm^2 inner stratum corneum: 69.0 µg/cm^2 remaining epidermis: 1510.6 µg/cm^2	[66]
tea tree oil; terpinen-4-ol content, 42.7%	100%	vertical Franz cells	female (n = 1) abdominal skin; SCE	degassed mixture of ethanol/water (50:50 v/v)	The effect of excipients on the permeability of tea tree oil was determined using infinite	**tea tree oil only** lag time – 59 min flux – 0.02 ± 0.00 mg/cm^2/h K_p – 5.6 ± 1.1 x 10^{-5} cm/h amount permeated – 0.56 ± 0.14 mg/cm^2	[67]

Table 11. In vitro dermal penetration studies of tea tree oil using skin samples

Test Article	Concentration	Diffusion Cell	Skin Sample	Receptor Fluid	Procedure	Penetration/Absorption/Other Parameters	Reference
					dosing conditions. Terpinen-4-ol was used as a marker. 500 µl (~ 700 mg/cm^2) tea tree oil, alone or with a 1 ml mixture (1:1 v/v) with isopropyl myristate, oleic acid, PEG400, or diethylene glycol ethyl ether, was added to the donor compartment, which was covered with wax film to avoid evaporation. Samples were taken at various intervals for up to 24 h, and assayed for 4-terpinen-ol using CGC/FID. Three replicates were used.	retained in skin sample – 0.14 ± 0.00 mg/cm^2 **tea tree oil with isopropyl myristate** lag time – 30 min flux – 0.05 ± 0.01 mg/cm^2/h K_p –23.5 ± 6.3 x 10^{-5} cm/h amount permeated – 1.18 ± 0.31 mg/cm^2 retained in skin sample – 0.04 ± 0.02 mg/cm^2 **tea tree oil with oleic acid** lag time – 12 min flux – 0.70 ± 0.25 mg/cm^2/h K_p – 325.1 ± 119.3 x 10^{-5} cm/h amount permeated – 6.06 ± 2.15 mg/cm^2 retained in skin sample –0.36 ± 0.05 mg/cm^2 **tea tree oil with PEG400** lag time – 47 min flux – 0.04 ± 0.03 mg/cm^2/h K_p – 20.7 ± 13.0 x 10^{-5} cm/h amount permeated – 1.03 ± 0.67 mg/cm^2 retained in skin sample – 0.07 ± 0.01 mg/cm^2 **tea tree oil with diethylene glycol ethyl ether** lag time – 0 min flux – 0.06 ± 0.00 mg/cm^2/h K_p – 28.7 ± 3.0 x 10^{-5} cm/h amount permeated – 1.65 ± 0.24 mg/cm^2 retained in skin sample – 0.18 ± 0.17 mg/cm^2	

Table 12. Acute toxicity studies

Ingredient	Animals	No./Group	Vehicle	Concentration/Dose	Protocol	LD_{50} or LC_{50}/Results	Reference
				DERMAL			
Melaleuca Alternifolia (Tea Tree) Leaf Oil	rabbits	10 (sex not specified)	none	5 g/kg	A single 24-h occlusive patch was applied to clipped intact or abraded abdominal skin	> 5 g/kg 2 animals died; mottled livers were reported at necropsy; stomach and intestinal abnormalities were reported in 3 animals; the other 5 animals were normal	[72]
tea tree oil	NZW rabbits	5/sex	none	2 g/kg	Applied in accordance with OECD TG 402	> 2 g/kg 2 animals died (details not reported)	[8,9]
tea tree oil	dogs and cats	not stated	NR	"very high concentrations"	None stated.	Cases of tea tree oil toxicosis have been reported following topical application; onset of symptoms typically occurred 2-8 h after application; typically, the animals recovered; in one case, the cat died 3 d after exposure, and the cause of death was not determined	[73,74]
				ORAL			
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Swiss mice	not stated	not stated	0.5 - 2 g/kg	Preliminary dose-range-finding study; single dose by gavage	all animals dose with 2 g/kg exhibited a wobbly gait, prostration, and labored breathing at 30 min – 5 h after dosing	[8]
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Swiss mice	5/sex	corn oil	0, 1, 1.35, or 1.750 g/kg bw	Single dose by gavage, in accordance with OECD TG 474; animals were killed after 24 h; an additional vehicle control and high dose group, as well as a positive control group dosed with 40 mg/kg bw of 9,10-diemthyl-1,2-benzanthracene, was killed 48 h after dosing	A statistically significant decrease of PCE and PCE + NCE that was observed in the high dose group at 48 h was considered an indicator of toxicity. Reduced weight gain was noted in all high dose animals killed at 24 h	[8]
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Wistar rats	10 males	none	1.2, 3, or 5 g/kg	Animals were dosed orally	LD_{50} = 1.9 g/kg bw (calculated) One animal dosed with 1.2 g/kg, 9 animals dosed with 3 g/kg, and all animals dosed with 5 g/kg died Abnormalities (not described) in the lungs, heart, liver, stomach, urinary tract, and intestines were reported in the animals that died	[72]
tea tree oil	CRL:(NMRI)BR mice	3 females	PEG 400	2 g/kg bw	Single dose by gavage, in accordance with OECD TG 423	LD_{50} > 2 g/kg; no dose-related mortality Clinical effects, such as decreased activity, hunched back position, and piloerection in all animals, incoordination in 4 animals, and dyspnea in 3 animals	[9]
tea tree oil	Sprague-Dawley rats	5/sex	peanut oil	2.5 – 3.0 ml/kg (SPF rats) 1.7 – 2.4 ml/kg (non-SPF rats)	Single dose by gavage	LD_{50} (SPF rats - 2.6 ml/kg (calculated; equivalent to 2.3 g/kg bw); 30%, 90%, 70%, and 70% of rats dosed with 2.5, 2.6, 2.75, and 3.0 ml/kg, respectively, died within 14 d of dosing LD_{50} (non-SPF rats) - 1.9 ml/kg (calculated; equivalent to ~1.7 g/kg bw); 60%, 30%, 80%, 100%, and 100% of rats dosed with 1.7, 2.1, 2.15, 2.25, and 2.4 ml/kg, respectively, died within 14 d of dosing SPF and non-SPF animals exhibited lack of tonus in the forelimbs, weeping eyes, and bloodied noses	[9]

Table 12. Acute toxicity studies

Ingredient	Animals	No./Group	Vehicle	Concentration/Dose	Protocol	LD_{50} or LC_{50}/Results	Reference
				INHALATION			
tea tree oil	Wistar rats	5/sex	none	1.94, 3.7, and 5.04 mg/l	4-h exposure, nose-only MMAD, GSD, and inhalable fraction (< 4 µm) were: 1.94 mg/l: 2.31 µm; 2.09; 77.2% 3.7 mg/l: 3.40 µm; 2.42; 57.2% 5.04 mg/l: 3.51 µm; 2.0; 57.1%	LC_{50} (calculated) = 4.78 mg/l [males and females, combined]; 5.23 mg/l [males only]; 4.29 mg/l [females only] Mortality was 70% with 5.04 mg/l; no mortality reported in the other 2 groups	[9]
0.3% tea tree oil and 1.8% ethanol in carbon dioxide	Sprague-Dawley rats	5/sex	none	50 or 100 mg/l	1 h exposure under dynamic airflow conditions in a 100 l inhalation chamber that generated ~ 50 mg/l of air	No abnormal behavior or signs of toxicity observed during or after dosing	[8]

Table 13. Genotoxicity studies

Test Article	Concentration/Dose	Vehicle/Solvent	Test System	Procedure	Results	Reference
			IN VITRO			
tea tree oil	10 – 150 µl/plate		*S. typhimurium* TA 98, TA 100, TA 102	Ames test, with and without metabolic activation; appropriate positive controls were used	not mutagenic cytotoxic at ≥ 50 µl/plate	[9]
tea tree oil	*S. typhimurium*: up to 280 µg/plate (TA98) and 880 µg/plate (TA100) with metabolic activation, up to 2780 µg/plate without metabolic activation *E. coli*: up to 2000 µg/plate (tested at non-cytotoxic concentrations)	DMSO	*S. typhimurium* TA98 and TA100; *E. coli* WP2 *uvr* A	Ames test, with and without metabolic activation	not mutagenic	[76]
tea tree oil (and the component terpinen-4-ol)	up to 5000 µg/ml (tea tree oil) up to 2000 µg/ml (terpinen-4-ol)	acetone	*S. typhimurium* TA102, TA100, and TA98	Ames test, with and without metabolic activation	not mutagenic (tea tree oil and terpinen-4-ol	[77]
tea tree oil	9.76 – 58.59 µg/ml (3/20 h and 3/28 h treatment/sampling time, with activation; 3/20 h treatment/sampling time without activation) 4.88 – 39.06 µg/ml (20/28 h treatment/sampling time, without activation)	DMSO	Chinese hamster V79 cells	chromosomal aberration assay, with and without metabolic activation in accordance with OECD TG 473; solvent and positive controls	not clastogenic	[9]
tea tree oil	95, 182, and 365µg/ml; higher concentrations were cytotoxic	none	human lymphocytes	chromosomal aberration assay; negative (untreated culture) and appropriate positive controls were used	not genotoxic	[78]
tea tree oil	95, 182, and 365µg/ml	none	human lymphocytes	mammalian cells micronucleus assay; negative (untreated culture) and appropriate positive controls were used	not genotoxic	[78]
tea tree oil	5 – 275 µg/ml, with activation 5 – 120 µg/ml, without activation	DMSO	mouse lymphoma L5178Y cells	mammalian cell transformation assay, with (two 3-h assays) and without (one 3-h and two 24-h assays) metabolic activation, in accordance with OECD TG 476; negative, solvent, and positive controls were used	not genotoxic cytotoxicity was observed at ≥ 150 µg/ml with, and at ≥ 120 µg/ml (3 h) and ≥ 60 µg/ml (24 h) without, metabolic activation	[9]
tea tree oil	0 – 0.064%	none indicated	HaCaT cells	Comet assay to determine effect on DNA strand breaks (a % of tail DNA); hydrogen peroxide served as the positive control; 3 independent trials	did not induce DNA damage	[79]
			IN VIVO			
Melaleuca Alternifolia (Tea Tree) Leaf Oil	0, 1000, 1350, or 1750 mg/kg bw	corn oil	5 mice/sex/group	mammalian erythrocyte micronucleus test, performed in accordance with OECD TG 474 animals were given single dose by gavage, and killed 24 h after dosing; an additional vehicle control and high dose group, as well as a positive control group dosed with 40 mg/kg bw of DMBA, were killed 48 h after dosing	not clastogenic no significant increase in micronucleated erythrocytes at 24 or 48 h in any of the test groups when compared to the negative controls	[8]

Table 14. Anti-carcinogenicity studies

Test Article	Concentration/Dose	Test System	Procedure	Results	Reference
			IN VITRO		
tea tree oil	0 – 0.08%	murine AE17 mesothelioma cells and B16 melanoma cells	MTT assay; cells were treated for 24 and 48 h, and then measured for viability. Morphological fluorescent analysis was used to determine the primary mode of cell death.	A dose-dependent effect against both cell lines was observed. After 24 h, there was a greater effect against the AE17 cells compared to B16 cells; IC_{50} values were 0.03% and 0.05%, respectively. At 48 h, IC_{50} values were significantly reduced; values were 0.02% and 0.03% for AE17 and B16 cells, respectively. (An increase in exposure time to 72 h did not have a significant effect on the anti-proliferative effect against either cell line.) The primary mode of cell death in AE17 cells appeared to be necrosis; after 24 and 48 h exposure to 0.04% tea tree oil, necrosis levels were 36.2% and 55%, respectively, and apoptosis levels were 13.3% and 12.7%, respectively. Low levels of apoptosis and necrosis were observed with 0.04% tea tree oil in B16 cells at both exposure times (4.3% and 12.9% necrosis and 5.5% and 5.1% apoptosis at 24 and 48 h, respectively); significant necrotic cell death in B16 cells was only evident at concentrations > 0.06% tea tree oil. Cell cycle of B16 cells were significantly altered ().04% of the oil), with only modest changes in AE17 cells.	[80]
tea tree oil	0.005 – 0.03%	human melanoma M14 WT and ADR cells	Effect on cell growth was determined. Annexin V binding method was used to evaluate apoptosis. Migratory and invasive potential was evaluated using the transwell chamber invasion assay	A slight, but statistically significant decrease in the cell pool size of the ADR cells, but not the WT cells, was observed with 0.01% tea tree oil, and concentrations of 0.02% and 0.03% were strongly inhibitory in both the M14 WT and M14 ADR cells, with the effect being greater in the ADR cell line Caspase-dependent apoptosis of the cells, especially in the M14 ADR cells, was induced There was a significant decrease in the percentage of area occupied by the ADR cells migrated in the presence of tea tree oil, but no effect on migration and invasion of the WT cells	[81]
tea tree oil	0.004 – 2.0% (v/v) in DMSO	human malignant melanoma (A-375) and squamous cell carcinoma (Hep-2) cells	The viability of A-375 and HEp-2 cell lines was assessed using the MTT assay (24 h). Annexin V/PI staining was measured for apoptosis detection, cell cycle analysis was monitored using flow cytometry, and mRNA expression levels of the apoptosis-regulatory genes *P53*, *BAX*, and *BCL-2* were determined by real-time PCR and western blot analysis	tea tree oil markedly reduced viability in a dose-dependent manner, and exhibited a strong cytotoxicity towards both cell lines; IC_{50} values were 0.038% (v/v) for A-375 cells and 0.024% (v/v) for Hep-2 cells; cytotoxicity resulted from apoptosis in both cell lines. Cell cycle analysis showed that tea tree oil caused cell cycle arrest mainly at G2/M phase. Expression of proapoptotic genes (*P53* and *BAX*) was upregulated, while the anti-apoptotic gene *BCL-2* was downregulated	[82]

Table 14. Anti-carcinogenicity studies

Test Article	Concentration/Dose	Test System	Procedure	Results	Reference
tea tree oil	1 – 1000 µg/ml in DMSO	human MCF-7 and murine 4T1 breast cancer cells; HFF-1 fibroblast cells	MTT assay; 72 h Apoptosis was evaluated using flow cytometry (MCF-7 cells) Cell cycle analysis and a colony formation assay (after 10 d of treatment) were performed in MCF-7 cells	IC_{50} (72 h) was estimated to be 603 µg/ml for MCF-7 cells and 626 µg/ml for 4T1 cells; there was a significant decrease in MCF-7 and 4T1cell proliferation at concentrations > 300 and > 600 µg/ml, respectively. With HFF-1 cells, a significant decrease in cell proliferation was observed at 1000 µg/ml; however, with 300 µg/ml, cell proliferation of HFF-1 cells was induced at 72 h after treatment The increase in apoptosis in MCF-7 cells at 300 µg/ml was approximately 6x higher compared to untreated cells. 300 µg/ml significantly increased the number of cells in the S phase of the cell cycle In the colony formation assay, 300 and 600 µg/ml significantly decreased the number of cell colonies	[83]
tea tree oil	10 – 50 µg/ml (0.195 – 100%) in DMSO	human MDA MB breast cancer cells	MTT assay; 48 h incubation NIH3T3 mouse fibroblast cells were used as a control	IC_{50} = 25 µg/ml	[85]
tea tree oil	0.025 and 0.05 % in DMSO and Tween 80	human U87MG glioblastoma cells	MTT assay; cells were incubated for 24, 48 or 72 h Cell cycle and apoptosis assay were assessed by flow cytometry (0.025%, for up to 24 h or up to 72 h)	tea tree oil decreased cell viability in a dose- and time-dependent manner. Cell cycle arrest was triggered in the G0/G1 phase in a time- and dose-dependent manner; treatment (72 h) caused an increase of cells in the G0/G1 phase	[84]
tea tree oil	10 – 50 µg/ml (0.195 – 100%) in DMSO	human HT29 colon cancer cell line	MTT assay; 24 h incubation period Cisplatin served as the positive control	IC_{50} = 12.5 µg/ml	[86]
tea tree oil	0.0001% - 100%, in ethanol	human Hep G2 hepatocellular carcinomic human cell line	MTS assay; 4 h and 24 h exposure times Controls included ethanol; ethanol and cells; and ethanol and media	IC_{50} = 2800 µg/ml (4 h) IC_{50} = 20 µg/ml (24 h)	[87]
tea tree oil	0.0001% - 100%, in ethanol	HeLa epithelioid carcinomic cell line	as above	IC_{50} = 2800 µg/ml (4 h) IC_{50} = 2700 µg/ml (24 h)	[87]
tea tree oil	0.0001% - 100%, in ethanol	human MOLT-4 lymphoblastic leukemic T-cell line	as above	IC_{50} = 600 µg/ml (4 h) IC_{50} = 300 µg/ml (24 h)	[87]
tea tree oil	0.0001% - 100%, in ethanol	human K-562 chronic myelogenous leukemia cell line	as above	IC_{50} = 2800 µg/ml (4 h) IC_{50} = 270 µg/ml (24 h)	[87]
tea tree oil	0.0001% - 100%, in ethanol	CTVR-1; early B-cell line from bone marrow cells of a patient with acute myeloid leukemia	as above	IC_{50} = 310 µg/ml (24 h)	[87]

Table 14. Anti-carcinogenicity studies

Test Article	Concentration/Dose	Test System	Procedure	Results	Reference
			ANIMAL		
tea tree oil, or a solution of its components	10% in DMSO, acetone, or isopropanol (50 µl); neat (5 µl); 10% solution of components (40% ter-pinen-4-ol, 20% γ-ter-pinene, 10% α-terpinene, 5% 1,8-cineole, 5% p-cymene, in ethanol) in DMSO (50 µl))	C57BL/6J mice; 5 females/group	subcutaneous implantation with 5 x 10^5/100 µl PBS B16-F10 murine melanoma cells or 1 x 10^7/100 µl PBS AE17 murine mesothelioma cells; once tumors measured ~9 mm^2, mice were treated topically 1x/d for 4 d; 4 independent trials were performed Vehicle control received 10% water/DMSO; all animals were compared to untreated controls	10% tea tree oil in DMSO: regressed AE17 mesotheliomas in mice; untreated control growth levels resumed approximately 4 d after cessation of treatment. Significantly slowed the growth of B16-F10 melanomas; growth resumed at untreated control levels 2-3 d following cessation of treatment, rapidly reaching 100 mm^2 in size. Local skin irritation and inflammation (with an increased number of neutrophils and other immune cells including macrophages, mast cells, and lymphocytes, but not eosinophils) was observed with application undiluted tea tree oil;10% in acetone or isopropanol; vehicle control: no effect on tumor growth; no local effects with undiluted oil, or vehicle control; minimal local dermal irritation with 10% in acetone or isopropanol. 10% solution of components in DMSO: significantly inhibited the growth of AE17 tumors for a period of 5 d, and induced significant tumor regression in half of the test animals; growth resumed at untreated control levels 2 d following cessation of treatment.	[88]
tea tree oil	3.5%	nude CD1 mice; 8 males/group	subcutaneous implantation with 5 × 10^6 human glioblastoma cells /0.2 ml (matrigel and DMEM); after 7 d, tea tree oil was administered intratumorally, 2x/wk for 3 wk	Test mice had an 80% reduction in the tumor mass compared with control mice. Tumors treated with tea tree oil showed the same cell morphology as those that were untreated, but a marked reduction in cell density with large areas of necrosis was observed. Using the TUNEL assay, an increase in apoptotic tumor cells (DNA fragmentation) was found after treatment with tea tree oil.	[84]

Table 15. Effect on endocrine activity

Test Article	Concentration/Dose	Test System	Procedure	Results	Reference
			ESTROGENIC EFFECTS		
tea tree oil	0.025% (v/v) in DMSO	MCF-7 (ERα-positive) cells	Determined ERα-regulated gene expression, using quantitative PCR; cells were treated for 18 h, with or without 5 µM fulvestrant; vehicle controls and E2 (1 nM) controls were also used mRNA levels of ERα target genes (*GREB1*, *PGR*, and *CTSD*) were measured	All 3 genes showed significant induction when treated with tea tree oil; induction was blocked by co-treatment with fulvestrant	[89]
tea tree oil	0 – 0.05% (v/v) in DMSO	human MCF-7 breast cancer cells	MCF-7 cells that were positive for ER and were transiently transfected with an estrogen-inducible luciferase reporter plasmid containing 3 copies of an ERE (3X-ERE-TATA-luciferase) were treated for 18 h, with or without fulvestrant (an ER antagonist); 4 experiments were performed in duplicate. E2 (1 nM) served as the positive control.	ERE-dependent luciferase activity was stimulated in a dose-dependent manner, with the maximum activity observed at 0.025%; however, maximum activity corresponded to approximately 50% of the activity elicited by 1 nM E2. (Higher doses of tea tree oil were cytotoxic.) Fulvestrant inhibited tea tree oil-induced transactivation of the 3X-ERE-TATA-luciferase reporter plasmid; the researchers stated that this indicated that the activity observed with tea tree oil is ER-dependent. Additional testing in MCF-7 cells indicated that tea tree oil modulated the expression of the estrogen-regulated endogenous genes *MYC*, *CTSD*, and *IGFBP3*, that it increased the expression of mRNA for *MYC* and *CTSD*, and it decreased the expression of mRNA for *IGFBP3*, as compared with the DMSO controls; the researchers stated that these effects on mRNA were similar to the effect of 1 nM E2, in magnitude and timing.	[90]
tea tree oil; terpinen-4-ol; α-terpineol; 1,8-cineole	0.00075 – 0.1% (v/v)	MCF-7 BUS cells	E-screen assay; effect on cell proliferation was examined in the presence and absence of 0.00005 µM E2; proliferation results were expressed as the number of cells after 6 d of incubation, and given as the RPE compared to the maximum E2 response	Without E2, tea tree oil induced a weak, but significant, dose-dependent estrogenic response at concentrations ranging from 0.00075% - 0.025%, with a maximal response (corresponding to 34% of the maximal E2 response) induced by 0.0125% tea tree oil Terpinen-4-ol, α-terpineol, and 1,8-cineole, as well as an 8:1:1 mixture of these constituents, did not induce a significant estrogenic response (i.e., >10% of the maximal response induced by E2) at concentrations of 0.00075% - 0.1%. When tested in the presence of E2, < 0.025% tea tree oil reduced the RPE by 10%. Terpinen-4-ol produced a slight (~6%), and α-terpineol produced a significant and dose-dependent, inhibition of MCF-7 cell proliferation induced by E2; 1,8-cineole and the 8:1:1 mixture of the constituents did not have a significant effect. With all trials, the highest concentrations of tea tree oil and the constituents were cytotoxic.	[68]
ethanol extract of a hair conditioner product that contained tea tree oil	estrogenic activity assay: 1/100 - 1/100,000 dilution of the test material (i.e., 0.005 – 5 x 10^{-6} g/ml) anti-estrogenic activity assay: 1/333 - 1/729,000 dilution of the test material (i.e., 0.0015 - 6.85 x 10^{-7} g/ml)	MCF-7:WS8 cells (> 90% of the receptors are ER-α, and < 10% are ER-β)	E-screen cell proliferation assay (robotic version) Cells were treated with E2 or the test extract (0.5 g product/ml ethanol) for 6 d, and solutions were changed every other day. The vehicle control was 1% ethanol in estrogen-free medium, and fulvestrant (an ER antagonist) served as the positive control. Estrogenic activity was considered detectable if it produced a cell proliferation > 15% of the relative maximum % of E2, and anti-estrogenic activity was considered detectable if it suppressed low (set at 4.0 x 10^{-12} M) E2-stimulated cell proliferation by at least 3 standard deviations for at least one dilution of the extract.	The test material did not exhibit estrogenic activity, but it did exhibit anti-estrogenic activity. The normalized anti-estrogenic activity (as relative maximum % of the positive control) was 79%.	[91]

Table 15. Effect on endocrine activity

Test Article	Concentration/Dose	Test System	Procedure	Results	Reference
tea tree oil components (13.2% eucalyptol, 42.3% 4-terpineol, 1.3% dipentene/ limonene, 7.1% α-terpineol, 11.4% α-terpinene, 24.7% γ-terpinene)	0.005 – 0.025% (v/v) in DMSO	human HepG2 hepatocellular cancer cells (ERα negative)	Luciferase reporter assay with ERα; transfected cells were treated for 18 h; vehicle controls and E2 (1 nM) controls were also used	Activation observed at all concentrations of tea tree oil, with a maximum of an ~20-fold increase in ERα ERE-mediated promotor activity; E2 produced an ~50-fold increase Components produced up to a 10-fold increase in activation; 0.005% did not produce a significant effect	89
tea tree oil	0.025% (v/v) in DMSO	HepG2 cells	Mammalian two-hybrid binding assay to determine binding activity to the ERα LBD by analyzing ligand dependency of hERα, LBD, and SRC-2-NR element interactions; transfected cells were treated for 18 h; vehicle controls and E2 (1 nM) controls were also used	Significant induction of ERα ERE-mediated activity with 0.01% tea tree oil (and with E2) Tea tree oil recruited SRC-2-NR and demonstrated binding to the LBD of ERα.	89
			ANTI-ANDROGENIC ACTIVITY		
tea tree oil	0.001 – 0.01% (v/v) in DMSO	MDA-kb2 breast cancer cells (positive for the AR)	Evaluation of effect on androgenic activity. The cells were stably transfected with an androgen-inducible and glucocorticoid-inducible MMTV-luciferase reporter plasmid, and were treated for 24 h tea tree oil in the presence and absence of DHT; 3 experiments were performed, in quadruplicate. Flutamide served as a positive control for androgen-receptor antagonism.	Tea tree oil did not transactivate the MMTV-luciferase reporter plasmid at any concentration tested, while 0.1 nM DHT produced an ~4-fold increase in luciferase activity when compared to DMSO controls. Transactivation of the MMTV-luciferase reporter plasmid by 0.1 nM DHT was inhibited in a concentration-dependent manner by tea tree oil (as well as by flutamide); upon simultaneous treatment of the cells with DHT and tea tree oil, maximum inhibition occurred with 0.005% tea tree oil, corresponding to a decrease in luciferase activity of 4% in the presence of 0.1 nM DHT. Additional experiments indicated that the anti-androgenic properties of tea tree oil extended to inhibition of DHT-stimulated expression of the androgen-inducible endogenous genes *CYP4F8*, *C1orf116*, *UGT2B28*, and *SEC14L2*. The researchers stated that because the amount of androgen-receptor mRNA or protein was not altered, the anti-androgenic effect of the oil is not caused by down-regulation of the expression of the AR.	90
tea tree oil	0.01% (v/v) in DMSO	MDA-kb2 cells	Luciferase reporter assay with AR using MMTV; cells were co-treated with 1 nM testosterone and tea tree oil for 18 h; DMSO, 1 nM testosterone, and 1 nM testosterone + 1 µM flutamide were used as controls	Increasing concentrations of tea tree oil, co-treated with testosterone, significantly inhibited AR MMTV-mediated activity at concentrations ≥ 0.0005% (v/v); change in AR MMTV-mediated activity, as compared to testosterone, was 36%	89
tea tree oil	0.025% (v/v) in DMSO	MDA-kb2 cells (AR-positive)	Determined AR-regulated gene expression using quantitative PCR; cells were co-treated with 1 nM testosterone and tea tree oil for 18 h; DMSO, 1 nM testosterone, and 1 nM testosterone + 1 µM flutamide were used as controls; mRNA levels of AR target genes (*CTP4F8*, *UGT2B28*, and *SEC14L2*) were measured	Tea tree oil, co-treated with testosterone, significantly inhibited all 3 target genes	89

Table 16. Dermal irritation and sensitization studies

Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
IRRITATION					
ANIMAL					
Melaleuca Alternifolia (Tea Tree) Leaf Oil	undiluted; 0.5 ml	4 NZW rabbits	single 4-h semi-occlusive patch applied to clipped dorsal skin; the test site was evaluated at 1, 24, 48, and 72 h and 7 d after patch removal	irritant effects; average scores were 2.0 for erythema and 1.7 for edema	[102]
Melaleuca Alternifolia (Tea Tree) Leaf Oil	undiluted; 5.0 g/kg	10 rabbits	single 24-h occlusive patch on clipped intact and abraded abdominal skin (see acute dermal toxicity study)	irritant effects; skin abnormalities at necropsy (details not provided)	[72,103]
tea tree oil (conformed to ISO standards)	0.625, 1.25, 2.5, 5, and 10%; 50 µl	5 female Wistar rats	single 4-h application (type of patch not specified) applied to shaved skin; application was rinsed with distilled water; test site was evaluated 24 and 48 h after application	no irritation was observed with ≤ 2.5% 5% produced very slight erythema and edema at 24 and 48 h 10% produced well-define erythema and very slight edema at 24 and 48 h	[24]
tea tree oil	undiluted; 0.5 ml	6 NZW rabbits	Draize study; test material was applied to intact and abraded skin for 72 h (type of patch not specified)	Draize irritation index = 5.0; severe irritant	[8,9]
tea tree oil	12.5, 25, 50, and 75% (vehicle not specified)	rabbits; number not provided	semi-occlusive patch test performed according to OECD 404 (acute dermal irritation/corrosion study)	applications of 12.5 and 25% were not irritating; 50% was minimally irritating; 75% was slightly irritating	[8]
tea tree oil	25% in paraffin oil	rabbits; number not provided	repeated applications for 30 d to shaved skin	initial minor irritations declined with time; microscopic skin changes were observed	[8]
HUMAN					
Melaleuca Alternifolia (Tea Tree) Leaf Oil	1% in pet.	22 subjects	48-h occlusive patch (conducted as a pre-test for a maximization test)	no irritation	[103,104]
tea tree oil	0, 1, 2.5, 5, and 10% in a 0.05 ml sorbolene cream	28 subjects	occlusive patches applied to the back, 5x/wk for 3 wk, for a total of 15 applications; duration of dosing not stated	5 subjects reported slight irritation: 1 to 1%; 1 to 2.5%; 2 with 5%; 2 with 10% slight irritation was observed for 1 subject on 11 of the 15 d with 10% tea tree oil; for the others, irritation was reported only for 1 or 2 d	[18]
tea tree oil	25% in soft white paraffin (8 samples; contained 1.5-28.8% 1,8-cineole and 22.6-40.3% terpinen-4-ol)	28 initial subjects; 25 subjects completed the study	24-h occlusive patches were applied to the upper arm or back, 5x/wk for 3 wk - 1,8-cineole (3.8-21%) was tested for comparison	no irritation to the oil or 1,8-cineole was observed - an allergic, but not irritant response (erythema with marked edema and itching), was observed in 3 subjects to all 8 samples: 1 subject had a +3 response at day 3; 1 had a +3 reaction to on day 8; and 1 subject had a +2 reaction on day 14. These subjects were withdrawn from the trial and tested for sensitization (described under 'Sensitization')	[105-107]
tea tree oil	undiluted; 10 samples	219 subjects	48-h occlusive application	prevalence of marked irritancy was 2.4-4.3% prevalence of any irritancy (mild to marked) was 7.2-10.1%	[8,14]

Table 16. Dermal irritation and sensitization studies

Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
			SENSITIZATION		
ANIMAL					
tea tree oil (purity, ISO Standard 4730-2004; GLP-compliant)	0, 5, 25, and 50% in PEG 400	female CBA mice, 5/group	LLNA Ear thickness was measured prior to application on day 1, after 48 h and prior to 3rd (and last) application on day 3, and on day 6; mice were injected with BrdU 5 d after initial application, and lymph nodes were isolated at necropsy B:T cell ratio was measured in lymph node preparations by immunotyping 25% HCA was used as the positive control	EC3 value of 8.3% (categorized as weak[9] or moderate[8] sensitization potential) Sensitizing response at 25 and 50% (SI of 2.1, 7.7, and 7.9 at 5, 25, and 50%, respectively); the sensitizing effect was supported by immunotyping (B cells and B:T cell ratio increased by >25% compared to controls[3]) No dermal irritating response (as determined by change in ear thickness)	3,8,9
tea tree oil (purity, ISO Standard 4730-2004; GLP-compliant)	0, 2, 20, and 100% in PEG 300	female CBA mice, 5/group	LLNA; no positive control	EC3 value of 4.4% (moderate skin sensitizer) SI were 2.4, 6.9, and 16 at 2, 20, and 100%, respectively	8
tea tree oil (non-oxidized, undegraded; purity, ISO Standard 4730; GLP-compliant)	0, 2, 20, and 100% in PEG 300	female CBA mice, 5/group	LLNA; no positive control	EC3 value of 24.3% (moderate sensitization potential) SI were 1.8, 2.8, and 6.5 at 2, 20, and 100%, respectively	8
tea tree oil (non-oxidized, undegraded; purity, ISO Standard 4730; GLP-compliant)	0, 2, 20, and 100% in PEG 300	female CBA mice, 5/group	LLNA; no positive control	EC3 value of 25.5% classified as weak[9] or moderate[8] sensitization potential) SI were 1.6, 2.8, and 5.7 at 2, 20, and 100%, respectively (a comment was made that PEG is not a recommended vehicle for the LLNA[8])	8,9
tea tree oil	induction, intradermal: 5% in paraffin oil B.P. and 1:1:1 mixture of the oil, saline, and FCA; epidermal: 100% challenge: 30% in pet	albino guinea pigs, 20/group	GPMT; induction consisted of 2 intradermal injections, followed 1 wk later by a 48-h occlusive patch; the challenge was conducted 2 wk later with a 24-h occlusive patch	not sensitizing	3,9
tea tree oil	induction: not stated challenge: 10% and 30%	10 Pirbright white guinea pigs	Adjuvant maximization protocol (FCA method; details not provided) reacting animals were cross-challenged with terpinen-4-ol	10% challenge: no reactions 30% challenge: positive reactions in 3/10 animals at 48 h no response to cross-challenge with terpinen-4-ol	3,109
tea tree oil (freshly distilled)	"pure" 30 mg for induction 0.05 ml for challenge	10 female Pirbright white guinea pig	modified FDA technique; the material was dissolved in 4 ml FDA, and emulsified with 4 ml physiological saline (30 mg); challenge was performed 11 d after induction, with an open epicutaneous application of pure test material; test site scores were recorded at 24 and 48 h, according to the ICDRG	mean response: 0.4 (24 h); 0.5 (48 h) low sensitizing capacity	108
oxidized tea tree oil (exposed to light, warmth, moisture, and oxygen)	"pure"	10 guinea pigs	challenge material; oxidized tea tree oil	mean response: 0.45 (24 h); 1.78 (48 h)	
		10 guinea pigs	challenge material: oil stored for 2 mo in a transparent flask challenge material: oil stored for 2 mo in a brown flask challenge material: oil stored for 2 mo in a closed flask challenge material: oil stored for 2 mo in an open flask	mean response: 0.8 (24 h); 1.0 (48 h) mean response: 0.55 (24 h); 1.1 (48 h) mean response: 0.62 (24 h); 0.65 (48 h) mean response: 1.0 (24 h); 1.58 (48 h)	
		10 guinea pigs	challenge material: monoterpene fraction challenge material: sesquiterpene fraction challenge material: thujene/pinene-free fraction	mean response: 0.85 (24 h); 0.9 (48 h) mean response: 0.2 (24 h); 0.18 (48 h) mean response: 1.3 (24 h); 1.7 (48 h)	

Table 16. Dermal irritation and sensitization studies

Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
		10 guinea pigs	challenge materials (in acetone) – at 5%: *p*-cymene; 1,8-cineole; myrcene; sabinene; α-terpinene at 10%: viridiflorene; aromadendrene; α-terpinene; ascaridole; terpinen-4-ol; α-pinene; β-pinene; α-terpineol; terpinolene	mean response with *p*-cymene: 1.25 (24 h); 1.13 (48 h) for all others mean response varied from 0.0 – 0.3 (24 h) to 0.0 0 0.53 (48 h)	
HUMAN					
Melaleuca Alternifolia (Tea Tree) Leaf Oil	1% in pet	22 subjects	Kligman maximization test occlusive patch applied to the volar 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	not a sensitizer	103,104
Melaleuca Alternifolia (Tea Tree) Leaf Oil	10% in caprylic/capric triglycerides; 200 µL, volatilized for 30 min	102 subjects	modified HRIPT 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	not an irritant or sensitizer	110
tea tree oil (conformed to ISO standards; peroxide content was 9.5 mEq O_2/kg)	5% in a cream base; 25% in a cream, ointment, and gel base; 100% negative control; cream base	309 subjects	Draize sensitization study induction: 48-h occlusive applications were made with Finn chambers (11 mm) containing 100 µl of the liquid formulation or 100 µg of the solid-phase preparation to the 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	Scoring for irritation was based on 306 subjects because 3 subjects were not included because they developed grade 3 vesicular reactions during induction); allergenicity was evaluated with all 309 subjects During induction; the maximum mean irritancy score was 0.2505/4, with undiluted tea tree oil Of the 3 subjects that developed grade 3 vesicular reactions, only one subject (day 8 reaction) returned for challenge, in which a positive grade 3 reaction was confirmed; because different samples were tested simultaneously, it was not possible to determine which specific concentration was responsible for inducing sensitization at challenge; no other subjects had reactions at challenge	111

Table 16. Dermal irritation and sensitization studies

Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
tea tree oil	"varying concentrations" (not specified)	3 sensitized subjects (from the irritation study described above)	tested 2 wk after initial study	all 3 had positive results at 3 and 7 d	105-107
major component of tea tree oil	25% in soft white paraffin; similar dilutions as above		major components of tea tree oil were also patch-tested (24 - 48 h)	one subject had an allergic response to α-terpinene (tested at 5.9% in soft white paraffin) none of the subjects reacted to α-pinene, β-pinene, limonene, p-cymene, 1.8-cineole, γ-terpinene, terpinolene, terpinen-4-ol, or α-terpineol	
crude sesquiterpenoid fractions; sesquiterpene hydrocarbon concentrate; sesquiterpene alcohol concentrate	crude fraction - 10.7%; sesquiterpene hydrocarbon fraction – 1.5%; 98% sesquiterpene alcohol –tested at 0.03% 5.3% sesquiterpene alcohol –tested at 1.4% vehicle – soft white paraffin			all 3 sensitized subjects reacted positively to the sesquiterpenoid fractions and sesquiterpene hydrocarbons; 1 subject reacted to the 0.03% sesquiterpene alcohol sample	

Table 17. Retrospective, multicenter, and cross-sectional patch test studies with tea tree oil

Years/Testing Group	Concentration/Vehicle	# patients	# Positive (%)	Relevance	Comments	Reference
NORTH AMERICA						
2000 – 2007; Mayo Clinic *	oxidized, 5% pet**	869	18 (2.1%)	not stated	macular erythema – 3 (0.3%); weak reaction – 9 (1%); strong reaction – 5 (0.6%); extreme reaction – 1 (0.1%)	115
2003 - 2004; NACDG	oxidized, 5% pet	5137	45 (0.9%)	not stated		113
2003 - 2006; NACDG***	oxidized, 5% pet	9569	all rxn:101 (1.0%) "+ "only: 55 (0.6%)	not stated	positivity ratio (percent of weak (+) reactions among the sum of all positive reactions) – 54.5% reaction index (number of positive reactions minus questionable and irritant reactions/sum of all 3) – 0.73 85 allergic reactions (not irritant; not questionable) 117 allergic reactions (with irritant; with questionable)	121
2003 - 2007; NACDG	oxidized, 5% pet	11,649 (ages 19 – 64)	35 (0.3%)	22 (0.2%)		128
2005 - 2006; NACDG	oxidized, 5% pet	4435	1.4%	definite - 8.2% probable - 27.9% possible - 36.1%		116
2007 - 2008; NACDG	oxidized, 5% pet**	5078	1.4%	definite – 5.7% probable – 31.4% possible – 40.0% past – 5.7%	SPIN - 55	117
2009 - 2010; NACDG	oxidized, 5% pet	4299	1.0%	definite - 14.3% probable - 35.7% possible - 21.4%	SPIN – 45 (rank 36)	118
2011 - 2012; NACDG	oxidized, 5% pet (Melaleuca Alternifolia (Tea Tree) Leaf Oil)	4231	36 (0.9%)	definite - 11.1% probable - 41.7% possible - 22.2%	reaction severity: 17 +++; 8 ++; 10 +; 1 +/- SPIN – 41 (rank 41)	119

Table 17. Retrospective, multicenter, and cross-sectional patch test studies with tea tree oil

Years/Testing Group	Concentration/Vehicle	# patients	# Positive (%)	Relevance	Comments	Reference
2015 - 2016, NACDG	oxidized, 5% pet (tea tree leaf oil)	5593	66 (1.2%)	definite – 7 (10.6%) probable – 20 (30.3%) possible – 19 (28.8%) past – 8 (12.1%)	SPIN – 47 (rank 36)	[120]
2003; NACDG	oxidized (5% pet)**	1603	5 (0.3%)	definite - 0% probable – 1 (20%) possible – 3 (60%) unknown – 1 (20%)	only 1/5 patients that reacted to tea tree oil also reacted to the fragrance makers fragrance mix and *Myroxilon pereirae* in the test population, younger patients were more likely to be allergic to tea tree oil	[122]
2009 – 2014; NACDG	oxidized, 5% pet	13,398	123 (0.92%)	not stated	63 of the patients that reacted to oxidized tea tree oil did not react to any of the fragrance mixes that were tested; half of the reactions to tea tree oil were strong (13 ++ and 19 +++ reactions), and of definite (8; 12.7%) or probable (25, 39.7%) clinical relevance	[123]
2014 - 2017; Northwestern Medicine patch-testing clinic; 48-h patch	oxidized, 5% pet (Melaleuca Alternifolia (Tea Tree) Leaf Oil)	502 (total) *current AD?:* yes, 108; no, 394 *past AD?:* yes, 109; no, 209	current AD:0 no current AD: 1 (0.2%) past AD: 0 (both groups)	not stated		[124]
CROSS-SECTIONAL STUDIES						
formulation type-specific						
2001 - 2004; NACDG	5% (oxidized) associated with a moisturizer	835 529 female/ 306 male with moisturizer-associated positive reactions	1.2% 1.5% (F) 0.7% (M)	not stated	test group comprised a subgroup of patients with moisturizer-associated positive reactions from a parent group of patients (n = 2193; 1582 females and 611 males) with allergic reactions to cosmetics; the percent of male patients with a positive allergic reaction to moisturizers (50.1%) was greater than female patients (33.4%)	[125]
site-specific						
2003 - 2004; NACDG	oxidized, 5% pet*	1959 hand dermatitis patients	4 (0.2%)	3 (75%)	test group was a subgroup of patients with hand-only reactions and final diagnosis code that included ACD; parent group n = 5148	[126]
		959 hand dermatitis patients	4 (0.4%)	2 (50%)	test group was a subgroup of patients with hand-only reactions and final diagnosis code was only ACD; parent group n = 5148	
2001 - 2004; NACDG	oxidized, 5% pet	60 lip ACC patients	3 (5%)	not stated	of 10.061 patients, 196 had a skin condition limited to the lips that was ACC; the test group consisted of subjects from the "lip" group that had at least one clinically relevant reaction to an NACGD series allergen	[127]
age specific - children						
2003 - 2007; NACDG***	oxidized, 5% pet	1007 ≤18 yr	4 (0.4%)	4 (0.4%)		[128]
2003 – 2004, NACDG***	oxidized, 5% pet	age 0 – 5 y (n not specified)	14.3%	14.3%		[129]
		age 0 – 18 yr (n not specified)	1.1%	1.1%		

Table 17. Retrospective, multicenter, and cross-sectional patch test studies with tea tree oil

Years/Testing Group	Concentration/Vehicle	# patients	# Positive (%)	Relevance	Comments	Reference
2005 – 2012, NACDG	oxidized, 5% pet	n = 40, age 0 – 5 yr	0%	0%		130
		n = 836, age 6 – 18 yr	0.8%	0.4%		
		n = 876, age 0 – 18 yr	0.8%	0.3%		
age-specific – older individuals						
2003 - 2007; NACDG***	oxidized, 5% pet	2409 ≥65 yr old	8 (0.3%)	6 (0.3%)		128
EUROPE						
2001, Sept – 2002, Jan; Denmark	5% in a commercial lotion; 10% in pet. also tested with the European standard series	217	5% lotion: 1.4% weak positive; 20.3% weak irritant reactions 10% pet: 0.5% (++ reaction)		Finn chambers were applied to the upper back for 2 d; the test sites were scored on day 3 using ICDRG criteria 3 subjects had weakly positive reactions to the lotion (categorized as non-relevant) 44 subjects had weak irritant reactions to the lotion 1 subject had a "++" reaction to the test substance in pet. and the lotion (this subject had previously experienced dermatitis following application of a cosmetic product that contained tea tree oil)	131
2003, June – Aug; Denmark	5% (4 lotions) also tested with the European standard series	160	3.1% had irritant reactions 0 allergic reactions		Finn chambers were applied to the upper back for 2 d; the test sites were scored on day 3 using ICDRG criteria no allergic reactions to the lotions were reported 5 subjects (3.1%) had irritant reactions: 1 subject reacted to all 4 lotions and all substances in the European standard series; 3 had weak irritant reactions to 3 of the lotions; 1 subject had a weak irritant reaction to all 4 lotions	131
pre-2004 (yr not stated; 15 mo study) Sweden (4 clinics)	5% in alcohol	1075	2.7% 3.0 (F)/1.9 (M) 3.1% irritant/doubtful	not stated	509/1075 have/had adverse reactions to cosmetics or skin care products	132
1999-2000; Germany and Austria (11 labs); DKG	standardized, 5% in diethyl phthalate	3375	36 (1.1%)	56%	readings were taken on days 2 and 3 positive patch test reactions ranged from 0 to 2.3% among the centers 36 patients (1.1%) with reactions; 14 of these patients also had a positive response to oil of turpentine regional differences in frequencies were noted	4,8,133
1998-2003; Germany	oxidized, 5% (contained 16 identified allergens)	6896	70 (1.0%)		38 of the patients with positive results were tested with the 16 single allergens; reactions were observed with the following: terpinolene (23); ascaridole (21); α-terpinene (18); 1,2,4-treihydroxymenthane (14); α-phellandrene (10); (+)-limonene (5); myrcene (4); viridiflorene (S) (3); aromadendrene (S) (1) No reactions were observed with (+) or (-)-carvone; sabinene; terpinen-4-ol; *p*-cymene; 1,8-cineole, or α-pinene	134
1999 – 2003, Germany	oxidized, 5% (contained 16 identified allergens)	2284	21 (0.9%)		20 of the patients with positive results were tested with the 16 single allergens; reactions were observed with the following: terpinolene (17); ascaridole (15); α-terpinene (16); 1,2,4-treihydroxymenthane (13); α-phellandrene (7); (+)-limonene (11); myrcene (7); viridiflorene (S) (1); aromadendrene (S) (1); (+)-carvone (4); (-)-carvone (4); sabinene (2); terpinen-4-ol (1) No reactions were observed with *p*-cymene; 1,8-cineole, or α-pinene	134

Table 17. Retrospective, multicenter, and cross-sectional patch test studies with tea tree oil

Years/Testing Group	Concentration/Vehicle	# patients	# Positive (%)	Relevance	Comments	Reference
2012, Feb – 2013, Mar; Netherlands	5% oxidized tea tree oil	221	2 (0.9%; +)		no irritant reactions reported	[135]
2012, Nov – 2013, Feb	1, 2, and 5% ascaridole and 5% oxidized tea tree oil	additional 29 re-patch patients from a different ascaridole study (250 total)			co-sensitization was evaluated: in 30 patients that had positive reactions to any concentration of ascaridole, 6 tested positive to tea tree oil in 220 patients that did not react to any concentration of ascaridole, none reacted to tea tree oil	
1990-2016; Belgium	oxidized, 1 and 5%, pet	105, from a total of 15,980 patients tested (125 had tested positive to a botanical)	11(10.5%)		Retrospective analysis of patients who had attended a patch test clinic (tertiary referral center) because of contact dermatitis, and were identified as being allergic to herbal medicines and/or botanical ingredients Patch tests were applied to the back, and readings were performed according to ESCD guidelines	[136]
2000-2009; Belgium	not stated	301 reactions to a fragrance mix	1/88 (1.1%) reactions to skin care products	not stated	study of "presence confirmed" fragrance allergens in cosmetic products to which patients reacted positively a reaction was only observed in a skin care product, and not the other 14 cosmetic product categories, containing tea tree oil	[137]
2000-2010; Belgium	not stated	621 reactions to non-fragrance allergens	5/212 (2.4%) reactions to skin care products	not stated	study of non-fragrance allergens in cosmetic products to which patients reacted positively reactions were only observed in skin care products, and not the other 10 cosmetic product categories, containing tea tree oil	[138]
2011-2012; Italy (multicenter)	5% pet	19 patients that had positive reactions to botanicals	2 (10.5%)	100%	original test group consisted of 1274 patients that used botanicals; 139 had cutaneous reactions; 122/139 were patch tested with the botanical integrative series; 19 had positive reactions, 2 of which were to tea tree oil	[139]
1997; Swiss clinic	5, 10, 50, and 100% in arachis oil	1216	7 (0.6%)	not stated	14 eczema patients tested used products that contained tea tree oil; the elicitation concentrations were not given the study authors stated that allergic potential to low concentrations is presumed to be low on healthy skin; photoaged tea tree oil is the stronger sensitizer	[8,140]
pre-2015 (5 yrs ; years not specified); Spain	5% pet	not stated	5 (0.4%)	100%	strong reactions were observed in all patients 3/5 also reacted to limonene	[141]
1996-1997, UK	neat	29 patients thought to have a cosmetic dermatitis; plant series had been applied	7 (24.1%)	not stated	Patch tests were performed with a standard and plant series as well as the patient's own cosmetic products; in addition, where there was a strong suspicion of fragrance allergy, patients were also tested to an extended fragrance series Site of contact dermatitis was variable, but was primarily involved face, neck, or fingertips; 23 (79%) of the patients had a positive reaction to fragrance mix Reactions were mainly seen in people who had been using tea tree oil, and who gave a history of worsening dermatitis on use of the product; 5 of the 7 patients recalled use of products containing tea tree oil; one additional patient may have been exposed via aromatherapy; reactions were not thought to be irritant The researchers stated that although no controls were formally tested, the same concentration of tea-tree oil was tested routinely in their plant series, and over the same 2-yr period, 9/165 patients tested positively to the oil, including those reported in this study 23/29 patients had a positive reaction to the fragrance mix included in the standard series; 17 patients had a positive reaction to at least 1 component of the plant series	[142]
2001, UK	neat, oxidized	550	13 (2.4%)	definite: 4 (30%) possibly: 5 (38.5%)	irritant reactions – 38%	[4]

Table 17. Retrospective, multicenter, and cross-sectional patch test studies with tea tree oil

Years/Testing Group	Concentration/Vehicle	# patients	# Positive (%)	Relevance	Comments	Reference
2008-2014, UK	5% pet	2104	+/++/+++: 11 (0.5%) ?+: 2 (0.1%) irritant: 3 (0.1%)	not stated	Patients were also tested with a fragrance series; the researchers noted that 4 of the subjects with a positive reaction to tea tree oil did not react to any of the fragrance series ingredients, oxidized linalool, or oxidized limonene	[143]
2016, UK	5% pet	1019	0.29%	0.29%		[144]
2016-2017, UK/Ireland	oxidized, 5% pet	4224	0.45%			[114]
AUSTRALIA						
not stated	10%	219	2.9% - 4.8%	not stated	prevalence increased to 4.6-7.7% using only patients with prior tea tree oil exposure	[145]
1999	not stated	477	12 (2.5%)	not stated		[4]
2000-2004; Skin and Cancer Foundation	oxidized, 5% pet; oxidized, 10% in white soft paraffin	2320	41 (1.8%)	41%	17 of 41 patients with positive reactions recalled prior use of tea tree oil; 8 specified prior application of neat tea tree oil	[145]
2001-2010; Skin and Cancer Foundation	oxidized, 5% pet**	794	28 (3.5%)	43%		[146]
	10% pet	5087	129 (2.5%)	33%		

*NACDG procedures (48-h occlusive patches using Finn chambers o Scanpor tape) were followed
** patches obtained from Chemotechnique Diagnostics, which are supplied as oxidized tea tree oil, 5% pet
*** total testing period was 1994 – 2006; however, tea tree oil (pet, oxidized) was added to the NACDG test tray in 2003[113]

Table 18. Cross-reactivity with tea tree oil

Test Substance	Years/Location (if known)	positive reactions /# subjects	Cross Reactivity	Comments (if applicable)	Reference
5, 10, 50, and 100% tea tree oil in arachis oil	1997; Swiss clinic	7/1216 (described previously)	2 of the 7 patients also exhibited a type IV hypersensitivity towards fragrance mix or colophony	study authors stated there was a possibility of an allergic group reaction caused by contamination of the colophony with the volatile fractions of turpentines	[8,140]
5% tea tree oil in diethyl phthalate	1999-2000; Germany and Austria (11 labs)	36/3375 (described previously)	14/36 patients (38.9%) also had positive patch test reactions to oil of turpentine		[133]
5% tea tree oil in alcohol	pre-2004 (15 mo study); Sweden	2.7% (1075 subjects) (described previously)	no correlation was reported between positive reactions to tea tree oil and colophony		[132]
Other Compounds as the Test Substance					
compound tincture of benzoin	1999; Melbourne, Australia	45/477 patients with reaction to the tincture (there were 14 strong and 25 weak positive reactions on days 2 and 4, and 6 weak reactions on day 4 only))	9/45 patients (20%) also had positive reactions to tea tree oil 5/14 patients with strong (++) reactions to the tincture had ++ or +++ reactions to tea tree oil	patch testing with compound tincture of benzoin was occlusive	[148]
Cross-Reactions Described in Case Reports (see Table 19 for case report details)					
tea tree oil, undiluted		patient with atopic dermatitis	positive reactions to the tea tree oil and eucalyptol (+/+++)		[38]
tea tree oil, undiluted		patient had a 1-wk history of dermatitis on the forehead and around the mouth	an erythematopapular reaction (++) was reported at the application site of 20% colophony in pet		[149]
tea tree oil		patient with pruritic ery-thematous rash	positive reactions to tea tree oil and colophony		[150]
5% oxidized tea tree oil, pet 1, 2, and 5% ascaridole, pet		patient with periorbital dermatitis	"?" reaction to oxidized tea tree oil (days 3 and 7) + reactions to 1 and 2% ascaridole; irritant reaction to 5% ascaridole (days 3 and 7)	patient had used an herbal remedy containing tea tree oil to treat dermatitis, and a soap that contained tea tree oil	[151]
5% oxidized tea tree oil, pet 1, 2, and 5% ascaridole, pet		patient with periorbital dermatitis and folliculitis barbae	+ reaction to oxidized tea tree oil (days 3 and 7) + reactions to 1, 2, and 5% ascaridole (days 3 and 7)	patient had used a shaving cream that contained tea tree oil	[151]

Table 19. Case reports with tea tree oil

Test Substance	Subject(s)/Symptoms	Testing	Results/Comments	Reference
DERMAL EXPOSURE				
used in treatment of dermatitis and/or psoriasis				
tea tree oil, undiluted	a patient with long-standing atopic dermatitis was treated with undiluted tea tree oil; the dermatitis initially improved, but then worsened; the patient was then advised to ingest oil mixed with honey	patch testing was first performed with the European standard series, additional series (not described), and the patient's own products; additional testing was then performed with the main components of the oil all at 5% pet, except linalool was tested at 10% pet)	Initial patch testing produced positive reactions (++/++) to tea tree oil only Subsequent testing resulted in positive reactions to the oil and eucalyptol (+/+++) 20 controls had negative results	[38]
tea tree oil	subject treated atopic eczema with tea tree oil		became sensitized within 3 mo; also reacted to fragrances, turpentine, and several Compositae plants.	[108]
melaleuca oil (tea tree oil), undiluted	7 patients in a 3-yr period with eczematous dermatitis consisting of ill-defined plaques of erythema, edema, and scaling after application to compromised skin; vesiculation was present in 3 patients	48-h applications (Finn chambers) were made to the upper back with a standard battery of 20 allergens, and a 1% (v/v) solution of melaleuca oil, 1, 5, or 10% (v/v) solution of 11 primary constituents of *Melaleuca alternifolia*, and 5% d-carvone in in anhydrous ethanol (except myrcene was dissolved in olive oil); patches with ethanol and olive oil and a blank chamber were used as controls 20 control patients with unrelated dermatoses were patch tested with 1% melaleuca oil 10 control patients were patched with 1% of the 11 constituents and 5% d-carvone and 7 control patients were patched with 5 or 10% of the constituent compounds	- All patients reacted to 1% melaleuca oil (1 had a score of +2, 5 with a score of +3, 1 with a score of +4) - All patients reacted to 1% of: d-limonene (6 patients), α-terpinene (5 patients), and aromadendrene (5 patients) - 1% terpinen-4-ol, p-cymene, and α-phellandrene each caused a reaction in 1 patient - 1 subject had a reaction during testing with the routine battery controls: both groups had negative results to the test articles at 1%; most of the 7 controls reacted to 5 or 10% d-limonene, α-terpinene, aromadendrene, α-phellandrene, α-pinene, and aromadendrene	[109]
tea tree oil, 5% (pet, or own product)	5 patients presented with strong, relevant, reactions (on the eyelids, hands, arms, feet, or legs) after using tea tree oil to treat what was presumed to be dermatitis		All 5 subjects reacted (++ or +++) to tea tree oil; this corresponds to 0.4% of all patients studied over a 5-yr period 3 of the patients also reacted to oxidized d-limonene	[141]
tea tree oil	the patient presented with periorbital dermatitis; she had used an herbal remedy containing tea tree oil to treat dermatitis, and a soap that contained the oil	patch testing was performed with the local extended European baseline series and a cosmetic series; oxidized tea tree oil, 5% in pet was also tested	the patient did not react to the standard series a "?" reaction was observed on days 3 and 7 with oxidized tea tree oil	[151]
tea tree oil, undiluted	a patient with history of psoriasis applied the oil to psoriatic lesions on the leg and reported immediate, intense erythema of the legs, throat constriction, changes in phonation, pruritus, flushing and light-headedness. The subject had used tea tree oil shampoos, but had never applied oil to the lesions before.	Skin-prick and intradermal tests were conducted with 0.01, 01, and 1% dilutions in phenol saline solution. An ELISA for specific IgG and IgE against tea tree oil was performed. Five control subjects were also tested.	The patient did not react to the skin prick testing, and did not react to the low or mid-dose with intradermal testing, but there was a positive wheal and flare reaction within 20 min with 1% tea tree oil. No specific IgG or IgE was detected. Control results - negative	[152]
tea tree oil	used to treat psoriasis vulgaris		subject became sensitized within 3 mo; also reacted to fragrance mix, balsam of Peru, and turpentine	[108]
tea tree oil, 5% pet.	five patients had occupational contact dermatitis caused by limonene	these patients were patch-tested with tea tree oil	2 of the patients had a strong reaction (++) and 2 had a very strong reaction (+++) to tea tree oil, results were negative in the fifth subject	[153]
other direct skin applications				
wart paint containing tea tree oil (concentration not stated)	the patient had a 4-mo history of blistering dermatitis over the right temple that occurred 24 h after treatment of 2 seborrheic warts with a wart paint that contained tea tree oil	patch testing was performed using Finn chambers with the European standard series, 1% aq. tea tree oil, and other compounds	at d 3, a papulovesicular reaction (+++) was observed at the site of an open patch to the tea tree oil and an erythematopapular reaction (++) to 1% tea tree oil reported 50 controls were negative with 1 and 5%	[154]
tea tree oil	patient treated warts on his hands		became sensitized in 3 mo	[108]

Table 19. Case reports with tea tree oil

Test Substance	Subject(s)/Symptoms	Testing	Results/Comments	Reference
tea tree oil	the patient had a 9-yr history of large, painful, red lesions occurring on the face and neck; she had been using the oil for several skin conditions, including acne and tinea pedis	patient was instructed to discontinue using the oil on her face; a usage test was conducted with application of a small amount of the oil to the back of her neck 2x/d for 2 d	a large, ill-defined, erythematous eruption with severe pain and pruritus occurred at the site of the usage test patient was instructed to discontinue using products with the oil; incidental use of a tea-tree oil toothpaste cause lesions in the mouth; otherwise, no lesions were observed	155
tea tree oil, undiluted	the patient had a 1-wk history of dermatitis on the forehead and around the mouth; she had used the oil for years without any similar reactions; the symptoms worsened with topical treatment with corticosteroids and erythromycin	patch testing was performed with the European standard series and the oil using Finn chambers	at day 3, a papulovesicular reaction (+++) was observed with the tea tree oil, and an erythematopapular reaction (++) was reported at the application site of 20% colophony in pet	149
tea tree oil	6-wk history of papulo-vesicular eruption affecting the left forearm; condition had worsened with application of tea tree oil	patch testing was performed with the oil	strongly positive reaction after 48 h of patch testing The condition cleared with discontinuation of oil and application of topical corticosteroids	156
tea tree oil, 5%	bullous eruption resulting from allergic contact dermatitis caused by application of Burnshield®, a tea tree oil-containing hydrogel, and a Burnshield® dressing	occlusive 48-h patch testing was conducted on the upper back using the British Contact Dermatitis Society baseline series, a cosmetic/facial series, a fragrances/ essential oils series, and the patient's own products, including the Burnshield® products	Positive reactions to tea tree oil were recorded on day 2 (+) and day 4 (++). Positive reactions (+++) also were observed at both time periods with both Burnshield® products. (Positive results were also reported with a number of other test substances.)	157
tea tree oil, 5%	applied to treat chronic, recurrent tinea versicolor	testing was not done; the patient was instructed to apply hydrocortisone	patient suddenly developed a pruritic confluent erythematous rash on the anterior neck and upper back; the rash completely resolved within 1 wk of discontinuing application of the oil	158
tea tree oil	plaster applied to breast skin after an operation, and treated with tea tree oil; the oil was also applied due to insect bites		irritant reaction to tea tree oil; also reacted to turpentine	108
tea tree oil (concentration not stated; assumed undiluted)	The patient applied the oil to the umbilicus area following piercing, and after 2 wk of exposure developed a pruritic erythematous rash over the umbilical region, which gradually spread, with the development of blisters; the patient was prescribed erythromycin and was advised to continue applying the oil, which resulted in an increase in the size and number of the blisters and a separate vesicular eruption on the left flank at the site of contact with medical tape	patch testing was performed with the European standard series, tea tree oil, and "Ster-Zac" powder, which she also used a histological exam was also performed	patch testing reported positive reactions to tea tree oil and colophony The histological examination showed subepidermal blistering with edematous dermal papillae containing numerous neutrophils; direct immunofluorescence showed a bright linear band of IgA at the basement membrane zone in peri-lesional skin; these results were reported to be characteristic of linear IgA disease	150
tea tree oil	used to treat sunburn		no reactions at site of application, but reacted to tea tree oil at patch testing	108
tea tree oil	10-yr old male with irritating eruption on the left knee and an itch on the sole of the right foot; the oil had been applied 3x/d. Upon examination, the patient had an acute vesiculo-bullous eruption affecting the lower thigh and upper lower leg in the region of the left knee, and a bulla was also present on the sole of the right foot near the metatarso-phalangeal joint	Patch testing was performed with the oil	A bullous reaction appeared after 24 h, necessitating removal of the patch. The lesions cleared with application of cold compresses and topical corticosteroids. .	156
tea tree oil (and other herbal extracts)	patient solely used herbal extracts for hygiene and cosmetic purposes, including at least 500 ml of tea tree oil		became sensitized and had to be admitted to the hospital for treatment of skin lesions reacted to colophony, Compositae plants, fragrances, turpentine, and 10 different plant oils	108

Table 19. Case reports with tea tree oil

Test Substance	Subject(s)/Symptoms	Testing	Results/Comments	Reference
tea tree oil	The patient presented with a severe and widely scattered dermatitis of 1 wk duration; the left shin displayed an 8 x 20 cm, scarlet, annular plaque with a purpuric margin; numerous other erythematous papules and plaques, ranging in size from 0.5 - 3 cm, were scattered on the trunk and the extensor aspect of the extremities; no involvement of the palms, soles, or mucous membranes. 3 wk prior, the patient treated a superficial abrasion of the left shin with tea tree oil under an occlusive dressing; after 2 wk, the treated area became red and itchy. Applications were discontinued, but lesions on the left leg enlarged in an annular pattern and spread to distant sites on the trunk and extremities.	Patient was treated medically, and lesions cleared within 2 wk. After 5 mo, patch testing was performed with the North American standard series, tea tree oil, abitol, abietic acid, and turpentine peroxides, as well as with the patient's aged (oxidized) sample of tea tree oil.	at 96 h, the patient reacted to both tea tree oil samples, with a stronger reaction the aged preparation. (He also had positive reactions to colophony, balsam of Peru, and abitol.) The researchers stated that although, clinically, the case mimicked erythema multiforme, that diagnosis was not supported by the histological findings, which were those of a spongiotic dermatitis. The researchers stated that erythema multiforme–like id-reaction described the eruption.	[159]
tea tree oil products (and creams containing lavender oil)	marked erythema and lichenification of the groin, suprapubic area, and perianal and vulval mucosa; eczema of the right (dominant), but not left, hand; eczema of the periorbital area and axillae4 6-mo history of these symptoms; had used tea tree oil products extensively (and had also used creams containing lavender oil).	Patch testing was performed with the European standard series, tea tree oil, and aromatherapy lavender gel.	positive reactions at days 2 and 4 (++) with tea tree oil; also with lavender gel (++) and quaernium-15 (+)	[160]
5% tea tree oil, oxidized, in pet	patient had periorbital dermatitis and persistent follicular barbae		+ reaction to 5% oxidized tea tree oil patient used a shaving oil that contained tea tree oil; skin problem resolved with discontinued use	[151]
1 and 5% tea tree oil, in pet	patient was an aromatherapist with eczema on arms and upper trunk, which later spread to the legs, face, and hands; hand eczema became chronic and was associated with handling several different substances, including essential oils, which she diluted herself	Patch testing was performed with the European standard, a perfume series, and several essential oils	+ reaction with 1%, and ++ reaction to 5%, tea tree oil, on day 3 Also had positive reaction to the fragrance mix, some oils from the perfume series, and 17 of 20 essential oils that were tested	[161]
from hand wash or shampoos				
hand wash containing 3% tea tree oil	patient developed raised red lesions at the sites of contact within 5 min of application; the reaction occurred on 3 separate occasions; she had regularly used a tea tree oil shampoo without adverse effects	Patch testing was performed using IQ chambers with 3% (same oil as in the wash), 10 different samples of 10%, and the same 10 samples of 100% tea tree oil.	no reactions occurred with 3 or 10% tea tree oil; mild erythema and pruritus occurred with 6 of the oils in 1 test, and in 4of the oils in a second test testing with the individual component of the wash produced inconsistent results	[162]
shampoo containing tea tree oil	patient used the shampoo, and tea tree oil for blisters on his face	epicutaneous testing	patient became sensitized use of the products reacted to tea tree oil only (other test substances were not identified)	[108]
shampoo, to which tea tree oil was added			also reacted to fragrances, turpentine, and tiger balsam, which he had used against the side effects of the oil	[108]

Table 19. Case reports with tea tree oil

Test Substance	Subject(s)/Symptoms	Testing	Results/Comments	Reference
tea tree oil transfer to sunglasses	the patient presented with a 12-mo history of intermittent eye-lid dermatitis; she had a history of scalp psoriasis and no history of atopy; the patient was using a shampoo containing tea tree oil; the patient had previously applied pure tea tree oil to acne papules	48-h patches were applied using an extended European standard series, cosmetic series, ingredients of creams and a variety of her own samples (appropriately diluted); readings were taken on day 2 and day 4	0n day 4, there were positive results to nickel (++), tea tree oil (+), and scrapings from the frame of her sunglasses (+) (the sunglasses did not contain nickel) the rash resolved with avoidance of the shampoo and the sunglasses, but flared within 48 h of wearing the glasses. The glasses were thoroughly cleaned, and the rash did not reappear; the patient frequently placed her glasses on her wet hair, and it was assumed that sufficient residue of the tea tree oil shampoo was transferred to the sunglasses, precipitating the recurrent flares of eyelid dermatitis, even after the shampoo was no longer used	163
CASE REPORTS WITH OXIDIZATION COMPONENTS				
7 typical constituents (5 or 10%) and 2 degradation products (5%) of tea tree oil	15 patients sensitive to tea tree oil from both dermal and oral routes of exposure	Readings were taken at 72 h.	# of patients with reactions to constituents: 5% α-terpinene (10); 5% α-phellandrene (6); 10% terpinolene (15); 5% myrcene (2); d/l-carvone (1); 5% aromadendrene (1); 5% viridiflorene (2) # of patients with reactions to degradation products: 5 5% 1,2,4-trihydroxymenthane (11); 5% ascaridole (10)	164
EXPOSURE TO VAPORS				
tea tree oil, aq. solution	a patient with hand eczema and a known allergy to turpentine inhaled vapors from a hot aq. solution of the oil (concentration and duration of exposure not stated); after 2 successive days, he developed an acute exudative edematous dermatitis of the face and eyelids, which spread to his trunk and arms	Patch testing (Finn chambers) was first performed with the European standard series, a cosmetic series, several essential oils, and the patient's own products.	positive reactions were observed with tea tree oil, as well as colophony, fragrance mix, several oils, and methylchloroisothiazolinone	165

Table 20. SED of tea tree oil, assuming 3% absorption [8]

Product Type	Concentration of tea tree oil (%)	Amount applied (mg)	Retention Factor	SED (mg/kg/d)
tea tree oil (undiluted)	100	200	1	3.33
bath additive	15	10,000	0.01	0.25
cleansing face wash	0.7	5000	0.01	0.006
anti-dandruff shampoo	2.0	8000	0.01	0.027
deodorant stick/roller	2.5	500	1	0.21
foot powder	1.0	2000	1	0.33
foot spray	2.0	2000	1	0.67
body lotion	1.25	8000	1	1.67
hand wash	0.7	3000	0.01	0.0035
mouthwash	0.2	10,000	0.1	0.033
hand wash /solid soap	2.0	500	0.01	0.0017

Table 21. SED and MOS of tea tree oil, assuming 100% absorption [29]

Product Type	Concentration of tea tree oil (%)	Calc relative daily exposure (mg/kg bw/d)	SED (mg/kg bw/d)	MOS (NOAEL/SED)*
mouthwash	0.2	32.54	0.065	1798
shampoo	2.0	1.51	0.030	3900
deodorant stick/roller	2.5	22.03	0.55	213
foot powder**	1.0	1.67	0.033	3545
body lotion (total body)	1.25	123.20	1.54	76
hand wash /solid soap	2.0	3.33	0.067	1757
neat (nails)	NS	NS	1.67	
overall***			2.22	53

* NOAEL = 117 mg/kg bw/d (for renal effects, derived based on repeated dose systemic toxicity of tea tree oil constituents)
**2 applications/d
**shampoo + deodorant stick + foot powder + body lotion + hand wash soap + neat tea tree oil (nails)
NS – not stated

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MELALEUCA ALTERNIFOLIA (TEA TREE) EXTRACT	Baby Lotions, Oils, Powders, and Creams	01B	1
MELALEUCA ALTERNIFOLIA (TEA TREE) EXTRACT	Other Baby Products	01C	1
MELALEUCA ALTERNIFOLIA (TEA TREE) EXTRACT	Bath Oils, Tablets, and Salts	02A	1
MELALEUCA ALTERNIFOLIA (TEA TREE) EXTRACT	Shampoos (non-coloring)	05F	1
MELALEUCA ALTERNIFOLIA (TEA TREE) EXTRACT	Tonics, Dressings, and Other Hair Grooming Aids	05G	3
MELALEUCA ALTERNIFOLIA (TEA TREE) EXTRACT	Lipstick	07E	1
MELALEUCA ALTERNIFOLIA (TEA TREE) EXTRACT	Other Makeup Preparations	07I	1
MELALEUCA ALTERNIFOLIA (TEA TREE) EXTRACT	Basecoats and Undercoats	08A	1
MELALEUCA ALTERNIFOLIA (TEA TREE) EXTRACT	Bath Soaps and Detergents	10A	3
MELALEUCA ALTERNIFOLIA (TEA TREE) EXTRACT	Other Personal Cleanliness Products	10E	3
MELALEUCA ALTERNIFOLIA (TEA TREE) EXTRACT	Cleansing	12A	1
MELALEUCA ALTERNIFOLIA (TEA TREE) EXTRACT	Face and Neck (exc shave)	12C	16
MELALEUCA ALTERNIFOLIA (TEA TREE) EXTRACT	Body and Hand (exc shave)	12D	1
MELALEUCA ALTERNIFOLIA (TEA TREE) EXTRACT	Moisturizing	12F	15
MELALEUCA ALTERNIFOLIA (TEA TREE) EXTRACT	Paste Masks (mud packs)	12H	5
MELALEUCA ALTERNIFOLIA (TEA TREE) EXTRACT	Skin Fresheners	12I	1
MELALEUCA ALTERNIFOLIA (TEA TREE) EXTRACT	Other Skin Care Preps	12J	7

MELALEUCA ALTERNIFOLIA (TEA TREE) FLOWER/LEAF/STEM EXTRACT	Shampoos (non-coloring)	05F	4
MELALEUCA ALTERNIFOLIA (TEA TREE) FLOWER/LEAF/STEM EXTRACT	Tonics, Dressings, and Other Hair Grooming Aids	05G	1
MELALEUCA ALTERNIFOLIA (TEA TREE) FLOWER/LEAF/STEM EXTRACT	Other Hair Preparations	05I	2
MELALEUCA ALTERNIFOLIA (TEA TREE) FLOWER/LEAF/STEM EXTRACT	Other Manicuring Preparations	08G	2
MELALEUCA ALTERNIFOLIA (TEA TREE) FLOWER/LEAF/STEM EXTRACT	Bath Soaps and Detergents	10A	1
MELALEUCA ALTERNIFOLIA (TEA TREE) FLOWER/LEAF/STEM EXTRACT	Other Personal Cleanliness Products	10E	1
MELALEUCA ALTERNIFOLIA (TEA TREE) FLOWER/LEAF/STEM EXTRACT	Aftershave Lotion	11A	1
MELALEUCA ALTERNIFOLIA (TEA TREE) FLOWER/LEAF/STEM EXTRACT	Cleansing	12A	4
MELALEUCA ALTERNIFOLIA (TEA TREE) FLOWER/LEAF/STEM EXTRACT	Face and Neck (exc shave)	12C	9
MELALEUCA ALTERNIFOLIA (TEA TREE) FLOWER/LEAF/STEM EXTRACT	Moisturizing	12F	3
MELALEUCA ALTERNIFOLIA (TEA TREE) FLOWER/LEAF/STEM EXTRACT	Paste Masks (mud packs)	12H	1

MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF	Other Eye Makeup Preparations	03G	1
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF	Tonics, Dressings, and Other Hair Grooming Aids	05G	1
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF	Foundations	07C	2
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF	Other Manicuring Preparations	08G	2
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF	Cleansing	12A	2
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF	Face and Neck (exc shave)	12C	3
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF	Moisturizing	12F	4
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF	Paste Masks (mud packs)	12H	1
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF	Other Skin Care Preps	12J	1

MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF EXTRACT	Bath Soaps and Detergents	10A	1
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF EXTRACT	Cleansing	12A	2
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF EXTRACT	Face and Neck (exc shave)	12C	10
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF EXTRACT	Body and Hand (exc shave)	12D	1
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF EXTRACT	Moisturizing	12F	1
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF EXTRACT	Paste Masks (mud packs)	12H	1
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF EXTRACT	Other Skin Care Preps	12J	1

MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF OIL	Baby Shampoos	01A	1
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF OIL	Baby Lotions, Oils, Powders, and Creams	01B	5
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF OIL	Other Baby Products	01C	3
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF OIL	Bath Oils, Tablets, and Salts	02A	15
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF OIL	Bubble Baths	02B	2
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF OIL	Other Bath Preparations	02D	4
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF OIL	Eye Lotion	03D	2
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF OIL	Other Eye Makeup Preparations	03G	2
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF OIL	Perfumes	04B	5
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF OIL	Other Fragrance Preparation	04E	18
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF OIL	Hair Conditioner	05A	29
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF OIL	Rinses (non-coloring)	05E	1
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF OIL	Shampoos (non-coloring)	05F	55
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF OIL	Tonics, Dressings, and Other Hair Grooming Aids	05G	32
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF OIL	Wave Sets	05H	1
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF OIL	Other Hair Preparations	05I	16
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF OIL	Face Powders	07B	5
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF OIL	Foundations	07C	11
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF OIL	Lipstick	07E	3
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF OIL	Makeup Bases	07F	2
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF OIL	Other Makeup Preparations	07I	5
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF OIL	Basecoats and Undercoats	08A	2
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF OIL	Cuticle Softeners	08B	3
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF OIL	Other Manicuring Preparations	08G	6
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF OIL	Dentifrices	09A	8
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF OIL	Mouthwashes and Breath Fresheners	09B	3
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF OIL	Other Oral Hygiene Products	09C	5
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF OIL	Bath Soaps and Detergents	10A	72
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF OIL	Deodorants (underarm)	10B	27
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF OIL	Douches	10C	2
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF OIL	Other Personal Cleanliness Products	10E	15
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF OIL	Aftershave Lotion	11A	2
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF OIL	Beard Softeners	11B	14
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF OIL	Preshave Lotions (all types)	11D	3
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF OIL	Shaving Cream	11E	3
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF OIL	Shaving Soap	11F	2
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF OIL	Other Shaving Preparation Products	11G	3
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF OIL	Cleansing	12A	68
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF OIL	Depilatories	12B	1
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF OIL	Face and Neck (exc shave)	12C	64
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF OIL	Body and Hand (exc shave)	12D	25
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF OIL	Foot Powders and Sprays	12E	6
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF OIL	Moisturizing	12F	81
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF OIL	Night	12G	6
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF OIL	Paste Masks (mud packs)	12H	13
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF OIL	Skin Fresheners	12I	7
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF OIL	Other Skin Care Preps	12J	63
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF OIL	Suntan Gels, Creams, and Liquids	13A	2
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF OIL	Other Suntan Preparations	13C	1

MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF POWDER	Other Personal Cleanliness Products	10E	1
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF POWDER	Cleansing	12A	1
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF POWDER	Paste Masks (mud packs)	12H	1

MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF WATER	Face Powders	07B	2
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF WATER	Face and Neck (exc shave)	12C	1
MELALEUCA ALTERNIFOLIA (TEA TREE) LEAF WATER	Moisturizing	12F	4

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review

FROM: Carol Eisenmann, Ph.D.
Personal Care Products Council

DATE: April 11, 2019

SUBJECT: Concentration of Use byFDA Product Category: *Melaleuca alternifolia* (Tea Tree)-Derived Ingredients

Concentration of Use by FDA Product Category – Tea Tree-Derived Ingredients*

Melaleuca Alternifolia (Tea Tree) Leaf Oil
Melaleuca Alternifolia (Tea Tree) Extract
Melaleuca Alternifolia (Tea Tree) Flower/Leaf/Stem Extract
Melaleuca Alternifolia (Tea Tree) Flower/Leaf/Stem Oil
Melaleuca Alternifolia (Tea Tree) Leaf
Melaleuca Alternifolia (Tea Tree) Leaf Extract
Melaleuca Alternifolia (Tea Tree) Leaf Powder
Melaleuca Alternifolia (Tea Tree) Leaf Water

Ingredient	Product Category	Maximum Concentration of Use
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Hair conditioners	0.0072-0.01%
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Shampoos (noncoloring)	0.01-0.1%
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Tonics, dressings and other hair grooming aids	0.01-0.3%
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Lipstick	0.02%
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Cuticle softeners	0.63%
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Nail polish and enamel	0.005%
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Other manicuring preparations	0.01-0.4%
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Dentifrices	0.017%
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Mouth washes and breath fresheners	0.01%
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Other oral hygiene products	0.00025%
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Bath soaps and detergents	0.00025-0.3%
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Deodorants Not spray Aerosol	 0.2% 0.5%
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Other personal cleanliness products	0.005-0.01%
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Other shaving preparations	0.05-0.2%
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.01%
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Foot powders and spray	0.03%
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Moisturizing products Not spray	 0.003%
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Other skin care preparations	0.05%
Melaleuca Alternifolia (Tea Tree) Flower/Leaf/Stem Extract	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.001%
Melaleuca Alternifolia (Tea Tree) Flower/Leaf/Stem Extract	Other skin care preparations	0.01%
Melaleuca Alternifolia (Tea Tree) Leaf Extract	Skin cleansing (cold reams, cleansing lotions, liquids and pads	0.001%
Melaleuca Alternifolia (Tea Tree) Leaf Extract	Other skin care preparations	0.0001%

*Ingredients included in the title of the table but not found in the table were included in the concentration of use survey, but no uses were reported.

Information collected in 2019; Table prepared April 10, 2019

SAFETY DATA SHEET

Version 1.04 Issued 04 May 2018

Tea Tree (*Melaleuca alternifolia*) leaf oil

1. IDENTIFICATION of the SUBSTANCE and the COMPANY

Product Name: Tea Tree (*Melaleuca alternifolia*) leaf oil
Other Names: Tea Tree Oil, Melaleuca oil, *Melaleuca alternifolia* oil, Teebaumöl
Recommended Use: Topical antibacterial, antiseptic and anti-inflammatory agent
Australian AHECC Code and Name: 3301.29.60, Essential Oil of Tea Tree (*Melaleuca alternifolia*)

Suppliers Product Name (as Labelled)	

Supplier	
ABN	
Street Address	
Telephone	
Facsimile	
Email	

Emergency Telephone Number	

2. HAZARD IDENTIFICATION

UN Proper Shipping Name: TERPENE HYDROCARBON, N.O.S. (Tea Tree Oil)
UN Number: 2319
UN Packing Group: III
GHS Classification: Flammable liquids 3, Acute toxicity 4, Acute inhalation 4, Skin irritation 2, Aspiration toxicity 1, Aquatic chronic 2. [1]

GHS Pictograms:

GHS Signal word: Danger [1]
Hazard Statements: **H226** Flammable liquid and vapour, **H302** Harmful if swallowed, **H304** May be fatal if swallowed and enters airways, **H315** Causes skin irritation, **H332** Harmful if inhaled, **H411** Toxic to aquatic life with long lasting effects. [1]

GHS Precautionary Statements [1]

Prevention:	**P210, P233, P235, P240, P241, P242, P243, P261, P264, P270, P280, P280, P353, P361.**
Response:	**P301, P302, P303, P304, P310, P312, P313, P321, P330, P331, P332, P340, P352, P353,P361, P362, P364, P370, P378, P391, P403**
Storage:	**P235, P403, P405**
Disposal:	**P501** (For full precautionary statements see Section 15 on page 7)

Poisons Schedule: S6 - Poison

Health Hazards: Flammable liquid and vapour, Harmful if swallowed, May be fatal if swallowed and enters airways, Causes skin irritation, Harmful if inhaled. [1]

Reactivity Hazards: None known

Environmental Hazards: Toxic to aquatic life with long lasting effects. [1]

Emergency Considerations: Emergency responders must wear proper personal protective equipment and have appropriate fire suppression equipment suitable for the situation to which they are responding

EU Labelling and Classification: For further information under CLP Regulation (EC) 1271/2008 refer to section 15 on page 7

Health Hazards or Risks from Exposure:

Acute: Causes irritation to the skin, Harmful if swallowed, May be fatal if swallowed and enters airways, Causes skin irritation, Harmful if inhaled. [1]

Chronic: Toxic to aquatic life with long lasting effects. [1]

3. COMPOSITION/INFORMATION ON INGREDIENTS

Chemical Identity: Oil of Melaleuca, Terpinen-4-ol type ISO 4730:2017 [1]

Common Names: Tea Tree Oil, Melaleuca oil, *Melaleuca alternifolia* oil, Teebaumöl

HAZARDOUS INDREDIENTS	CAS Number	EINECS Number (EC No.)	ICSC Number	Weight %	HAZARD CLASSIFICATION; RISK PHRASES
Tea Tree Oil	85085-48-9 or 68647-73-4	285-3771	Not Established	100%	**Hazard Classification:** Flammable liquid 3, Acute toxicity 4, Acute inhalation 4, Skin Irritation 2, Aspiration toxicity 1, Aquatic Chronic 2. **Hazard Statements:** H226, H302, H304, H315, H322, H411
Balance of water and other components. Each of the other components is present in less than 1% concentration (0.1% concentration for potential carcinogens, reproductive toxins, respiratory tract sensitisers and mutagens)					Hazard Classification: Not classified Hazard Statements: None

NOTE: All Canadian WHMIS required information is included in appropriate sections based on GHS format. This product has been classified in accordance with hazard criteria of the GHS and the SDS contains all the information required by the GHS, EU Directives and the Japanese Industrial Standard JIS Z 7250: 2000

See Sections 2 and 15 for full text of **Hazard Classification**, **Signal Words** and **Hazard Statements**

4. FIRST AID MEASURES

Individuals contaminated by chemical exposure must be taken for medical attention if any adverse effect occurs. Rescuers should be taken for medical attention if necessary. Take a copy of the label and this SDS to the health professional with contaminated individual.

Symptoms caused by exposure

Human adult: Hallucination, distorted perception, coma, diarrhoea, allergic dermatitis

Human child: Hallucination, distorted perception, sleep, ataxia, coma, somnolence, diarrhoea

Medical Attention and Special Treatment

Skin Contact: Wash contacted area thoroughly with soap and water. Remove exposed or contaminated clothing, taking care not to contaminate eyes. Seek medical attention if irritation develops

Inhalation: If fumes or vapours are inhaled, or breathing difficulty is experienced, remove victim to fresh air. If necessary, use artificial respiration to support vital functions. Seek immediate medical attention if breathing difficulty persists

Ingestion: If the chemical is swallowed, call a physician or poison control centre for the most current information. If no professional advice is available, DO NOT induce vomiting, rinse the mouth. Never induce vomiting or give diluents (milk or water) to someone who is unconscious, having convulsions or who cannot swallow. Victims of chemical exposure must be taken for medical attention. Take a copy of the label and this SDS with the victim to a health professional.

Medical Conditions aggravated by exposure: Pre-existing skin, eye or respiratory problems may be aggravated by prolonged contact

Recommendation to Physicians: Treat symptoms and eliminate exposure

5. FIRE FIGHTING MEASURES

Flash Point: 59 °C (138 °F) [11]

Suitable fire extinguishing materials: Carbon dioxide, foam, dry chemical, halon or water fog/ mist.

Unsuitable fire extinguishing materials: Do not use full water jet

Unusual fire and explosion hazards: This product is flammable & vapours may travel some distance and flash back if ignited

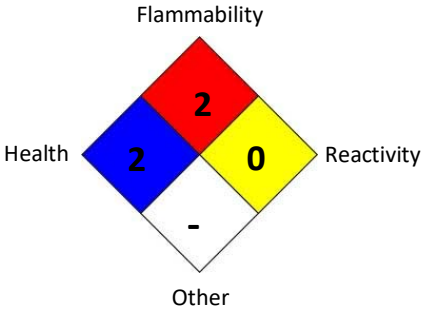
Explosion sensitivity to mechanical impact: Not sensitive

Explosion Sensitivity to static discharge: Sensitive

Specific hazards arising from the substance: May produce toxic fumes of carbon monoxide and/or carbon dioxide and hydrocarbons if burning.

Special firefighting procedures: Incipient fire responders should wear eye protection. Structural fire fighters must wear self-contained breathing apparatus and full protective equipment. Isolate materials not yet involved in the fire and protect personnel. Move containers from fire area if this can be done without risk; otherwise keep containers cool with carefully applied water spray/mist. If possible, prevent runoff water from entering storm drains, bodies of water or other environmentally sensitive areas

NFPA RATING:



Hazard Scale:

0 = Minimal
1 = Slight
2 = Moderate
3 = Serious
4 = Severe

6. ACCIDENTAL RELEASE MEASURES

Personal Precautions: Proper protective equipment should be used (see Section 8: Personal Protection). Personnel should be trained for spill response operations.

Emergency Procedures: Trained personnel following pre-planned procedures should handle non-incidental releases.

Spill Containment/Clean-up: Contain spilled material using poly-pads or other suitable absorbent material. Avoid generating mists or sprays. Place all spill residues in an appropriate container and seal. Ventilate area and wash spill area after material pickup is complete.

Environmental Precautions: Prevent run-off into drains and waterways. Decontaminate area thoroughly. Do not mix with wastes from other materials. Dispose of in accordance with applicable Federal, State and Local procedures (see Section 13).

7. HANDLING and STORAGE

Work Practices and Hygiene Practices: Read all labels before use. As with all chemicals; avoid getting this product on you or in you. Wear personal protective equipment (see Section 8) and wash thoroughly after handling this product. Do not eat, drink, smoke or apply cosmetics while handling this product. Avoid breathing mists or sprays generated by this product. Use in a well ventilated location. Remove contaminated clothing immediately.

Storage and Handling Practices: Observe all Federal and State regulations pertaining to the storage and handling of flammable liquids. Store in a cool, dry, well ventilated area away from direct sunlight. Keep containers tightly closed when not in use. Store away from sources of heat or ignition (sparks, open flame, hot surfaces). Store away from incompatible materials (oxidising agents and acids). Inspect regularly for damage and leaks. Take precautionary measures against static discharge: Ground container and receiving equipment, use only non-sparking tools and use explosion-proof electrical and other equipment.

This product is listed in the Australian Scheduling of Drugs and Poisons as a Schedule 6 Poison; storage and handling procedures must be in accordance with the relevant regulations.

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Ventilation and Engineering Controls: Use with adequate ventilation to ensure exposure levels are maintained below the limits provided below

Chemical Name	CAS Number	ACGIH-TLV's	OSHA PEL's	NIOSH-TLV's	Other
Tea Tree Oil	85085-48-9 or 68647-73-4	Not Established	Not Established	Not Established	Not Established

Currently, international exposure limits are not established for the components of this product. Please check with a competent authority in each country for the most recently established limits

The following information on Personal Protective Equipment (PPE) is provided to assist employers in complying with OSHA regulations found in 29 CFR sub-part I (beginning at 1910.132) or equivalent standard of Australia and Canada, or standards of EU member states (including EN 149 for respiratory PPE and EN 166 for face/eye protection), and those of Japan. Please reference applicable regulations and standards for full relevant details

Eye/Face Protection: Splash goggles or safety glasses with side shields are recommended. If necessary, refer to US OHSA Standard 29 CFR 1910.133, the European Standard EN 166, the appropriate Australian Standards, Canadian Standards, or the relevant Japanese Standards

Hand Protection: Compatible protective gloves are recommended. Wash hands after removing gloves. If necessary, refer to US OHSA 29 CFR 1910.138, the European Standard DIN EN 374, the appropriate Australian Standards, Canadian Standards, or the relevant Japanese Standards

Body Protection: Use body protection appropriate to the task. Coveralls, rubber aprons or chemical protective clothing made from natural rubber are generally acceptable depending on the task. If a hazard of injury to the feet exists due to falling objects, rolling objects or where objects may pierce the soles of the feet or where an employee's feet may be exposed to electrical hazards, use foot protection in accordance with US OSHA 29 CFR 1910.136. If necessary refer to the appropriate Australian Standards, Canadian Standards, or the relevant Japanese and European Standards

Respiratory Protection: If exposure limits are exceeded, use only respiratory protection authorised in the US Federal OSHA Respiratory Standard 29 CFR 1910.134, equivalent US State standards, Canadian CSA Standard Z94.4-93, the European Standard EN 149 or equivalent EU member State Standards

9. PHYSICAL and CHEMICAL PROPERTIES

Appearance: Colourless to pale yellow liquid [11]
Odour: Myrtistic, characteristic [11]
Odour threshold: Mild [11]
pH: Not established
Melting point: Not applicable (liquid at room temperature)
Freezing point: -22 °C
Boiling point/range: 97 °C – 220 °C
Flash point: 59 °C (Penksy-Martin closed cup) [11]

Evaporation rate: Not established
Flammability: 55 °C (Cleveland open cup) [11]
Upper flammability: Not established
Lower flammability: Not established
Vapour pressure: 2100 Pa [18]
Vapour density: Not established
Relative density: 0.885-0.906 [11]
Solubility: Insoluble in water, 1 part miscible with 2 parts ethanol (85% v/v) at 20 °C [11]
Partition coefficient: Log_{10} P_{ow} = 3.4 – 5.5 [18]
Auto-ignition temp: 269 °C [1]
Decomposition temp: Not established
Viscosity (Kinematic): 2.86 mm^2/s at 20 °C and 1.71 mm^2/s at 40 °C [18]
(Dynamic): 2.54 mPa.s at 40 °C & 1.52 mPa.s at 40 °C [18]
VOC content (% volatile): 100% or 866-906 grams per litre (g/l)
Optical rotation: +7° to +12° at 20 °C [11]
Saturated vapour concentration: Not established
Release of invisible flammable vapours and gases: This product is flammable & vapours may travel some distance and flash back if ignited

10. STABILITY and REACTIVITY

Reactivity: None known
Chemical stability: Stable under ordinary conditions of use and storage
Conditions to avoid: Excessive heat, sparks, flames and other sources of ignition
Incompatible materials: Strong oxidising or reducing agents. Protect from air
Hazardous depolymerisation: Will not occur
Hazardous decomposition products: When heated, decomposition may produce hydrocarbons, CO and/or CO_2

11. TOXICOLOGICAL INFORMATION

Likely routes of exposure and symptoms related to exposure

Skin contact: Causes skin irritation. May cause erythema, irritation or oedema if oil is oxidised Repeated or prolonged skin contact may lead to allergic contact dermatitis in sensitised individuals.

Inhalation: Potential irritant. Over-exposure at high levels may result in mucous membrane irritation of the nose and throat with coughing

Ingestion: May be fatal if swallowed or enters airways. May result in allergic dermatitis, hallucination, ataxia, diarrhoea, central nervous system depression, sleep or coma

Measures of toxicity

Acute oral toxicity:	Oral LD_{50} rat:	1900 mg/Kg [9]
Skin corrosion/irritation:	Dermal LD_{50} rabbit:	>5000 mg/Kg [9]
Eye damage/irritation:	HET-CAM	Mild irritant [15]
Dermal Toxic Dose :	Feline:	5-7 mL/Kg [14]
Dermal Toxic Dose:	Canine:	0.143 – 0.164 g/Kg [14]
Dermal Toxic Dose:	Human adult:	> 25% (in white paraffin applied for 21 days) [20]
Oral Toxic Dose:	Human adult:	0.5 – 1.0 mL/Kg after repeat low dose exposure [18]
Oral Toxic Dose (1):	Human child:	0.5 mL/Kg [7, 12]
Oral Toxic Dose (2):	Human child:	Approx. 0.6 mL/Kg [16]

Toxic effects

Rat:	Somnolence, muscle weakness, ataxia, partial paralysis
Feline:	Ataxia, change to leukocyte count

Canine:	Somnolence, ataxia, partial paralysis
Human adult:	Hallucination, distorted perception, coma, diarrhoea, allergic dermatitis
Human child:	Hallucination, distorted perception, sleep, ataxia, coma, somnolence, diarrhoea

Sensitisation potential

Skin:	Low (modified FCA method, guinea pig model); LLNA [10]
Eye:	Category 2B for reversible eye effects [17]

Germ cell mutagenicity: Not mutagenic as determined by the Ames test [5]
Micronucleus Assay OEDC 474 [3]

Carcinogenicity: The components of this product are not listed by agencies tracking the carcinogenic potential of chemical compounds as follows:

NTP Regulated:	No
IARC Regulated:	No
OSHA Regulated:	No

Reproductive Toxicity Effects of this product and its components on the human reproductive system:

Mutagenicity:	The components of this product are not reported to produce mutagenic effects in humans
Embryotoxicity:	The components of this product are not reported to produce embryotoxic effects in humans
Teratogenicity:	The components of this product are not reported to produce teratogenic effects in humans
Reproductive Toxicity:	The components of this product are not reported to produce reproductive effects in humans

STOT: single exposure: No valid data
STOT: repeated exposure: No valid data
Aspiration hazard: No valid data

12. ECOLOGICAL INFORMATION

All work practices must be aimed at eliminating environmental contamination

Environmental Toxicity: Not acutely toxic to fish LC_{50} > 100 mg/L (OECD 203) [18]
Environmental Fate: May cause adverse side effects in an aquatic environment, biodegradable in seawater
Persistence and Degradability: This product is readily biodegradable (OECD 301F) [18]
Mobility in Soil: No data available
Other Adverse Effects: None known

13. DISPOSAL CONSIDERATIONS

Preparing waste for Disposal: Waste disposal must be in accordance with the appropriate Australian Federal, State and Local regulations as well as those of Canada, USA, EU Member States and Japan
Disposal methods: Dispose of containers and small amounts at an approved landfill site. For larger quantities contact a licensed professional waste disposal service
Precautions: Prevent contamination of drains and/or waterways

14. STORAGE and TRANSPORT INFORMATION

UN Proper Shipping Name: TERPENE HYDROCARBONS, N.O.S. (Tea Tree Oil)
UN Number: 2319
UN Transport Hazard Class: Flammable liquids category 3
UN Packing Group: III
GHS Packing Groups: P001, IBC02, LP01
GHS Labelling requirements
GHS Signal word: Danger
GHS Classifications: Flammable liquids 3, Acute toxicity 4, Acute inhalation 4, Skin irritation 2, Aspiration toxicity 1, Aquatic chronic 2

GHS Pictograms:

GHS Hazard Statements: **H226** Flammable liquid and vapour, **H302** Harmful if swallowed, **H304** May be fatal if swallowed and enters airways, **H315** Causes skin irritation,

H332 Harmful if inhaled, **H411** Toxic to aquatic life with long lasting effects.

Hazchem EAC Code: **3Y**

US DOT Labelling Requirements: Flammable Label (Flame pictogram)

Environmental Hazards: May cause adverse effects in aquatic environments. This product is biodegradable

Special Precautions during Transport

IATA and IMO Labelling Requirements: Flammable Label (Flame pictogram)

Aircraft Restrictions: Passenger Aircraft 60 l, Cargo Aircraft 220 l

Australian National Transport Commission: This produce is classified as **Dangerous Goods** under the Australian Dangerous Goods Code (ADG7).

US Dept. of Transport (DOT) Shipping Regulations: This product is classified as **Dangerous Goods** per DOT regulations under 49 CFR 172.101.

Transport Canada, Transport of Dangerous Goods Regulations: This product is classified as **Dangerous Goods** as per regulations of Transport Canada (Canadian Transport of Dangerous Goods).

International Air Transport Association (IATA): This product is classified as **Dangerous Goods** *requirements under IATA DG Regulations which are based in part on the UN Recommendations for the Transport of Dangerous Goods*

International Maritime Organisation (IMO) Designation: This product is classified as **Dangerous Goods** under IMO *DG Code which is based in part on the UN Recommendations for the Transport of Dangerous Goods*

European Agreement concerning the international carriage of Dangerous Goods by Road (ADR): This product is classified as **Dangerous Goods** by the United Nations Economic Commission for Europe

15. REGULATORY INFORMATION

Note: All countries have specific requirements for labelling depending on a wide variety of factors. The following regulatory information is provided to assist in complying with some common regulations for major export destinations including Australia, the USA, Canada, EU member states and Japan. Please reference applicable regulations and standards for full relevant details for destinations

Australia

AICS Status: All components of this product are listed or exempt

Standard for the Uniform Scheduling of Drugs and Poisons: Schedule 6 (**S6**) Poison

Classification & Labelling: UN GHS for classification and labelling of chemicals.

Classification: Flammable liquids 3, Acute toxicity 4, Acute inhalation 4, Skin irritation 2, Aspiration toxicity 1, Aquatic chronic 2

GHS Pictograms:

GHS Signal Word: Danger

GHS Hazard Statements: **H226** Flammable liquid and vapour, **H302** Harmful if swallowed, **H304** May be fatal if swallowed and enters airways, **H315** Causes skin irritation, **H332** Harmful if inhaled, **H411** Toxic to aquatic life with long lasting effects.

GHS Precautionary Statements *For full details refer to the appropriate section of this SDS*

Prevention:
P210: Keep away from heat/sparks/open flames/hot surfaces.-No Smoking, **P233**: Keep container tightly closed
P240: Ground/bond container and receiving equipment, **P242**: Use only non-sparking tools
P241: Use explosion proof electrical/venting/lighting equipment
P242: Use only non-sparking tools
P243: Take precautionary measures against static discharge
P261: Avoid breathing fumes, mist or vapours
P270: Do not eat, drink or smoke when using this product, **P264**: Wash thoroughly after handling
P280: Wear protective gloves//protective clothing/eye protection/face protection

Response:
P301 + P312: IF SWALLOWED immediately call a POISON CENTRE or doctor/physician if you feel unwell.
P302+P361+P353: IF ON SKIN (or hair): remove/take off immediately all contaminated clothing. Rinse skin with water/shower
P305+P351+P338: IF IN EYES: Rinse cautiously for several minutes, remove contact lenses if present & easy to do, continue rinsing.
P313+P317: If eye irritation persists get medical attention, **P330**: Rinse mouth
P331: Do NOT induce vomiting, **P332 + P313**: If skin irritation occurs: Get medical advice/attention.
P370+ P378: In case of fire: Use Carbon dioxide, foam, dry chemical for extinction
P304 + P340 IF INHALED: Remove person to fresh air and keep comfortable for breathing.
P362 + P364: Take off contaminated clothing and wash it before reuse, **P391**: Collect spillage.

Storage: **P403+P235**: Store in a well-ventilated place, keep cool
P405: Store locked up

Disposal: **P501**: Dispose of contents/container in accordance with local/regional/national/international regulations.

United States

SARA Reporting Requirements: None
Marine Pollutant: This product contains no component listed as a Marine Pollutant under 49 CFR 172.101 Appendix B
TSCA: All components in this product mixture are listed on the US TSCA inventory of chemicals or are exempt from listing
SARA 31/312: Acute Health: Yes; Chronic Health: No; Fire: Yes; Reactivity: No
US CERCLA (RQ): None
California (Proposition 65): This product does not contain any component above the 0.1% level which is listed as a California Proposition 65 Chemical

Canada

Canada DSL Inventory Status: All of the components of this product are on the Revised in Commerce List (ICL). This product is listed on the ICL as *Oils, tea-tree* under identifier 1600 and CAS # 68647-73-4
CEPA Substance List: No component of this product is on the CEPA First Priorities Substance List
Canadian WHMIS Classification and Symbol: Class B-2 Flammable Liquid. (Flame pictogram):
Canadian federal Hazardous Products Act (HPA) and associated Controlled Products Regulations (CPR)

European Union

EINECS: This material is listed on the European Inventory of Existing Chemical Substances (EINECS).
Classification & Labelling: CLP Regulation (EC) 1271/2008 classified as a substance of "Unknown or Variable composition, Complex reaction products or Biological materials" (UVCB substance).

International Chemical Inventories Summary

Listing of the components on individual country Chemical Inventories:

Asia-Pacific:	Listed or exempt	**Australian ICS:**	Listed or exempt
Korean ECL:	Listed or exempt	**Japanese ENICS:**	Listed or exempt
Philippines ICCS:	Listed or exempt	**Swisse Giftliste:**	Listed or exempt
USA TSCA:	Listed or exempt	**Canadian DSL:**	Listed or exempt

16. OTHER INFORMATION

Abbreviations

ACGIH American Conference of Governmental Industrial Hygienists, **ADG7** Australian Dangerous Goods 7th Edition, **AHECC** Australian Harmonized Export Commodity Classification, **AICS** Australian Inventory of Chemical Substances, **California (Proposition 65)** The Safe Drinking Water and Toxic Enforcement Act of 1986, **CAS** Chemical Abstracts Service, **CEPA** Canadian Environmental Protection Act, **CERCLA** Comprehensive Environmental Response Compensation and Liability Act, **CFR** Code of Federal Regulations, **CLP** Classification, Labelling & Packaging, **DSL** Domestic Substances List, **DIN** Deutsches Institut für Normung, **DOT** Department of Transport, **DPD** Dangerous Preparations Directive, **ECL** Existing Chemicals List, **ENICS** Existing national Inventory of Chemical Substances, **EU** European Union, **FCE** Formal Concept Analysis, **GHS** Globally Harmonised System, **HET-CAM** Hen's Egg Test Chorioallantoic Membrane, **IATA** International Air transport Association, **ICCS** Inventory of Chemicals and Chemical Substances, **ICS** Inventory of Chemical Substances, **IMO** International Maritime Organisation, **JIS** Japanese Industrial Standards, **LD_{50}**, Lethal Dose 50%, **LLNA** Local Lymph Node Assay, **MITI** Minister of International Trade and Industry, **NFPA** National Fire Protection Association, **NIOSH** National Institute for Occupational Safety and Health, **NOS** Not Otherwise Specified, **OECD** Organisation for Economic Cooperation and Development, **OSHA** Occupational Safety & Health Administration, **PELs** Permissible Exposure Limits, **PPE** Personal Protective Equipment, **RQ** Reportable Quantity, **SARA** Superfund Amendments and Reauthorization Act 1986, **SDS** Safety Data Sheet, **STOT** Single Target Organ Toxicity, **TLV** Threshold Limit Value, **TSCA** Toxic Substances Control Act, **UN** United nations, **UVCB** Unknown or Variable Composition, Complex reaction products or Biological Materials, **VOC** Volatile Organic Compound, **WHMIS** Workplace Hazardous Materials Information System.

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Disclaimer

This SDS was prepared using the data sources and references provided. The information in this document is believed to be correct at the date of issue but does not claim to be all inclusive and shall be used only as a guide. Users should consider these data as a supplement to other information gathered by them. Independent determination of suitability and completeness of information from all sources must be made to assure proper storage, handling and use of the material having regard to the health and safety of employees, customers and the environment.

Author: P Bryant

Version 1.0 (New) Issued 21 Feb 2020

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Lillian Gill, D.P.A.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: Beth A. Lange, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: March 2, 2016

SUBJECT: Melaleuca Alternifolia (Tea Tree) Leaf Oil

Product Investigations, Inc. 2016. Determination of the irritating and sensitizing propensities of 10% Melaleuca Alternifolia (Tea Tree) Leaf Oil (in Caprylic/Capric Triglyceride) on human skin.

1620 L Street, N.W., Suite 1200 | Washington, D.C. 20036 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

PRODUCT INVESTIGATIONS, INC.

151 East Tenth Avenue
Conshohocken, PA 19428
610-825-5855 • fax 610-825-7288

[redacted]

REPORT: PII Nº 35747

DETERMINATION OF THE IRRITATING AND SENSITIZING PROPENSITIES OF MT#2700253 ON HUMAN SKIN

10% Melaleuca Alternifolia (Tea Tree) Leaf Oil in Caprylic / Capric Triglyceride

PREPARED FOR

[redacted]

10 February 2016

TABLE OF CONTENTS

DETERMINATION OF THE IRRITATING AND SENSITIZING PROPENSITIES OF MT#2700253 ON HUMAN SKIN

1.00 OBJECTIVES:

.01 To identify and characterize the skin-damaging propensities that MT#2700253 can be induced to exercise under the conditions of this modified patch test procedure.

.02 To adjudge whether the exercise of such propensities under the test conditions contraindicates the kind of skin contact that would be occasioned during the appropriate use of the product.

2.00 DESIGN:

.01 A modified version of the Repeated Insult Patch Test (cf. Protocol M[redacted]) was conducted on a panel whose total was greater than one hundred subjects at the outset.

.02 The regimen comprised nine sequential 24-hour induction applications and two concurrently conducted 24-hour challenge applications, one on the initial induction site and one on a naive site.

.03 During the initial phase, the skin of the contact sites was graded and the grades recorded on Wednesdays, Fridays (i.e. twenty-four hours after patches had been removed), and Mondays (i.e. forty-eight hours after patches had been removed).

.04 During the challenge phase, the skin of the contact sites was graded within moments after the patches had been removed (24 hours post application) and again twenty-four hours later. Follow-up examinations were conducted thereafter only if adverse effects were present.

.05 This study was conducted in compliance with the standards of good clinical practices generally applicable for the protection of the privileges and well-being of individuals who participate in patch test procedures.

3.00 SPONSOR: [redacted]

[redacted]

4.00 STUDY PRODUCT: [redacted]

Sponsor Identification:	MT#2700253 [redacted]
Date received:	11/25/15
Quantity rec'd:	>665 g. gross wt.
Form used in study:	Volatilized
PI N[redacted]	35747

[redacted]

7.00 SELECTION OF SUBJECTS:

.01 RECRUITING:

Prospective subjects were recruited from surrounding localities via phone, posters and personal contact.

.02 INFORMED CONSENT:

All individuals who expressed interest in participating were given an informed consent document to read. This document, which each candidate had to read and sign before being entered into the study, presented the following information:

a. How many subjects were to be enrolled in the study;
b. The intended use of the product;
c. Why the product was being tested;
d. How the test was to be performed;
e. That the regimen was not intended to benefit a subject's health, well being, or quality of life.
f. The different ways that participation may be detrimental to a subject's health, well being, or quality of life.
g. That not all detrimental effects could be foreseen and made known at the time the informed consent was presented for the prospective subject's signature.
h. What commitments a subject had to make to be in compliance; and
i. What considerations a subject was entitled to receive and the conditions for receiving them.

.03 DETERMINATION OF ELIGIBILITY:

Information concerning a prospective subject's qualifications was obtained from the answers the subject gave in filling out a medical history form and in responding to specific questions. Those who did not meet the following criteria were rejected.

a. **Inclusion Criteria: Satisfaction of all the following items was obligatory:**
   i. The candidate was at least eighteen years old, and
   ii. agreed to comply fully with the scheduled study regimen, and
   iii. expressed awareness that a participant would incur risks that would affect her/his well-being, and
   iv. denied that the amount of the stipend had induced her/him to participate against her/his better judgment, and
   v. had read the informed consent agreement, and
   vi. had assured the interviewer that she/he had no questions about the informed consent's contents that had not been answered to her/his satisfaction, and
   vii. had signed the consent form willingly and without reservation.

b. **Exclusion Criteria:** Any one of the following items was cause for rejection:
   i. The candidate had an illness that contraindicated participation; or
   ii. a condition that rendered the skin unsuitable for use in this study; or
   iii. was using dosages of medications that could alter the skin's tolerance; or
   iv. had a documented history of intolerance to the category of products submitted for study; or
   v. was a female who was pregnant or was breast feeding an infant.

.04 PANEL INFORMATION:

a. **Panel N^{os}:** 15496

b. **Demographics:**

SEX	Number	Age Range
Female	58	18 – 73
Male	52	18 – 80

c. **Dedication:** This was a shared panel, i.e. the subjects were engaged in the evaluation of materials submitted by sponsors other than }

8.00 SITE INFORMATION:

.01 LOCATION:

MT#2700253 was assigned Band #6 on the left side of the back of each subject.

.02 IDENTIFICATION OF A CONTACT SITE:

At each visit the skin around the contact site was marked to facilitate examinations after the device was removed and positioning of subsequently-applied devices as precisely as was feasible on the same site.

9.00 PATCHING DEVICES:

.01 TYPE OF DEVICE:

Partially-occlusive patching devices consisting of a 2cm x 2cm absorbent pad centered on the adhesive-coated surface of a 2cm x 4cm plastic film were used to convey and maintain the product on the skin.

.02 PREPARATION OF A PATCHING DEVICE:

The webril pad of a patching device was infused with 200 µL of the test material and allowed to volatilize for 30 minutes prior to application.

.03 POSITIONING AND REMOVING A PATCHING DEVICE:

a. A prepared device was positioned on its designated site on each subject with the product-treated surface of the pad in contact with the skin.
b. Firm pressure was applied to the backing of the device to affect intimate contact of the pad with the skin and to bond the flanges of the device securely to the skin.
c. When the time came for removing the device, the device was peeled off the skin as gently as was feasible under the circumstances.

10.00 DATA ACQUISITION:

.01 GRADING PROCEDURE:

a. Examinations of the contact sites to grade the effects elicited by the product were conducted on Mondays, Wednesday and Fridays. When a subject came in on a scheduled examination day, the technician examined the skin of the contact site.
   i. If no adverse effect was detected, a "0" was recorded in the subject's Case Report Form.
   ii. If an adverse effect was detected, the technician entered a grade indicating her assessment of the response's intensity.
b. The subject was then sent into the patching room where the site was examined again by a second technician to ascertain independently whether or not the site should be used again. If she disagreed with the first technician's assessment, the application was held in abeyance until the issue could be resolved with the help of the supervisor and/or the investigator.
c. The supervisor or the investigator was called in not only when a disagreement had to be resolved, but also to validate substantial sudden changes, e.g. when a response is deemed to merit a grade ≥3 or when a response has been judged to have decreased by two or more points from the previous day's status.

.02 CRITERIA FOR GRADING RESPONSE INTENSITY:

The following scale was used in this procedure to designate the intensities of those gross skin changes that may be occasioned by exposing the surface of the skin to a product.

Morphology	Visible Change	Grade
Subclinical Stage	None	0
Inflammation		
Vascular Dilation:	Faint redness with poorly defined margins	1
	Redness with well-defined margins	2
Infiltration:	Redness plus well-defined edema	3
	Redness plus papules, or vesicles or ulceration	4

.04 SITE CHANGES:

a. **Switch to a Naive Site:**
   i. If the product had elicited a Grade 2 response on a subject, application of the product would have been switched immediately to a naive site on the subject.
b. **Discontinuation of Applications:**
   i. If the product had elicited a second Grade 2 on a subject, application of the product would have been discontinued immediately for the remainder of the initial phase on the affected subject.
   ii. If the product had elicited a Grade 3 response on a subject, application of the product would have been discontinued immediately for the remainder of the initial phase on the affected subject.

11.00 OVERVIEW OF STUDY REGIMEN:

	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Week #1	Apply-	Remove	Grade/Apply	Remove	Grade/Apply	–	–
Week #2	Grade/Apply	Remove	Grade/Apply	Remove	Grade/Apply	(Removed)	–
Week #3	Grade/Apply	Remove	Grade/Apply	Remove	Grade/Apply	(Removed)	–
Week #4	Grade	–	–	–	–	–	–
Week #6	Apply	Remove/Grade	Grade	Grade*	Grade*	–	–

*If necessary

12.00 STUDY REGIMEN:

.01 INITIAL/INDUCTION PHASE-

Week #1:

Monday:

i. As each subject presented herself/himself at the clinic, the skin of the contact site assigned to the product submitted for study was examined and ascertained to be suitable before applications were begun.
ii. A freshly-prepared patching device was applied on its assigned site.
iii. The skin around the device was marked and the subject was instructed to return on Tuesday/Wednesday.

Tuesday:

i. As each subject returned, the site-identifying marks were reinforced.
ii. The patching device was removed by a technician and the subject was instructed to return on Wednesday

Wednesday:

i. As each subject returned, the skin of the contact site was graded. The grade was recorded.
ii. A freshly-prepared patching device was applied on the same site.
iii. The site-identifying marks were reinforced and the subject was instructed to return on Thursday

Thursday:

i. As each subject returned, the site-identifying marks were reinforced.
ii. The patching device was removed by a technician and the subject was instructed to return on Friday.

Friday:

i. As each subject returned, the skin of the contact site was graded. The grade was recorded.
ii. A freshly-prepared patching device was applied on the same site.
iii. The site-identifying marks were reinforced.
iv. The subject was dismissed with instructions to remove the patching device on Saturday, to record the time of removal, and to return to the clinic on the following Tuesday for resumption of the regimen.

Week #2:

Monday:

i. As each subject returned, the skin of the contact site was graded. The grade was recorded.
ii. The time at which the patch was removed on Saturday was recorded.
iii. A freshly-prepared patching device was applied on the same site.
iv. The site-identifying marks were reinforced and the subject was instructed to return on Tuesday.

Tuesday:

i. As each subject returned, the site-identifying marks were reinforced.
ii. The patching device was removed by a technician and the subject was instructed to return on Wednesday

Wednesday:

i. As each subject returned, the skin of the contact site was graded. The grade was recorded.
ii. A freshly-prepared patching device was applied on the same site.
iii. The site-identifying marks were reinforced and the subject was instructed to return on Thursday

Thursday:

i. As each subject returned, the site-identifying marks were reinforced.
ii. The patching device was removed by a technician and the subject was instructed to return on Friday.

Friday:

i. As each subject returned, the skin of the contact site was graded. The grade was recorded.
ii. A freshly-prepared patching device was applied on the same site.
iii. The site-identifying marks were reinforced.
iii. The subject was dismissed with instructions to remove the patching device on Saturday, to record the time of removal, and to return to the clinic on the following Monday for resumption of the regimen.

Week #3:

Monday:

i. As each subject returned, the skin of the contact site was graded. The grade was recorded.
ii. The time at which the patch was removed on Saturday was recorded.
iii. A freshly-prepared patching device was applied on the same site.
iv. The site-identifying marks were reinforced and the subject was instructed to return on Tuesday.

Tuesday:

i. As each subject returned, the site-identifying marks were reinforced.
ii. The patching device was removed by a technician and the subject was instructed to return on Wednesday

Wednesday:

i. As each subject returned, the skin of the contact site was graded. The grade was recorded.
ii. A freshly-prepared patching device was applied on the same site.
iii. The site-identifying marks were reinforced and the subject was instructed to return on Thursday

Thursday:

i. As each subject returned, the site-identifying marks were reinforced.
ii. The patching device was removed by a technician and the subject was instructed to return on Friday.

Friday:

i. As each subject returned, the skin of the contact site was graded. The grade was recorded.
ii. A freshly-prepared patching device was applied on the same site.
iii. The site-identifying marks were reinforced.
iv. The subject was dismissed with instructions to remove the patching device on Saturday, to record the time of removal, and to return to the clinic on the following Monday for resumption of the regimen.

Week #4:

Monday:

i. As each subject returned, the skin of the contact site was graded. The grade was recorded.
ii. The site-identifying marks were reinforced and the subject was instructed to:
    i) report back to the clinic on the second Monday following to receive the challenge applications, and
    ii) to notify the investigator without delay should any significant changes occur in the skin of the contact site before Monday of the challenge week.

HIATUS PHASE: Week 4, Tuesday through Friday, all of Week 5.

.03 **CHALLENGE PHASE-**

Week #6:

Monday:

i. As each subject returned, the skin of the initial induction site was examined and ascertained to be free of any conditions that would have rendered it unfit for undergoing the challenge applications.
ii. A prepared device was applied on the initial induction site.
iii. A second prepared device was applied on a naive site.
iv. The skin around both devices was marked and the subject was instructed to return on Tuesday.

Tuesday: (Note: If a subject was absent on Monday, she/he was patched on Tuesday.)

i. As each subject returned, the site-identifying marks around both contact sites were reinforced.
ii. Both patching devices were removed by a technician.
iii. The skin of both contact sites was graded; the grades were recorded.
iv. The subject was instructed to return on Wednesday.

Wednesday:

i. As each subject returned, the skin of both contact sites was graded; the grades were recorded.

ii. If follow-up was indicated, the subject was instructed to return on Thursday, otherwise the subject was dismissed from the study of this material.

.04 FOLLOW-UP PHASE:

Week No. 7 and Week No. 8:

During the two weeks following the exit examination, the subjects were given the opportunity to relay any information concerning effects that were relevant to the characterization of the product as well as to communicate the need for treatment of persistent or newly-occurring responses.

13.00 PROCEDURE DEVIATIONS:

None were necessary

14.00 COMPLIANCE

PHASE	No. Of AEC's Required	EXCUSED	COMPLIANT YES	COMPLIANT NO
Induction	8	0	106	4
Challenge	1/1	0	102	8

106 of the 110 Subjects were in compliance with the number of required application/examination cycles during induction.
102 of the 110 Subjects were in compliance with the number of required application/examination cycles during challenge.

15.00 INCIDENCE OF RESPONSES:

GRADE	TYPE OF RESPONSE	INDUCTION PHASE	CHALLENGE PHASE: ORIGINAL CONTACT SITE	CHALLENGE PHASE: NAIVE CONTACT SITE
0	NO VISIBLE CHANGE	110 SUBJECTS	102 SUBJECTS	102 SUBJECTS
1	FAINT REDNESS, UNDEFINED BORDER	0 "	0 "	0 "
2	INTENSE REDNESS, DEFINED BORDER	0 "	0 "	0 "
3	REDNESS + DEFINITE EDEMA	0 "	0 "	0 "
4	REDNESS + PAPULES, OR VESICLES, ETC.	0 "	0 "	0 "
	NO. OF RESPONDERS	0 SUBJECTS	0 SUBJECTS	0 SUBJECTS
	NO DATA ACQUIRED	0 SUBJECTS	8 SUBJECTS	8 SUBJECTS

16.00 SIGNIFICANCE OF THE RESPONSES:

.01 INITIAL/INDUCTION PHASE:

No responses were noted on any of the 110 subjects who underwent at least one post-application examination. The absence of responses characterizes the product as one which is devoid of clinically significant skin-irritating propensities.

.02 CHALLENGE PHASE:

a. **Original Contact Sites:**
No responses were noted on any of the 102 subjects who participated in this phase of the study. The absence of responses characterizes the product as one which is devoid of clinically significant skin sensitizing propensities.

b. **Naive Contact Sites:**
No responses were noted on any of the 102 subjects who participated in this phase of the study. The absence of responses characterizes the product as one which is devoid of clinically significant skin sensitizing propensities.

17.00 CONCLUSIONS:

.01 MT#2700253 was found to be neither a clinically significant skin irritant nor a skin sensitizer under the conditions of this study.

.02 MT#2700253 is not contraindicated for usages entailing repeated applications on human skin under conditions appropriate for such products.

PRODUCT INVESTIGATIONS, INC.

2/10/10
Date

Sophia E. Shelanski
Sophia E. Shelanski
Clinical Research Associate

2/12/10
Date

Joseph E. Nicholson III
Director, Dermatological Studies

18.00 COMPLIANCE WITH GOOD QUALITY ASSURANCE STANDARDS:

I have audited the results presented in this report and believe that, to the best of my knowledge, they accurately reflect the raw data acquired during the course of this study.

Samuel Charles
Director, Quality Assurance

Subj #	Panel: 15496					Sample No. 2700253 (AV)										PI No: 35747					Site: L6					
	INDUCTION PHASE															HIATUS/MAKEUPS					CHALLENGE PHASE					
	WEEK 1					WEEK 2					WEEK 3					WEEK 4					WEEK 6					
	M	T	W	TH	F	M	T	W	TH	F	M	T	W	TH	F	M	T	W	TH	F	M	T	W	TH	F	
1	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
2	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
3	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
4	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
5	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
6	B		0		0	0		0		A	0		0		0	0		0			B	0/0	0/0			
7	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
8	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
9	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
10	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
11	B		0		0	0		0		0	0		0		0	0					A	A	A			
12	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
13	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
14	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
15	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
16	B		0		0	0		0		0	0		0		0	0					A	A	A			
17	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
18	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
19	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
20	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
21	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
22	B		0		0	0		0		0	0		0		0	0					A	A	A			
23	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
24	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
25	B		0		0	0		0		0	0		0		0	0					A	B	0/0	0/0		
26	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
27	B		0		0	A		0		0	0		0		0	0		0			B	0/0	0/0			
28	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
29	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
30	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			

(Grade) / (Grade) = Original Site / Naive Site

Subj #	Panel: 15496					Sample No. 2700253 (AV)										PI No: 35747					Site: L6					
	INDUCTION PHASE															HIATUS/MAKEUPS					CHALLENGE PHASE					
	WEEK 1					WEEK 2					WEEK 3					WEEK 4					WEEK 6					
	M	T	W	TH	F	M	T	W	TH	F	M	T	W	TH	F	M	T	W	TH	F	M	T	W	TH	F	
31	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
32	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
33	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
34	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
35	B		0		0	0		0		0	0		0		0	0					A	A	A			
36	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
37	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
38	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
39	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
40	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
41	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
42	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
43	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
44	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
45	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
46	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
47	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
48	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
49	B		0		A	0		0		0	0		0		0	0		0			B	0/0	0/0			
50	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
51	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
52	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
53	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
54	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
55	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
56	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
57	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
58	B		A		0	0		0		0	0		0		0	0		0			B	0/0	0/0			
59	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
60	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			

(Grade) / (Grade) = Original Site / Naive Site

Subj #	Panel: 15496					Sample No. 2700253 (AV)										PI No: 35747					Site: L6					
	INDUCTION PHASE															HIATUS/MAKEUPS					CHALLENGE PHASE					
	WEEK 1					WEEK 2					WEEK 3					WEEK 4					WEEK 6					
	M	T	W	TH	F	M	T	W	TH	F	M	T	W	TH	F	M	T	W	TH	F	M	T	W	TH	F	
61	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
62	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
63	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
64	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
65	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
66	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
67	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
68	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
69	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
70	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
71	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
72	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
73	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
74	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
75	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
76	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
77	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
78	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
79	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
80	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
81	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
82	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
83	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
84	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
85	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
86	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
87	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
88	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
89	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
90	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			

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Subj #	Panel: 15496					Sample No. 2700253 (AV)										PI No: 35747					Site: L6					
	INDUCTION PHASE															HIATUS/MAKEUPS					CHALLENGE PHASE					
	WEEK 1					WEEK 2					WEEK 3					WEEK 4					WEEK 6					
	M	T	W	TH	F	M	T	W	TH	F	M	T	W	TH	F	M	T	W	TH	F	M	T	W	TH	F	
91	B		0		0	0		0		A	0		0		0	0		0			B	0/0	0/0			
92	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
93	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
94	B		0		0	0		0		Dropped																
95	B		0		0	0		0		Dropped																
96	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
97	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
98	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
99	B		0		0	0		0		0	A		A		Dropped											
100	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
101	B		0		0	0		0		A	0		0		0	0		0			B	0/0	0/0			
102	B		0		0	0		0		Dropped																
103	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
104	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
105	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
106	B		0		0	0		0		0	0		0		0	0					B	0/0	A			
107	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
108	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
109	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			
110	B		0		0	0		0		0	0		0		0	0					B	0/0	0/0			

(Grade) / (Grade) = Original Site / Naive Site

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Lillian Gill, D.P.A.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: Beth A. Lange, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: February 8, 2016

SUBJECT: Concentration of Use by FDA Product Category: *Melaleuca alternifolia* (Tea Tree)-Derived Ingredients

Concentration of Use by FDA Product Category – *Melaleuca alternifolia* (Tea Tree)-Derived Ingredients*

Melaleuca Alternifolia (Tea Tree) Leaf Oil
Melaleuca Alternifolia (Tea Tree) Extract
Melaleuca Alternifolia (Tea Tree) Flower/Leaf/Stem Extract
Melaleuca Alternifolia (Tea Tree) Leaf
Melaleuca Alternifolia (Tea Tree) Leaf Extract
Melaleuca Alternifolia (Tea Tree) Leaf Powder
Melaleuca Alternifolia (Tea Tree) Leaf Water

Ingredient	Product Category	Maximum Concentration of Use
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Bath oils, tablets and salts	0.00099%
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Other bath preparations	0.2%
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Perfumes	9.9%
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Hair conditioners	0.005-0.2%
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Shampoos (noncoloring)	0.001-1%
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Tonics, dressings and other hair grooming aids	0.1-0.5%
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Other hair preparations (noncoloring)	0.005%
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Lipstick	0.02%
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Other makeup preparations	0.25-0.5%
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Cuticle softeners	1.8%
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Nail polish and enamel	0.01-0.6%
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Other manicuring preparations	0.0099-0.5%
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Dentifrices (aerosol, liquid, pastes and powders)	0.017%
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Mouth washes and breath fresheners	0.01%
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Bath soaps and detergents	0.00025-0.9%
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Deodorants Not spray	0.006%
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Other personal cleanliness products Leave-on	0.099%

Melaleuca Alternifolia (Tea Tree) Leaf Oil	Shaving cream (aerosol, brushless and lather)	0.02-0.2%
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.0006-0.9%
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Face and neck products Not spray	0.05-15%
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Body and hand products Not spray	0.0007-0.22%
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Foot products	0.01%
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Moisturizing products Not spray	0.1-0.2%
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Night products Not spray	0.7%
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Paste masks and mud packs	0.05-0.5%
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Skin fresheners	0.01-0.13%
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Other skin care preparations	0.01-0.5%
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Suntan products Not spray	0.35%
Melaleuca Alternifolia (Tea Tree) Extract	Body and hand products Not spray	0.0005%
Melaleuca Alternifolia (Tea Tree) Flower/Leaf/Stem Extract	Other bath preparations	0.0002%
Melaleuca Alternifolia (Tea Tree) Flower/Leaf/Stem Extract	Hair conditioners	0.0005%
Melaleuca Alternifolia (Tea Tree) Flower/Leaf/Stem Extract	Shampoos (noncoloring)	0.0005%
Melaleuca Alternifolia (Tea Tree) Flower/Leaf/Stem Extract	Tonics, dressings and other hair grooming aids	0.005%
Melaleuca Alternifolia (Tea Tree) Flower/Leaf/Stem Extract	Face powders	0.001%
Melaleuca Alternifolia (Tea Tree) Flower/Leaf/Stem Extract	Foundations	0.0005%
Melaleuca Alternifolia (Tea Tree) Flower/Leaf/Stem Extract	Other manicuring preparations	0.0001%
Melaleuca Alternifolia (Tea Tree) Flower/Leaf/Stem Extract	Deodorants Aerosol	0.0001%
Melaleuca Alternifolia (Tea Tree) Flower/Leaf/Stem Extract	Aftershave lotions	0.0002%
Melaleuca Alternifolia (Tea Tree) Flower/Leaf/Stem Extract	Other shaving preparations Rinse-off	0.0001%
Melaleuca Alternifolia (Tea Tree) Flower/Leaf/Stem Extract	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.000001-0.001%

Melaleuca Alternifolia (Tea Tree) Flower/Leaf/Stem Extract	Face and neck products Not spray	0.0001-0.001%
Melaleuca Alternifolia (Tea Tree) Flower/Leaf/Stem Extract	Body and hand products Not spray	0.005%
Melaleuca Alternifolia (Tea Tree) Flower/Leaf/Stem Extract	Foot products	0.0001%
Melaleuca Alternifolia (Tea Tree) Flower/Leaf/Stem Extract	Moisturizing products Not spray	0.005%
Melaleuca Alternifolia (Tea Tree) Flower/Leaf/Stem Extract	Paste masks and mud packs	0.001%
Melaleuca Alternifolia (Tea Tree) Flower/Leaf/Stem Extract	Skin fresheners	0.001%
Melaleuca Alternifolia (Tea Tree) Leaf Extract	Hair conditioners	0.00011%
Melaleuca Alternifolia (Tea Tree) Leaf Extract	Shampoos (noncoloring)	0.00011%
Melaleuca Alternifolia (Tea Tree) Leaf Extract	Tonics, dressings and other hair grooming aids Not spray	0.00011%
Melaleuca Alternifolia (Tea Tree) Leaf Extract	Bath soaps and detergents	2%
Melaleuca Alternifolia (Tea Tree) Leaf Extract	Shaving cream	0.1%
Melaleuca Alternifolia (Tea Tree) Leaf Powder	Hair conditioner	0.05%

*Ingredients included in the title of the table but not found in the table were included in the concentration of use survey, but no uses were reported

Information collected in 2015

Table prepared February 10, 2016

Submission

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review (CIR)

FROM: Tony Larkman
CEO – ATTIA Ltd

DATE: 29 September 2020

SUBJECT: Scientific Literature Review: Safety Assessment of *Melaleuca alternifolia*-Derived Ingredients as Used in Cosmetics (released 4th Aug 2020)

ATTIA Ltd respectfully submits the following comments on the scientific literature review: **Safety Assessment of *Melaleuca alternifolia*-Derived Ingredients as Used in Cosmetics.**

The CIR Scientific Literature Review for tea tree oil (TTO) has been converted to a Word document with page and line numbers inserted to assist the reviewers; a copy is attached.

Throughout the manuscript: you have used both terpinen-4-ol (47 times) and terpinene-4-ol (20 times) – numbers are approximate. The correct spelling for this compound is 'terpinen-4-ol'. Please consider using only this spelling throughout the manuscript.

Peroxide values: you have referred to the peroxide levels in TTO several times including a value of < 10 µeq O_2 for good quality, fresh oil referencing the EMEA (your reference 3). This data is likely derived from a Southwell (2006) paper [1] and I have attached a copy of this as it is hard to find. Please consider using the data and conclusions from this in the CIR.

Page 3, line 68: You state "For example, 1,8-cineole (also known as eucalyptol[2]) can be an allergen,[3]"

Consideration should be given to the significant contrary evidence available and as summarised in Southwell *et al* [2] that this is not the case. The researchers' state:

"The fact that cineole and eucalyptus oil have been used in chest rubs and other dermal application products for many years without adverse effects is, in itself, evidence that cineole is not a skin irritant. This has been verified by studies on animal and human subjects with neat and 16% preparations, respectively. 13 Full-strength eucalyptol was non-irritating to both intact and abraded rabbit skin for 24 hours under occlusion. A 16% formulation in petrolatum was also non-irritating on 25 human subjects after a 48-hour closed patch test. Consequently, it is unlikely that cineole in tea tree oil is responsible for skin irritancy.

To confirm this, and to assess any possible synergistic effect between cineole and tea tree oil constituents, we conducted further clinical studies. Pure 1,8-cineole in concentrations of 0.0, 3.8, 8.0, 12.0, 16.0, 19.9, 24.0 and 28.1 % in soft white paraffin did not produce skin irritancy when tested by occlusive patch on 25 human subjects Similarly, eight tea tree oil preparations containing 15, 3.1 , 5 7, 10.4, 15.0, 18.4, 24.4 and 28.8% cineole did not produce skin irritancy when tested as 2.5% formulations in soft white paraffin on 25 human subjects.

These results negate statements such as "cineole is a mucous membrane and skin irritant," "it is generally accepted that cineole is a skin irritant," "cineole is very low to help avoid irritation as well as increasing the expected effect" *and* "1,8-cineole, reputedly a skin irritant."

Page 3, Line 101: You state "native to northern New South Wales.[14]"

This is not strictly correct, the best description of the distribution of both *M. alternifolia* and *M. linariifolia* is given in Bejar E (2017) [3]:

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1.6 Distribution: *Melaleuca alternifolia* and *M. linariifolia* are both native Australian species endemic to the East coastal littoral of continental Australia from Maryborough, Queensland in the north to Port Macquarie, New South Wales in the south and west to the Great Dividing Range. The native habitat of *M. alternifolia* is low-lying, swampy, subtropical, coastal ground. *Melaleuca linariifolia* has a more limited distribution range, being endemic to the Australian states of Queensland and New South Wales. It grows in heath and dry sclerophyll forest in moist or swampy ground; on the East coast, Central and Southern Australia, and adjacent ranges.

Page 5, lines 119-120: You state "According to the ISO standard for tea tree oil … Available properties data for *Melaleuca alternifolia* (tea tree) oil are provided in Table 2."

On referring to Table 2 (page 34) it is my opinion that to state as you have "*According to the ISO standard…*" then use a medley of sources in the Table as you have done is confusing and potentially contradictory. Consideration should be given to the sources (references) as some of these are commercial entities and some of the information given is out of date, controversial or conflicted. Some examples are:

1. Reference 166 (The Good Scents Company) where there are several inconsistencies in the referenced URL and it appears the baseline data is taken from the BP and/or Ph Eur Standard (see note 3 below).
2. Reference 4 (de Groot *et al*) where "*…has a 'terpeny,' coniferous and 'minty–camphoraceus' odor*" is used. In my opinion and that of experts of long standing (including Ian Southwell) TTO does not have a 'camphoraceous' odour unless it has been adulterated when it is often 'piney' or 'pine-like' but can be 'camphoraceous' depending on what is used when adulterants are introduced; instead it is as described by ATTIA (reference 21) 'characteristic' or if this is not considered sufficient then 'myrtistic' could be used as ATTIA has done in the SDS (copy attached) commonly used by ATTIA members.
3. The use, at any time, of the **British Pharmacopeia** (BP) and/or **Pharmacopeia Europa** (Ph Eur) as an authoritative source (including references that use these Standards) is not recommended or at all advisable: These two Standards were last revised **24 years ago** in 1996 and both are deficient as they are still direct copies of ISO 4730-1996, a Standard that has since been revised twice (2004 and 2017).
   I could include here a full 'rant' on my decade long attempts to engage with these European Union (EU) authorities and, until very recently, their total lack of response (let alone engagement) to my repeated requests to revise the Standards but instead chose to send this separately as an email to you in late August 2020.
   A summary: in early 2019, in response to the enactment of REACH legislation in the EU in mid-2018, I received a request from the Ph Eur authorities requesting a line-by-line revision of the TTO Standard because REACH mandated the use of ISO 4730: 2017 or its successors over BP and/or Ph Eur. I have already sent a copy of my submission and the covering letter (dated July 2019) by email to you in August 2020 to show the proposed changes: effectively identical to ISO 4730: 2017 except for the inclusion of enantiomeric abundances for α-terpineol and limonene in addition to that of terpinen-4-ol.
   The BP and Ph Eur Standards are far too 'soft' and it is incredibly easy to significantly dilute (adulterate) TTO and still conform to these outdated Standards; my personal belief is this ability to adulterate with impunity is the principle reason why the EU may have consistently refused to allow a revision for so long. Only after the implementation of REACH legislation has this been allowed to proceed.

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In my opinion whenever possible a single or only a very few authoritative sources for the totality of the **chemical and physical properties** of TTO should be used and this is without a doubt in my mind the ISO 4730: 2017 Standard for TTO [4] and a seminal paper by Brophy *et al* (1989) [5].

Page 5 lines 121 to 125: this entire paragraph belongs in a separate section (or sub-section) which I suggest could be titled "Storage, Packaging & Transport" or similar although I understand that format options are restricted. Information to supplement this is available on the ATTIA website at URL https://teatree.org.au/teatree_about_packaging.php and in the attached SDS. Please consider reorganising the information in a more logical manner.

Page 5 line 130: you have a section titled "Melaleuca Alternifolia (Tea Tree) Leaf Water" followed by a single line on the manufacture of what is I assume tea tree hydrosol. Please consider including a foot note or similar indicating that this product is known in some markets as 'hydrosol' to improve clarity.
Page 5 lines 134-135: you state "…as well as the identical Australian standard AS 2782-2017, "Essential oil of Melaleuca, Terpinen-4-ol type" which is probably (no reference given) derived from the ATTIA website at this URL: https://teatree.org.au/standards.php which I recently changed. This is not the case and the AS body has failed to date to update AS2782 despite frequent prompting from ATTIA. Please consider removing this statement limiting it to ISO 4730: 2017.

Page 5 lines 136-137: you state"…steam distillation is required to conform to ISO standards" this is equally true for both Ph Eur and BP Standards which state "Essential oil obtained by steam distillation from...". Please consider rephrasing this, I suggest something like "…steam distillation is required to conform to all accepted normative Standards".

Page 5 lines 137-138: you state "…by hydrodistillation in a laboratory, usually with a Clevenger- type apparatus". While the essential oil may be largely similar to a steam distillation there can be subtle but significant differences depending on the time and other conditions in the Clevenger apparatus see **line 140** where a researcher ran the apparatus for 7 hours: please consider a caveat to this statement to this effect.

Page 5 line 159: you state "There are several varieties, or chemotypes, of *Melaleuca alternifolia*, and each produces oil with a distinct chemical composition" citing Carson et al (2005). A better direct source for the chemotypes of *M. alternifolia* is available in a paper by Keszei *et al* (2010) [6] where 3 cardinal and up to 4 intermediate chemotypes are described in detail. Please consider including this information here and also in the relevant section in your Summary (**line 794**) and in **Table 3**.

Page 5 lines 162-163: you state "The terpinen-4-ol chemotype is typically used in commercial tea tree oil production". This is not strictly true, the ISO 4730: 2017 and the preceding versions of this Standard states in section 1 **Scope**: "*This document specifies certain characteristics of the essential oil of Melaleuca, terpinen-4-ol type (Tea Tree oil), in order to facilitate assessment of its quality.*" Thus it can reasonably be stated that **only** the terpinen-4-ol chemotype should be used in commercial tea tree oil production. This is the case for all production in Australia without exception. It is likely only in other source countries where inadvertent use of the incorrect chemotype results in lower quality and sometimes non-conforming material. An example of this is in Kenya where there are credible though anecdotal reports of material with terpinen-4-ol levels as low as 22% which may result in adulteration to rectify this fault.

Page 6 lines 169-170: you state "one publication reported that over 220 constituents have been identified in tea tree oil samples, and the concentration of these constituents present in the oil can vary widely depending on the sample." This is from de Groot's book "Essential Oils: Contact Allergy and Chemical Composition" (your reference 4) and is on the face of the publication correct. However it is

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my strong opinion, with which Ian Southwell concurs and the authors also concede, that many of the components listed include a number of compounds that have never been reliably identified in known provenance 100% pure derived from *M. alternifolia* regardless of where produced. This has almost certainly been caused by the widespread systematic adulteration of TTO over many decades [7] which has allowed adulterants to creep into the list compiled by these authors and others they have referenced. Because of this I do not consider this list to be authoritative; please consider removing this statement and reference or alternatively add a caveat to the effect that the list is not definitive and very likely includes many compounds derived through adulteration of TTO.

Page 6 lines 173 to 174: you state in part "The components present in the greatest amounts are terpinen-4-ol (up to 48%), γ-terpinene (up to 28%), and 1,8-cineole (up to 15%)" and you have referenced ATTIA (your reference 21). I would far prefer that the ISO 4730: 2017 Standard [4] be used wherever possible as the definitive authoritative reference for the normative composition of TTO. Please also consider merging this section "Composition/Impurities" with the section "Chemical and Physical Properties' (above on the same page) and in so doing consolidate, sectionalize and therefore streamline the totality of information provided.

Page 6 line 178: you state "are identified in Table 4;[21]" once again I would prefer ISO 4730: 2017 [4] to be the authoritative reference and again as this contains compositional information please consider consolidating all of the information pertinent to this in a single section for clarity and ease of reference.
Page 6 lines 181-182: you state "Most of the specifications listed in the *European Pharmacopoeia* are similar to those specified in ISO standard" before listing only two differences. I respectfully disagree with this statement as there are several other significant differences in that neither the BP and Ph Eur

Standards include 4 of the 5 sesquiterpenes (ledene, δ-cadinene, globulol and viridiflorol) listed in ISO 4730: 2017; this is a **crucial difference** as the absence of these compounds in the prescribed ranges is just as telling of adulteration as all other parameters. Another **critical difference** is the Optical Rotation which is 7°-12° in ISO and 5°-15° in BP/Ph Eur. There are others.

Sabinene, which is listed twice in Table 4 is included incorrectly as "NS" in one and correctly as defined in ISO 4730: 2017 as "traces – 3.5%. To avoid this inadvertent error it would be good practice (and far easier for a reader to read and cross reference) if an identical order of listing for components were used throughout in all tables. Please consider rearranging Table 4 (and therefore Table 5 and all others as required) to match the generally accepted sequence used in ISO 4730: 2017 which is based on the elution sequence of the compounds. The order of listing I recommend is shown in the table below and I have used two decimal places for absolute clarity which I also recommend you use throughout:

Component	Min %	Max %
α-Pinene	1.00	4.00
Sabinene	traces	3.50
α-Terpinene	6.00	12.00
Limonene	0.50	1.50
p-Cymene	0.50	8.00
1,8-Cineole	traces	10.00
γ-Terpinene	140	28.00
Terpinolene	1.50	5.00
Terpinen-4-ol	35.00	48.00

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α-Terpineol	2.00	5.00
Aromadendrene	0.20	3.00
Ledene (syn. viridiflorene)	0.10	3.00
δ-Cadinene	0.20	3.00
Globulol	traces	1.00
Viridiflorol	traces	1.00

As part of the logical rearrangement of the information provided in the CIR it would be more informative to tabulate **all** normative information for each of the physical and organoleptic/observational characteristics as well as the compound ranges for each selected Standard (WHO/ISO/BP/Ph Eur) using the ISO listing as an initial reference as well including a row for the issue or revision date which will allow a reader to compare and contrast each. If it is acceptable to you I recommend contrasting the ISO to others and noting that it is 1) the most up-to-date and 2) the only normative Standard currently accepted by ATTIA Ltd. This would likely assist the entire TTO industry supply chain globally by reducing the incidence of adulteration especially if the enantiomeric ratio ranges for terpinen-4-ol was included in the table which I think should be in there please.

Page 6 lines 190-191: you state "…samples from Australia, Vietnam, and China that were analyzed between 1998 and 2013.[4]" once again referencing de Groot. Again I respectfully restate that this list likely includes, at the very least, some compounds that have crept in through decades of adulteration [7] and is therefore potentially misleading. A better, more authoritative listing is that found in "*Gas chromatographic quality control for oil of Melaleuca terpinen-4-ol type (Australian tea tree)*" [5] where 98 compounds from **known provenance 100% pure TTO** was analysed.

As justification for this I can categorically state that I have been analysing 'TTO' samples since 2009 and have **never, ever** seen a sample from China that was not adulterated with a single exception when the leaves and twigs from a plantation were taken from a Chinese plantation and analysed after lab-top distillation in the USA: the material extracted passed ISO 4730 with flying colours including the enantiomeric abundances for all 3 compounds listed earlier.

Page 6 lines 215-216: you state "…reported that levels of methyleugenol ranged from 0.01 - 0.06% (mean, 0.02%) for commercial distillations.[41]". There is no issue with this reference, however there is another paper [8] that is more rigorous in the statistical analysis and number of samples analysed for methyl eugenol content over a longer production period (5 production seasons) that you may consider either more suitable than your reference 41 or alternatively this could be included as an additional reference.

Page 7 line 224: you mention 'Heavy metal impurities" and use the US FDA as reference (44). Some good data is also available from a paper titled "*Trace determination of skin-irritating metals in tea tree oil by GFAAS*" [9] which you may wish to consider using either as an additional or a replacement reference.

Page 7 line 226: you mention "maximum pesticides residue limits" and correctly cite the WHO. It may be worth noting that ATTIA's Code of Practice (COP) tolerates no pesticide residues above limit of quantification (LOQ) in a standard assay of over 150 common pesticides.

Page 7 line 227- 229: here you mention possible adulterants; I would also far prefer, as requested earlier (and as detailed in a separate email sent on 1 September 2020), that a **section dedicated to adulteration**

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is included as part of the reorganisation to centralise and streamline the document is considered; indeed I **strongly recommend** that this be included. Other adulterants of significant concern are pesticides, endocrine disrupting compounds and some of the more bizarre adulterants, including on one memorable occasion, hashishene in a commercial sample of product labelled '100% pure TTO'.

Page 7 lines 239-245: Use metrics: ATTIA commissioned an independent survey titled "Survey of product categories, concentrations, and usage of tea tree oil in cosmetics" for both consumers and manufacturers in 2016/17 to gather metrics on use patterns, exposure levels and maximum inclusion levels of TTO in the EU resulting in some good metrics. Data was collected from 5 EU countries on 1,400+ individual products offered by 371 brands/suppliers with 2,903 of 17,575 consumers responding resulting in 2,535 validated users of TTO-containing products. While I am unable to provide a copy of this report it is highly likely that these metrics will closely parallel those in the USA.

Page 8 line 286: "Daily exposure of tea tree oil was calculated was calculated…" has a repeat phrase, please revise this.

Page 8 lines 283 – 289: This section deals with SED and NOAEL levels as calculated by the SCCS in their 2008 Opinion [10]. ATTIA has noted that the SCCP made a significant error in their calculations as shown below:

"*The SCCP 2008 Opinion has taken the ATTIA table of products and applied the simpler and conventional algorithm to determine daily SEDs for each of the products. [Based on a single application/day, an application weight, a retention factor and a known absorption percentage of the applied weight.] The SCCP has calculated quite different SEDs to the ATTIA values based on using this methodology. However, the algorithm applied by the SCCP neglected to apply the known 3% percutaneous absorption factor thereby resulting in all SED values being 33 times greater than they might have otherwise been reckoned.*"

ATTIA is waiting on the final part of a complex jigsaw of changes including the 2016/17 survey, inclusion of only 2 named species in the ISO 4730 Standard, data on methyl eugenol [8] and the final piece of the puzzle – dermal penetration data (due 2020 but currently under severe restraint due to COVID-19) to complement the work already done by Sgorbini *et al* (2017) [11] and Capetti *et al* (2020) [12].

The detail in these papers [11, 12] may assist in revaluation of the detail provided for SED and NOAEL in this section of the CIR and Table 12.

Page 9 line 341: you correctly quote Capetti *et al* [12] using "4-terpineol"; however you have throughout the paper used 'terpinen-4-ol' and while these are one and the same it may be confusing for a reader; please consider use of a footnote or similar to explain this.

Pages 11-12 lines 494 – 527: You extensively quote several publications stemming largely from a paper authored by Henley *et al* (2007) [13] without addressing the real concern that the inadvertent presence of phthalates or other ED substances may be the causal factor. Please consider balancing your reporting of this with information available in Carson *et al* (2014) [14]. Please also consider citing Hawkins *et al* (2020) [15] which largely refutes this claim. It may also be worth mentioning the study being undertaken by Hawkins *et al* (currently hampered by COVID-19) which I sent you information on back in June 2020 by email as this study will, in the fullness of time, likely definitively address the purported link between ED and TTO.

Page 14 line 264: you state "4-h semi-occlusive application[99]" citing an RIFM report (#5668). I do not have access to the data, can you please check if oxidised material was applied in the occlusive test (this is a certainty if patches from Chemotechnique, Malmo, Sweden were used) and if this is the case can you please ensure that this is clearly stated.

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Throughout this section on sensitisation/irritancy and in other areas: you have cited several papers, some have (I sent you full details on this by email in June 2020) clearly used oxidised material and published the data as "TTO" instead of 'oxidised TTO'. This is in my opinion a grossly unfair, indeed a negligent omission that causes confusion both to consumers and the medical community who take these papers at face value. Please consider addressing this negligence by the researchers more fully.

I sincerely thank you for your efforts in this section as well as in other sections and in Table 19 and elsewhere to address this issue however it saddens me to see that this the lack of differentiation between oxidised and unoxidised material appears to have been further inadvertently promulgated in this CIR. For example in Table 19 where work conducted by the Mayo clinic has been cited (your reference 112): Dr, Yiannias has confirmed to me (personal communication Aug 2020) "*We patch test to tea tree oil from Chemotechnique*" confirming that the **first entry** in Table 19 which is titled "**Retrospective, multicenter, and cross-sectional patch test studies with tea tree oil**" used oxidised TTO and not, as described in the title '…patch test studies with tea tree oil'; there are other examples:
Most, if not all NACDG series use oxidised TTO and report this as "TTO" with only a passing reference, buried in the literature, to the fact that they are in fact reporting on the skin sensitisation of oxidised material. Once again my sincere thanks to you for your significant effort in addressing this issue but there are other instances in the CIR and in Table 19 where this has not been addressed fully. Please very carefully consider all references and entries relating to patch testing, skin sensitisation etc to ensure that the CIR correctly reflects the material being used at all times.

I also wish to draw your attention to a paper by Ahlin *et al* (2012) [16] which you have not cited where they erroneously reported allergy to TTO ranging from 0-26%. This was addressed by Carson *et al* (2012) [17] and I have attached a copy of the manuscript in English. I include this because the paper is informative, authoritative and the inspiration for my own attempts to have this inequity addressed.

Page 14 line 645: I am highlighting the Aspres *et al* (2003) [18] citation here because it is one of the few studies where the TTO has been correctly described including a measure of the peroxide value (in this instance this was 9.5 mEq O2/kg which is on the cusp of being too high). It is noticeable that where 100% pure TTO that has been correctly stored and handled the incidence of irritancy (I acknowledge that there are some people, including my brother-in-law, who are severely allergic to TTO) is significantly lower leading Aspres *et al* to conclude: "*Topical application of tea tree oil is associated with negligible skin irritancy. In the group of subjects studied, the risk of developing an allergic dermatitis from topical tea tree oil usage was found to be< 1 %.*"

Page 15 line 692: you state "5% tea tree oil". As mentioned above and confirmed by Dr Yiannias the Mayo Clinic use Chemotechnique patches for TTO: this is without exception oxidised material. It is noteworthy that "a positive response was found in 18 patients (2.1%)" where the elevated response level is almost certainly due to the use of oxidised material.

Page 15 lines 698-699: you state "The NACDG also examined the frequency of positive patch test reactions with tea tree oil as compared to fragrance markers"; again this material is oxidised as acknowledged earlier (line 693) yet your statement refers to 'tea tree oil' and not 'oxidised tea tree oil'. If this statement is taken out of context, as has happened frequently with other documents, the assumption is that TTO and not oxidised TTO is being examined leading to assumptions that are then promulgated further. Similar to earlier comments please very carefully consider all references and entries relating to patch testing, skin sensitisation etc to ensure that the CIR correctly reflects the material being used at all times.

Page 15 line 705: you state "1.2% had positive reactions to oxidized tea tree oil". Thank you!

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Page 16 line 717 to 718: again "contained 5% tea tree oil, and 1 subject had a ++ reaction to the lotion and 10% tea tree oil in pet" is stated, I have read the Veien *et al* (2004) paper and the authors do not provide sufficient information to ascertain if the TTO was oxidised however being in pet I would bet they are oxidised so once more can you please ensure this is correctly reported.

Page 16 lines 739-743: you state "In Australia, positive reaction rates generally appear to be higher than those reported in the US or Europe' and correctly note that oxidised material was used in the first citation however in the second you state "5% and 10% tea tree oil" in this instance the authors note that Chemotechnique patched were used and that "The 10% patch test was prepared by a pharmacist by diluting neat tea tree oil (purchased from a pharmacy) that had first been allowed to oxidize by standing the open bottle on a window ledge for several days, with white soft paraffin". They also specifically title this report "Allergy to tea tree oil: Retrospective review of 41 cases with positive patch tests over 4.5 years" and appear, on the face of this, to be extraordinarily biased against TTO to the extent they also state "until recently there had been fewer than 50 cases of ACD to tea tree oil reported in the literature" before proceeding to deliberately oxidise material; I question this practice and wonder why it is done this way.

I will not include any further comments in this vein as there are too numerous but I again repeat my plea that each of these incidences be investigated and reported correctly, I have instead simply highlighted those I have noticed during my review without listing them here; there are likely others that I have missed.

Lines 782 – 1008 (Summary): Some of this section may need to be reviewed/rewritten in light of information provided above.

Page 17 line 794: you state "Six chemotypes"; please see earlier comment and recommendation (Page 5 line 159) on chemotypes of *M. alternifolia*.

Page 19 line 921: you state "Emulsions of tea tree oil in were cytotoxic to" there is something missing after the word 'in' or the word 'in' is superfluous, please revise this as required.

Tables: I simply do not have the resources to cross-check all of the information provided in Tables 1 to 21 inclusive; I have therefore skipped these except where suggestions on table construction, order and functionality have been specifically noted.

Sincerely,

Tony Larkman

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From:	Tony Larkman
To:	Bart Heldreth; Monice Fiume
Subject:	CIR review of tea tree-derived ingredients
Date:	Monday, August 31, 2020 7:23:02 PM
Attachments:	Ph. Eur. Monograph 1837 - Tea Tree Oil FINAL draft July 2019.pdf BP & Ph Eur Monograph Revision cover letter July 2019.pdf K175-1967.pdf How ISO 4730 - 2017 Helps Identify Fraudulent TTO Mar 2017.pdf The Australian TTO Industry Aug 2018.pdf

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Dear Bart and Monice,

I have started the review process for the draft CIR on tea tree and advise that the process is going to take a while, I will however ensure I have ATTIA's submission in before the deadline.

My main observation on first read through is that while the document is on the whole good, the information is presented in a somewhat fragmented manner and would significantly benefit from being rearranged more logically.

This is not easy to convey in a line-by-line review (which is what I am doing at the moment) but I will do my best.

I have also been corresponding with Dr Carol Eisenmann of the PCPC who was seeking information, amongst other things, on peroxide levels in TTO. I will address this within my review as well as is possible.

Carol also asked a specific question: "***Do you have an estimate of the amount of tea tree essential oil sold to the cosmetics industry conforms to the ISO standard? I am not sure this will be needed for the CIR report – but I am curious as this is the first essential oil that I have come across that has an ISO standard.***" My response (below and in the attachments) will, as much as possible, be addressed in ATTIA's submission.

Carol suggested that I send the information to you intact as it would be a good idea to provide you with this separately as a single, specific topic relevant to the TTO CIR:

1. The amount of TTO sold that conforms to ISO 4730: 2017 is a complete unknown as material sourced from countries other than Australia are often non-conforming, particularly anything that comes out of China: I have never, in 11 years of testing, seen a Chinese sample that is not adulterated and therefore conforms to ISO.
2. We know from a comprehensive 2017 survey that just over 50% of TTO sold into the EU is destined for cosmetic use. We also know the EU imports just over 300,000 kg of TTO annually of which Australian TTO comprises, since REACH was enacted, 83%. Prior to REACH it was 63%.
3. My best guess is that the total global supply and demand for TTO is between 1,800 and 2,00 metric tonnes (MT).
4. I can tell you that Australia produces, depending on environment conditions (drought, fire, flood), between 700 and 1,100 MT of TTO annually ~90% of which is exported. All of this TTO, without exception, conforms to the ISO Standard and we have records to prove this for every single batch as part of our Code of Practice (COP). In 2019 Australia produced 1,008 MT and exported 985 MT.
5. I have also been told (completely anecdotal) that much (all?) of the material from Kenya does not conform to ISO (or other Standards such as BP or Ph Eur) as it is the incorrect chemotype (low terpinen-4-ol, as low as 22% is common). This is likely adulterated with

terpinen-4-ol from China before being sold as 'ISO compliant'. This may also be happening (again anecdotal) with some of the South African/Zimbabwe origin material where they too have the wrong chemotype. Trouble is no one will tell me which is not surprising as they are adulterating. When I test TTO for adulteration up to 50% of the material that claims South African provenance is adulterated. Mind you about 30% of the material claiming Australian provenance is also adulterated, we know this is not happening here so it is being done overseas by unscrupulous traders.

TTO has both the British Pharmacopeia (BP) and *Pharmacopeia Europa* (Ph Eur) Standards but they are both from 1996 and therefore appallingly outdated (24 years actually) as well as the ISO 4730: 2017 Standard which we use.

The reason TTO uses ISO is complex and related to adulteration: Discounting the 1949 BP monograph for TTO which is descriptive the first functional Standard was the Australian Standard series (K175-1967, copy attached) which was absorbed into the ISO organisation and merged into AS2782 at the same time ISO 4730 was released. The BP and Ph Eur Standards copied the 1996 version of ISO 4730 verbatim and have not revised them since then.
The ISO was revised in 2004 than again in 2017 when I persuaded them to include the enantiomeric ratio for terpinen-4-ol (I asked for limonene and α-terpineol as well but they refused). This is a fine and definitive test for adulteration in TTO and identifies at least 95% of adulteration with a single analysis.

At the same time as I engaged with the ISO people I also asked the BP/Ph Eur committees to do the same, I have been doing this repeatedly since 2012 and they have consistently ignored me until very recently.

Then, in 2018 when the EU REACH legislation was enacted, the lead registrant (an ATTIA member) insisted on using the ISO 4730 Standard to define the substance against strong resistance (including recent legal threats and some actual action) because the BP/Ph Eur Standards are unrevised and therefore completely inadequate. This was justified by the fact that it is far too easy to adulterate to these two outdated Standards (I completely agree and I believe this is almost certainly why the EU consortium resisted the change so strongly and for so long).
Now the REACH substance identification is mandated as ISO 4730 and I am frankly so disillusioned by the BP/Ph Eur lack of action I have consistently refused to acknowledge these Standards (see attached "How ISO Standards…" and compare the parameters to the current BP and Ph Eur Standards). I have also summarised much of this in the attached paper "The Australian TTO Industry Aug 2018" and had a good bash at BP and Ph Eur in it.

The two classics in the Standards are limonene which is max 1.5% in ISO and 4% in BP (I have **never, ever** seen a real sample of TTO with limonene over 1.1%) and optical rotation (OR) which is 7-12 in ISO and 5-15 in BP (related to chirality) which makes it really, really easy to adulterate a low terpinen-4-ol TTO with material derived from pine or eucalyptus (sabinene → terpinen-4-ol is a common pathway) which has the opposite chiral ratio to TTO and then add a couple of percent of (+) limonene to correct the OR and still remain inside the BP/Ph Eur parameters. The material used is usually a frightening mix of crap: the most interesting I have seen was 0.9% hashishene in "pure

TTO"!. Phthalates, pesticides and other alarming crap is also detected in varying quantities from time to time.

BP is commonly used for trading TTO globally except in Australia where ATTIA has engaged with and used the ISO Standard for more than a decade. There is a notable exception: the Australian Therapeutic Goods Administration (TGA) who is mandated to use BP and it would take an amendment of a Parliamentary instrument to change this which is simply not going to happen.
The TGA also ignored my calls to help persuade the BP people until 2018 when even they realised (after I had been forced to use alternative legislation (Consumer Protection) to get an action filed against a local essential oil seller who was, like the 'EU adulteration consortium', hiding behind the BP anomaly to legally adulterate TTO and sell it here in Australia (see https://www.accc.gov.au/media-release/bosistos-pay-10800-penalty-for-allegedly-false-or-misleading-tea-tree-oil-claims).

This all came to a head after REACH was enacted in mid-2018 when, in early 2019 the UK's MHRA on behalf of BP and Ph Eur, asked me to give them a line-by-line revision of the Ph Eur (and therefore by default the BP) Standard with supporting data – basically what I had already provided the ISO committee with and suggested they use. This was delivered in late July 2019 and here we are waiting for the Ph Eur/BP committee to take action and revise the Standards - 14 months and counting.
I am hoping BP/Ph Eur get on with a revision soon because the ISO Standard is due for revision again in 2022 and I want to use the BP/Ph Eur revision to embarrass ISO into 1) including the other two enantiomeric compounds and 2) making chirality mandatory instead of advisory.
I have attached a copy of my line-by-line revision proposal and my covering letter which provides some explanations to the Ph Eur proposal noting that apart from the inclusion of the HPLC section (which is useless for TTO and many other essential oils but mandated for these Standards) the only substantive change from the ISO 4730: 2017 Standard is the proposed inclusion of the chiral ratios for all three compounds named earlier. There is of course reams of supporting data behind all of this which I can provide if it interests you.

Apologies for the rant but this is very close to my heart: I hate adulteration and falsification of any kind and will continue to campaign to achieve the end goal of a single, unified globally accepted and frequently revised TTO Standard that reduces fraud (I accept we will never completely stop it) to an acceptable level and give *bona fide* producers a level playing field. Any assistance you can provide in this arena will be beneficial to consumers as well as the entire TTO supply chain.

I could also provide you with a copy of the 2016/17 survey of cosmetic products/use patterns mentioned above if it is of interest; please advise.
Regards,

Tony Larkman
CEO - ATTIA Ltd
: 02 4017 1336
: 0434 263 664

Email: tlarkman@attia.org.au
Web: www.attia.org.au

[redacted]

Tea Tree Oil

General Notices

Melaleuca Oil *or*
Essential oil of *Melaleuca*, terpinen-4-ol type

CAS: 68647-73-4 or 85085-48-9 *INCI*: Tea Tree (*Melaleuca alternifolia*) Leaf Oil

(Ph. Eur. monograph 1837)

Ph Eur

This document specifies certain characteristics of **Tea Tree Oil** in order to facilitate assessment of its quality.

The whole oil, with nothing added or removed by any means after distillation, settling, separation from any water fraction and filtration constitutes the defined material.

DEFINITION

Essential oil obtained by steam distillation from the foliage and terminal branchlets of the terpinen-4-ol chemotype of *Melaleuca alternifolia* (Maiden et Betche) Cheel and/or *Melaleuca linariifolia* Smith.

CHARACTERISTICS

Appearance	Clear, mobile liquid
Colour	Colourless or pale yellow
Odour	Characteristic

TESTS

Characteristic	Requirement
Relative density (*2.2.5*)	Between 0.885 and 0.906
Refractive index (*2.2.6*)	Between 1.475 and 1.482
Optical rotation (*2.2.7*)	Between +7.00° and +12.00°

IDENTIFICATION

First identification: ***A***
Second identification: ***B***

A. Examine the chromatograms obtained in the test for chromatographic profile

Results: The characteristic peaks in the chromatograms obtained with the test solution are similar in retention time to those in the chromatogram obtained with the reference solution.

CHROMATOGRAPHIC PROFILE

Gas chromatography (*2.2.28*): use the normalisation procedure.

- *Test solution:* dissolve 0.15 mL of the substance to be examined in 10 mL of *ethanol R*.
- *Reference solution:* dissolve: 5 µL of *α-pinene R*, 5 µL of *sabinene R*, 15 µL of *α-terpinene R*, 5 µL of *limonene R*, 5 µL of *ρ-cymene* R, 5 µL of *ϐ-phellandrene R,* 5 µL of *1,8 cineole R*, 30 µL of *γ-terpinene R*, 5 µL of *terpinolene R*, 60 µL of *terpinen-4-ol R*, 5 µL of *α-terpineol R,* 5 µL of *aromadendrene R,* 5 µL of ledene R, 5 µL of δ-cadinene R, 5 µL of globulol R and 5 µL of viridiflorol R in 10 mL of *ethanol R*.

Non-polar Column conditions

- *Column*:
  - *material*: fused silica
  - *size*: *l* = 60 m (a film thickness of 0.25 µm may be used)
    Ø = 0.25mm
  - *stationary phase*: *5% Phenyl Polydimethylsiloxane*
  - *Carrier gas:* *helium for chromatography R*
  - *Flow rate:* 1.6 mL/min
  - *Split ratio:* 1:50.
- *Temperature*:

	Time (min)	Temperature (°C)
Column	0 - 1	50
	1 - 48	50 → 220
	48 – 52.5	220
Injection port		250
Detector		310

- *Detection:* Flame ionisation
- *Injection:* 1 µL of the solution
- *Elution order:* Order indicated in the composition of the reference solution, record the retention times of these substances.
- *System suitability:* Reference solution.
- *Resolution*: minimum 0.54 between the peaks due to β-phellandrene and 1,8-cineole

Using the retention times determined from the chromatogram obtained with the reference solution; locate the components of the reference solution in the chromatogram obtained with the test solution. Disregard the peak due to ethanol.

Figure 1: Typical chromatogram of the analysis by gas chromatography of Tea Tree Oil taken on an apolar column:

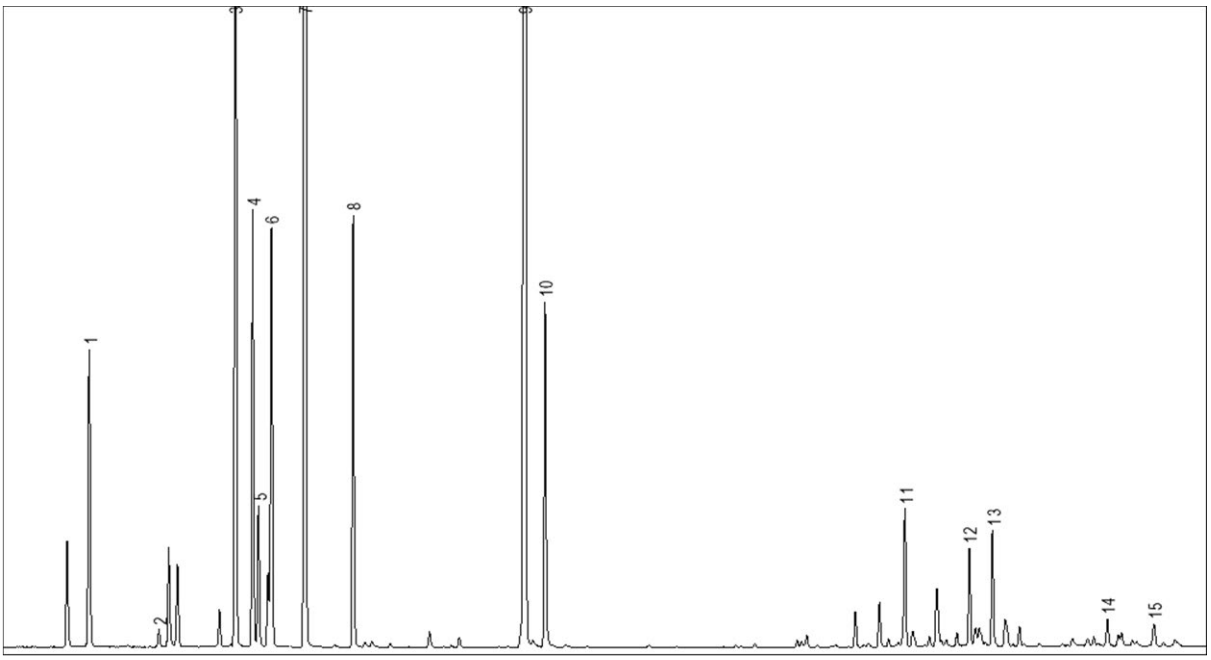


Figure 2: β-phellandrene elutes between peak 5 (limonene) and 6 (1,8-cineole). Resolution of >0.5:

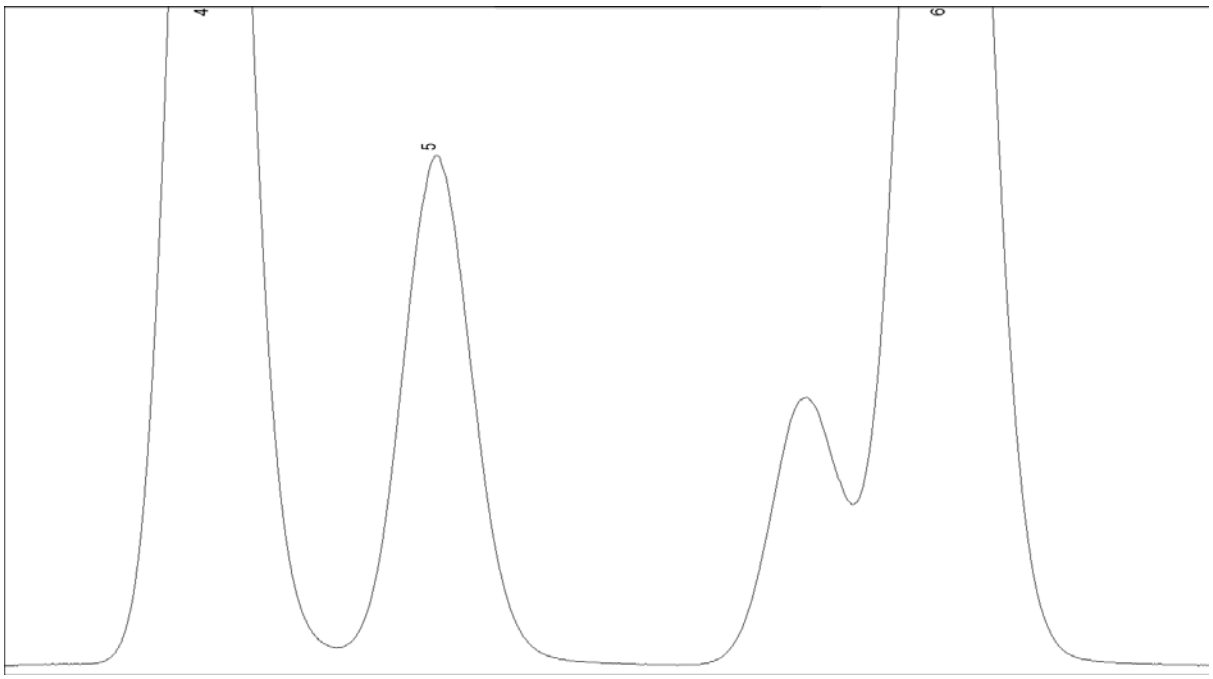
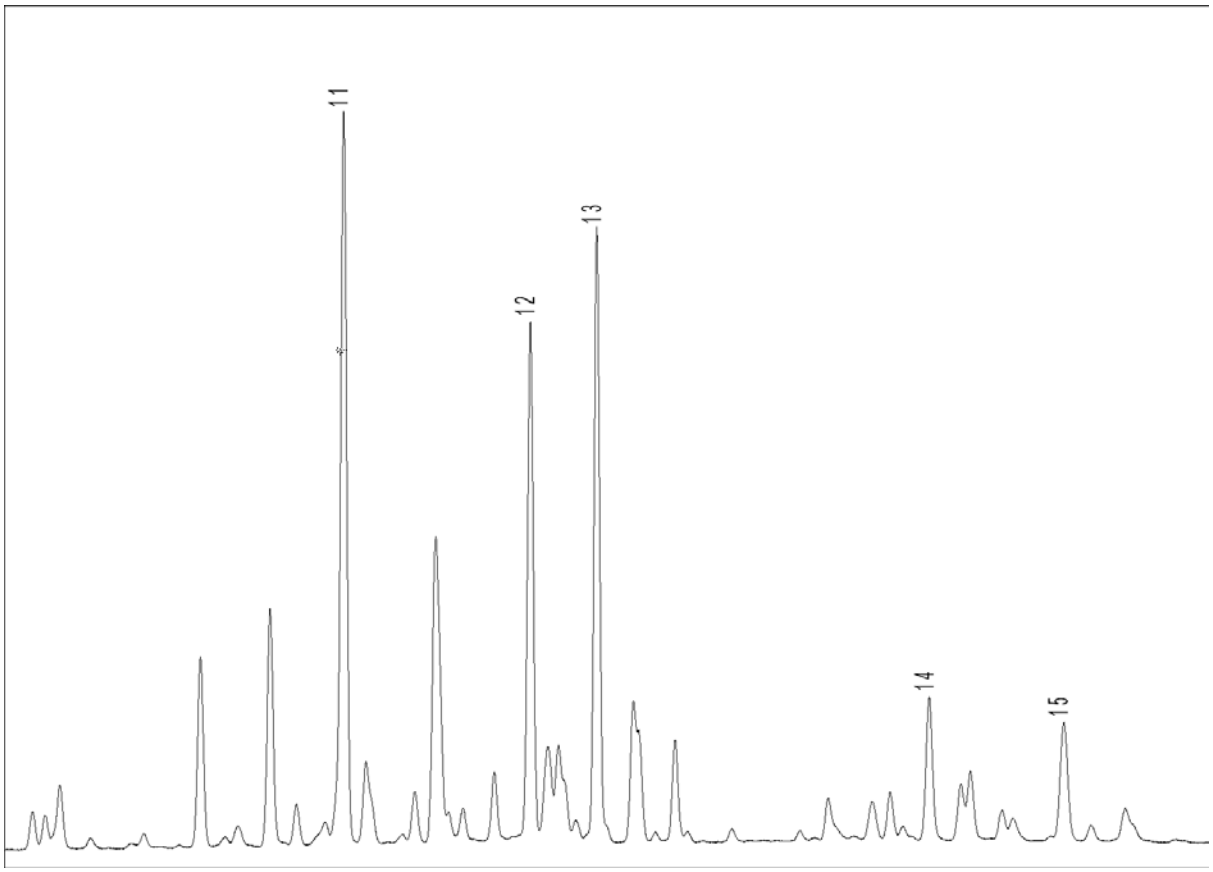


Figure 3: Characteristic sesquiterpene GC profile of Tea Tree Oil:



Mid-Polar Column conditions

- *Column*:
  - *material*: fused silica
  - *size*: *l* = 60 m (a film thickness of 0.25 µm may be used)
    Ø = 0.25 mm
  - *stationary phase*: *35% Phenyl Polydimethylsiloxane*
  - *Carrier gas:* *helium for chromatography R*
  - *Flow rate:* 1.6 mL/min
  - *Split ratio:* 1:50.
- *Temperature*:

	Time (min)	Temperature (°C)
Column	0 - 3	50
	1 - 30	50 → 240
	30 – 35.89	240
Injection port		250
Detector		300

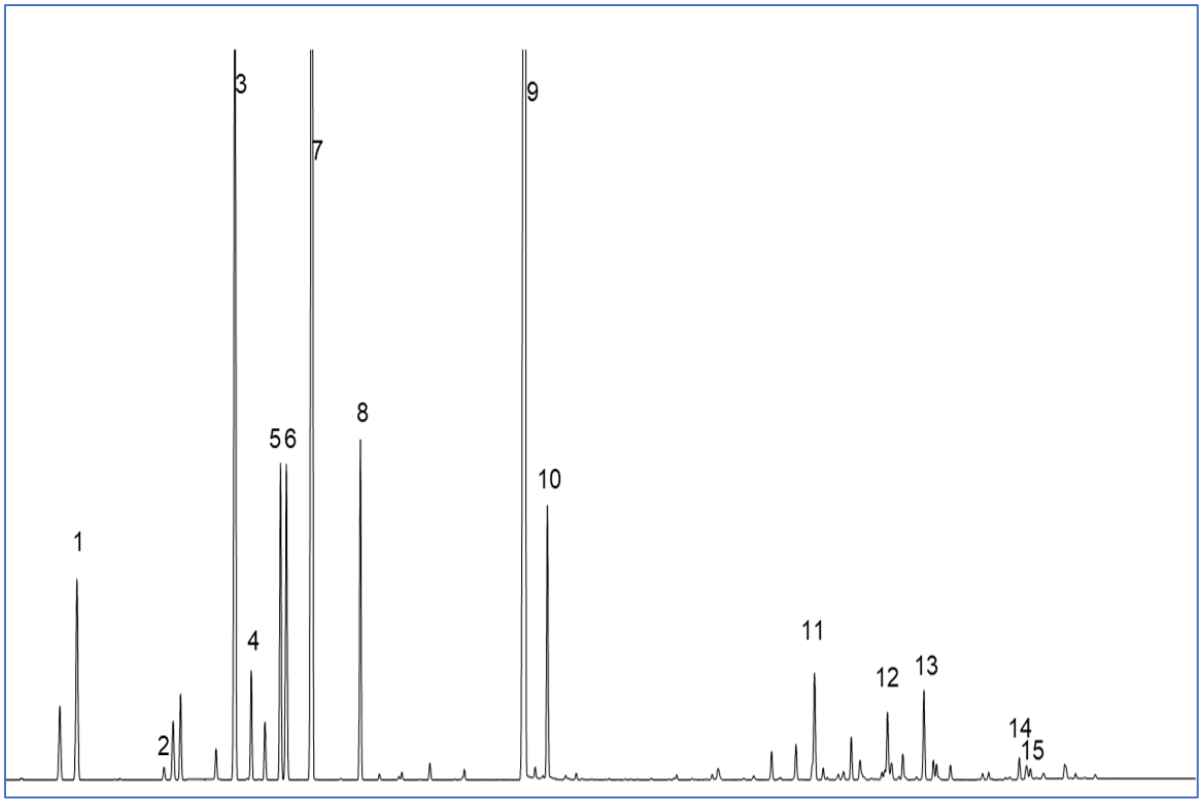
- *Detection:* Flame ionisation
- *Injection:* 1 µL of the solution
- *Elution order:* Order indicated in the composition of the reference solution. Record the retention times of these substances.
- *System suitability:* Reference solution.
- *Resolution*: minimum 1.04 between the peaks due to ρ-cymene and 1,8-cineole.

Using the retention times determined from the chromatogram obtained with the reference solution; locate the components of the reference solution in the chromatogram obtained with the test solution. Disregard the peak due to ethanol.

Determine the percentage content of these components. The percentages must be within the following ranges:

Components	Minimum percentage	Maximum percentage
α-pinene	1.00	4.00
sabinene	traces [a]	3.50
α-terpinene	6.00	12.00
limonene	0.50	1.50
ρ-cymene	0.50	8.00
1,8 cineole	traces [a]	10.00
γ-terpinene	14.00	28.00
terpinolene	1.50	5.00
terpinen-4-ol	35.00	48.00
α-terpineol	2.00	5.00
aromadendrene	0.20	3.00
δ-cadinene	0.20	3.00
ledene (syn. viridiflorene)	0.10	3.00
globulol	traces [a]	1.00
viridiflorol	traces [a]	1.00
[a] traces < 0.01percent		

Figure 4: Typical chromatogram of the analysis by gas chromatography of Tea Tree Oil taken on a mid-polar column:



Peak Identification

1. α-pinene
2. sabinene
3. α-terpinene
4. limonene
5. ρ-cymene
6. 1,8-cineole
7. γ-terpinene
8. terpinolene
9. terpinen-4-ol
10. α-terpineol
11. aromadendrene
12. ledene (viridiflorene)
13. δ-cadinene
14. globulol
15. viridiflorol

ENANTIOMERIC DISTRIBUTION

Some essential oil components exist in two enantiomeric forms designated as (R) or (S), D or L or (+) or (-) isomers. Many enantiomers have distinctly different properties and hence their presence in the right form is critical. Also, pure natural essential oils contain enantiomers in characteristic ratios.

This ratio is upset by the addition of adulterants including synthetic major components of different enantiomeric ratios.

Consequently, the measurement of enantiomeric excess or enantiomeric ratio as per *<insert Ph Eur Reference if available>* provides an extra measure of essential oil authenticity.

Gas chromatography (*2.2.28*): use the normalisation procedure.

- *Test solution:* dissolve 0.15 mL of the substance to be examined in 10 mL of *ethanol R*.
- *Reference solution:* dissolve: 3 µL of (+)-*limonene R*, 3 µL of (-)-*limonene R,* 30 µL of (+)-*terpinen-4-ol R*, 30 µL of (-)-*terpinen-4-ol R,* 5 µL of (+)-*α-terpineol R,* 5 µL of (-)-*α-terpineol R,* in 10 mL of *ethanol R*.

- *material*: fused silica
- *size*: *l* = 50 m (a film thickness of 0.25 µm may be used) Ø = 0.22 mm
- *stationary phase*: Permethylated Beta-Cyclodextrin
- *Carrier gas:* *helium for chromatography R*
- *Flow rate:* 1.3 mL/min
- *Split ratio:* 1:150.

- *Temperature*:

	Time (min)	Temperature (°C)
Column	0 - 1	50
	1 - 51	150
	51 – 59.8	220
Injection port		220
Detector		320

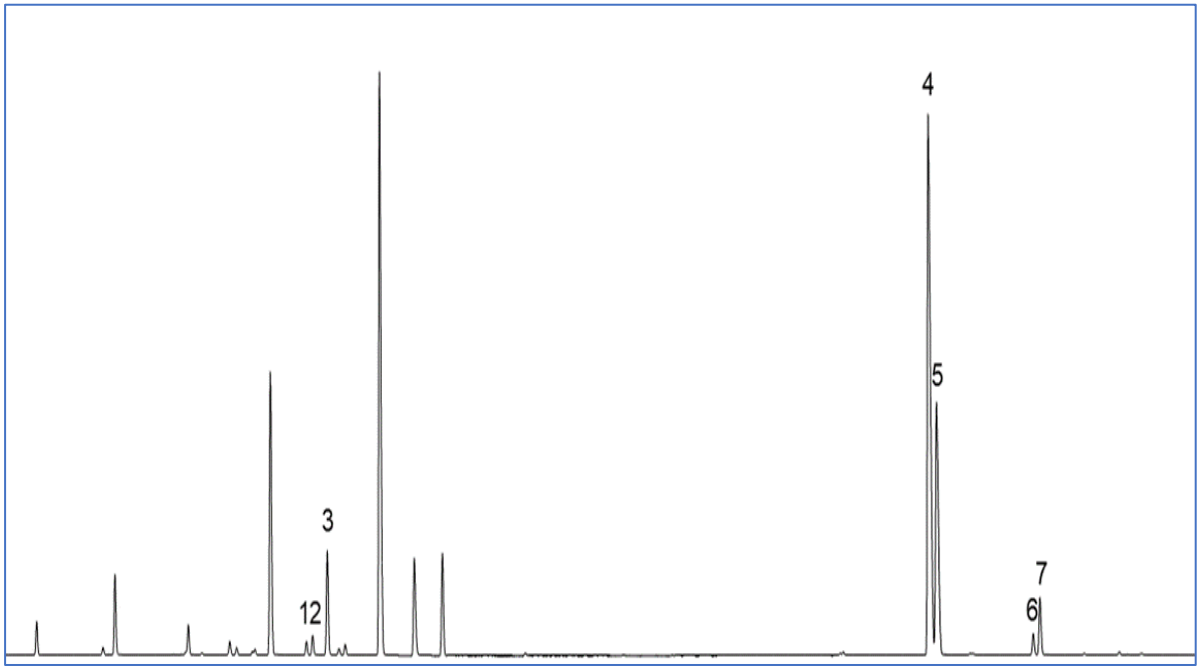
- *Detection:* flame ionisation
- *Injection:* 1 µL of the solution
- *Elution order:* Order indicated in the composition of the reference solution. Record the retention times of these substances.
- *System suitability:* Reference solution.
- *Resolution*: minimum 1.18 between the peaks due to (+)-limonene and (-)-limonene
  minimum 0.94 between the peaks due to (+)-terpinen-4-ol and (-)-terpinen-4-ol
  minimum 1.26 between the peaks due to (+)-α-terpineol and (-)-α-terpineol

Using the retention times determined from the chromatogram obtained with the reference solution; locate the components of the reference solution in the chromatogram obtained with the test solution. Disregard the peak due to ethanol.

Determine the percentage content of these components. The percentages must be within the following ranges:

Components	D (+) Maximum % and Minimum %	L (-) Minimum % and Maximum %
limonene	57.00 to 65.00	35.00 to 43.00
terpinen-4-ol	67.00 to 71.00	29.00 to 33.00
α-terpineol	69.00 to 79.00	21.00 to 31.00

Figure 5: Typical chromatogram of the analysis by gas chromatography of Tea Tree Oil taken on a chiral column:



Peak Identification

1. *(-) limonene*
2. *(+)-limonene*
3. *ρ*-cymene
4. *(+)*-terpinen-4-ol
5. *(-)*-terpinen-4-ol
6. *(-)-α*-terpineol
7. *(+)-α*-terpineol

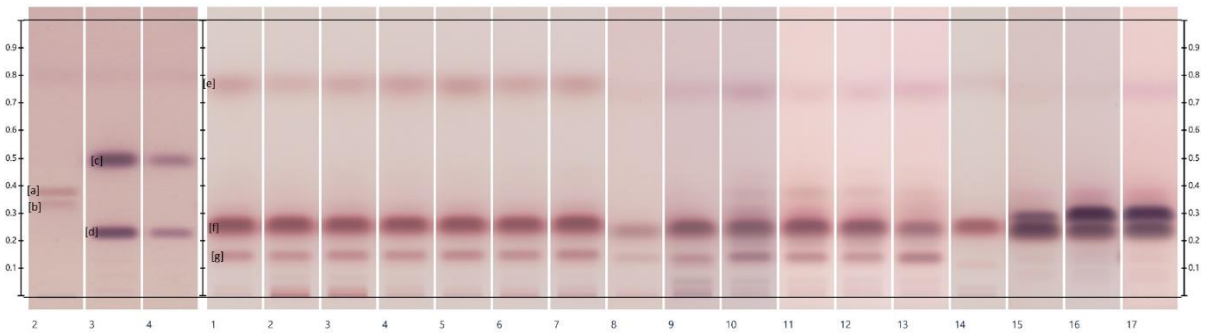
B. **Examine the chromatograms obtained using High-Performance Thin-layer Chromatography (HPTLC)**

HIGH PERFORMANCE THIN-LAYER CHROMATOGRAPHY (2.8.25)

Test solution:	Dissolve **30** µL of the essential oil to be examined in 1 mL of toluene *R*
Reference solution (*a*)	Dissolve 10 µL of ***linalool*** *R and 10* µL of ***linalyl acetate*** *R* in 10.0 mL of toluene *R.*
Reference solution (*b*)*:*	Dilute 2.5 mL of *reference solution (a)* to 10.0 mL with *toluene R*.
Intensity marker:	Linalool
Reference solution (*c*) *(SST):*	Dissolve 2.5 µL of isoeugenol *R* and 5 mg of isoeugenyl acetate *R* in 10.0 mL of toluene.
Plate:	*TLC silica gel plate F_{254} R (2-10 µm)*
Mobile phase:	Ethyl acetate *R*, toluene *R* (5:95 *V/V*).
Application:	2 µL, as bands of 8 mm
Development:	70 mm from lower edge of the plate.
Drying:	In a current of cold air
Derivatization reagent:	To 170 mL of cold methanol 20 mL of acetic acid and 10 mL of sulfuric acid are added and mixed well. After cooling to room temperature, 1 mL of anisaldehyde (*p*-methoxy benzaldehyde) is added to the mixture.
Detection A:	Treat the plate with the derivatization reagent and heat the plate at 100°C for 3 minutes. Examine the plate under white RT light.

- *System suitability:* the chromatogram obtained with reference solution (c) shows two clearly separated violet zones due to isoeugenol and isoeugenyl acetate (with increasing R_F)
- *Results:* See table and image below. The sequence of the zones present in the chromatograms obtained with the reference solution and the test solution. Other zones are present in the chromatogram obtained with the test solution.

Top of the plate		
		[e] A faint reddish zone
---	[c] Linalyl acetate (a violet zone)	---
[a] Isoeugenol acetate (a faint purple zone)		
[b] Isoeugenol (a faint purple zone)		[f] An intense purple zone (terpinen-4-ol)
---	[d] Linalool (a violet zone)	---
		[g] A purple zone (α-terpineol)
Reference solution (c) (SST)	***Reference solutions (a) and (b)***	***Test solution***



References: 2: (**c**) or (SST); 3: reference solution (**a**) 4: reference solution (**b**)

Samples: **1-7**: Tea tree oil commercial samples; **8-10**: oil distilled of *M. alternifolia*; **11-13**: oil distilled from *M. linariifolia*; **14**: terpinen-4-ol; **15-17**: oil distilled from *M. quinquenervia*

FLASHPOINT

The mean value is +59 °C using closed cup equipment.

SAMPLING

<Adapt from and insert Ph Eur Reference if available>

The organoleptic, physical and chemical characteristics of batches of essential oils are determined by means of examination of the samples. A satisfactory sampling operation therefore needs to provide, for analysis, samples representative of the batches from which they originate without modification of the original composition.

Apparatus: The sampling devices and the related instruments must be made of materials which do not affect the sampled essential oil (eg stainless steel or glass).

Mixing: Prior to any sampling, thoroughly mix or shake the essential oil using means suited to both the volume and the shape of the storage container.

Sampling method: All sampling operations must be performed immediately after appropriate mixing or shaking.

Take three increments per container, as follows:

- take the **first** increment from the section corresponding to 20 % of the container height
- take the **second** between 40 % and 60 % of the container height
- take the **third** at over 95 % of the container height

Gather together the three equal part increments and mix them in a non-reactive container minimising access to the atmosphere at all times. After mixing thoroughly take a minimum of 100 mL, which constitutes the sample. Pack, mark, record and store the sample as described below.

Packaging: When preparing for storage or despatch take the required aliquot from the sample held and use dark glass or inert material bottles (eg aluminium or stainless steel) which protect the essential oil against light. Pack the samples in clean, dry containers. The nature of the containers must not alter the essential oil. Close the containers with suitable non-reactive new stoppers or lids which do not affect the sampled product (eg PTFE/Teflon®-lined wadding). Ascertain the airtightness.

Marking: The information on the label must be marked in indelible ink and the label must be attached to each of the samples and must bear sufficient marking to enable the traceability of the product, as a minimum:

- the sampling date
- the nature of the product: goods and origin
- the name of the supplier
- the batch number
- the serial number of the sample out of the total number of containers

Conservation: Store the samples intended for the laboratory, protected from light, at a temperature which guarantees their quality.

Dispatch: The packaging must meet the requirements of the postal or other services involved in the transport of the sample within the relevant countries.

Sampling report: The sampling report must as a minimum indicate:

- the identification of the supplier
- the product identification marks
- the origin
- the batch number
- the quantity represented in grams, kilograms or tons
- the nature and the number of containers
- the presence or absence of the guarantee systems
- the date and time of sampling
- the name, signature and function of the person who carried out the sampling

The sampling report must clearly describe the physical condition of the sampled essential oil and must also indicate the technique employed, if different from that described in this Standard, as well as all circumstances which may have influenced the sampling.

STORAGE

For longer term (more than 24 months) storage: only stainless steel or amber glass vessels should be used at all times.

For shipment or short-term (less than 24 months) storage: aluminum flasks, appropriately fluorinated HDPE vessels or appropriate Intermediate Bulk Containers (eg SCHÜTZ MX-EX-OV) are suitable in addition to stainless steel and amber glass vessels.

Deployment of inert gas (Nitrogen or Argon) as a blanket or for sparging should be considered especially where the headspace is excessive in any storage vessel.

The wadding of any closure should be constructed to only allow PTFE/Teflon® to come into contact with the product.

Storage should not exceed ambient temperature. While data does not exist demonstrating product degradation, efforts should be made to maintain the temperature at or below 25°C.

Ph Eur

British Pharmacopoeia Secretariat
Attn: Himal Makwana and Hina Ashraf
C/O MHRA
10 South Colonnade
Canary Wharf
London E14 4PU UK

26th July 2019

Dear Himal and Hina,

RE: BP and Ph Eur Monograph Revision

As you are aware Catherine Lenihan wrote to me on Friday, 25 January 2019 saying in part:

"*I have forwarded EDQM the information provided by ATTIA to support a revision to the EP monograph. This has been discussed by the expert Group. The Group are willing to revise the monograph, and as you know the HPTLC work has been performed. However the Group have requested a more defined revision proposal to proceed.*

I think the best thing to do would be if you can provide a draft revised monograph, based on your data/methods, which we can submit to the Group. By this I mean use the current EP monograph as a basis, to prepare a draft revised monograph document to include the new methods/tests and proposed limits to outline exactly what a revision to the monograph should include. This does not need to be written in pharmacopoeial language, but just give a clear outline to the group of the expectations for the revision."

I have now finalised a draft revised Monograph and have attached this along with a number of supporting documents all of which form part of the revision proposal requested by Catherine.

I have used 'BP' liberally throughout the documents despite your advice "*The BP does not have a monograph for Tea Tree Oil based on the EP monograph superseding any previous monograph that we may have had*" because this initiative came from Australia's Therapeutic Goods Administration (TGA) who continue to refer to the BP Standard when managing and licensing tea tree oil (TTO). Please note that where I have used 'BP' throughout this is intended to be fully interchangeable with 'Ph Eur'.

The documents attached are:

1. This document: a **cover letter** and **brief explanation** of the full proposal
2. Ph. Eur. Monograph 1837 - Tea Tree Oil FINAL draft July 2019
3. BP Submission-Melaleuca Species in Standards July 2019
4. BP Submission - Change to some Component Ranges July 2019
5. BP Submission - Addition of Chiral Ratios for TTO July 2019
6. BP Submission-Inclusion of Methyl Eugenol in Chromatographic Profile of TTO July 2019
7. ATTIA BP + Ph Eur Dataset September 2018 (excel spreadsheet)
8. Chiral Median and Ranges TTO Jul 19 (Excel spreadsheet)

I have provided on the following pages a very brief explanation on the draft Monograph based on its headings with reference where necessary to the documents that form this submission.

PO Box 903, Casino NSW 2470 Tel: 02 4017 1336 Email: tlarkman@attia.org.au

This section, intended to provide guidance and a brief explanatory note for each section is based on the titles in the attached "**Ph. Eur. Monograph 1837 - Tea Tree Oil FINAL draft July 2019**".

The intention here is to produce a draft Monograph that I personally feel is fit-for-purpose noting that I do not have access to the resources in the hyperlinks to the relevant databases.

The expert committee will of course be able to make any structural, formatting or other changes as they see fit and to match the requirements for formatting etc.

General Notices

1. I have included two CAS numbers here as both are used in the EU's REACH database; the CAS numbers provide clear and unequivocal definition of the substance.
2. I have included the INCI name as this is mandated by the EU for use in manufactured products, again this provides clarity in relation to the definition of the substance.
3. I have included both '**Melaleuca oil**' and '**Essential oil of Melaleuca, terpinen-4-ol type**' as the latter is in my opinion a clearer definition of the substance universally traded as tea tree oil.
4. I have added the phrase "**The whole oil, with nothing added or removed by any means after distillation, settling, separation from any water fraction and filtration constitutes the defined material**" in this section in addition to what is already in the current Monograph. I anticipate that this may be deleted but urge the expert committee to consider its inclusion as the statement clearly defines what is 100% pure TTO and therefore provides absolute clarity on the definition of the substance.

Definition

I have removed the species ***Melaleuca dissitiflora*** from the definition along with the statement ***"…as well as other species of Melaleuca provided that the oil obtained conforms to the requirements given in this International Standard***". The attachment '**BP Submission-Melaleuca Species in Standards July 2019**' gives in-depth reasons for this and is supported by another attachment '**BP Submission-Inclusion of Methyl Eugenol in Chromatographic Profile of TTO July 2019**' which provides background data on the levels of methyl eugenol in a number of species of *Melaleuca* that is pertinent to this as well as a recommendation not to include Methyl Eugenol in the component table.

Characters

I have tabulated this to make it easier to read/use.

Tests

I have moved this section to the top and tabulated it with the intention of making it easier to read/use and to ensure that these physical parameters are separately identified for complete clarity.

1. The ranges for both Relative Density and Refractive Index have not been altered, the word 'between' has been added for absolute clarity.
2. The optical rotation (OR) range has been modified from '+ 5° to + 15°' to 'between +7.00° and +12.00°' with the deliberate inclusion of 2 decimal places for absolute clarity.

Data to support the proposed change to the OR is available in the attachment '**ATTIA BP + Ph Eur Dataset September 2018**' and detailed discussion and a recommendation is included in the attachment '**BP Submission - Change to some Component Ranges July 2019**'.

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Identification

I have changed the order of identification so the chromatographic profile is the first identification and HPTLC the second. This is because HPTLC is not particularly useful for identifying TTO and in particular it is not, in my opinion, of any use for quantitave or other differentiation between 100% pure TTO and adulterated (falsified) material masquerading as TTO; indeed I will be recommending later in this document that the entire HPTLC section be deleted from the Monograph. There have been several very significant changes to the gas chromatography section, these are summarised:

1. The solvent for the test solution has been changed to Ethanol to assist with OH&S obligations and also the environmental and economic impact of using Hexane or other solvents.
2. The reference solution has been modified to include components recommended for inclusion in the GC profile; some of these may not be easily available commercially. An alternative (not included in the draft) would be to source some known provenance 100% pure TTO and dilute this. The expert committee may wish to establish a reference standard for TTO and make this accessible as described in the article '*Ph. Eur. Reference Standards: Purpose and use*' available at https://www.edqm.eu/en/ph-eur-reference-standards-purpose-and-use. ATTIA would be delighted to collaborate in this as it will assist in avoidance of manipulation and/or inadvertent selection of falsified material.
3. The column length, film and diameter have been changed to 60 metres, 0.25 µm and 0.25mm respectively in line with commonly used columns in Australia and elsewhere.
4. The stationary phase (macrogol) has been changed to the most commonly used in commercial laboratories for this kind of work (5% Phenyl Polydimethylsiloxane) and operating parameters adjusted to suit. It is the considered opinion of many Australian experts in the field of GC that macrogol is particularly unsuited to performing analysis on essential oils in general and TTO in particular as the peak resolution and separation is poor.
5. Detail and images (high resolution copies of these are available on request) have been provided for both non-polar and mid-polar columns to provide suitable alternative methods depending on the columns used.
6. The component list has been tabulated and the altered and the minimum and maximum percentage values of each have been modified where appropriate. Wherever possible these include two decimal places for absolute clarity.
   The expert committee may have issues with the inclusion of 'traces' in the component table however this may be the only way to ensure inclusion of minima for some components.
   ATTIA has data to demonstrate that falsified samples sometimes (not always) dilute the levels of minor compounds which are always found in 100% pure TTO to below the level of quantification using GC-FID; their absence is just as telling of adulteration as an overabundance of a compound which is why they have been included in the component list in the manner presented. The use of 'traces [a]' with the notation '[a] traces < 0.01percent' is somewhat ambiguous but the best I can come up with.
   Data to support the proposed changes to the components table is available in the attachment '**ATTIA BP + Ph Eur Dataset September 2018**' and detailed discussion and a recommendation is included in the attachment '**BP Submission - Change to some Component Ranges July 2019**'

For convenience the proposed ranges and compounds is shown in Table 1 on page 4.

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Table 1: Proposed ranges and compounds

Components	Minimum percentage	Maximum percentage
α-pinene	1.00	4.00
sabinene	traces [a]	3.50
α-terpinene	6.00	12.00
limonene	0.50	1.50
ρ-cymene	0.50	8.00
1,8 cineole	traces [a]	10.00
γ-terpinene	14.00	28.00
terpinolene	1.50	5.00
terpinen-4-ol	35.00	48.00
α-terpineol	2.00	5.00
aromadendrene	0.20	3.00
δ-cadinene	0.20	3.00
ledene (syn. viridiflorene)	0.10	3.00
globulol	traces [a]	1.00
viridiflorol	traces [a]	1.00

[a] traces < 0.01percent

Enantiomeric Distribution

Enantiomeric (chiral) abundances have been included for the first time to assist with the detection of adulteration through fraudulent practices. This inclusion may be cross-referenced to the **Ph Eur Monograph 01/2008:2098** with particular reference to the heading '**Falsification**' where the use of a chiral column is recommended.

Similar to the section utilising a GC for compound identification and quantification a suitable column and parameters for operation have been included along with images. Tabulated maxima and minima to include two decimal places for absolute clarity have been provided.

The expert committee is requested to carefully consider the inclusion of the resolution for the named enantiomers as co-elution not only between enantiomers but also other compounds has been an issue in the past for some labs, this is easily resolved if the resolution is controlled. Some detail for this is included in the attachment '**BP Submission - Addition of Chiral Ratios for TTO July 2019**'.

Data to support the proposed addition of enantiomeric abundances is available in the attachments '**ATTIA BP + Ph Eur Dataset September 2018**' and "**Chiral Median and Ranges TTO Jul 19**". Detailed discussion and recommendations are included in the attachment '**BP Submission - Addition of Chiral Ratios for TTO July 2019**'.

High Performance Thin Layer Chromatography

This section was prepared and written by Dr. Eike Reich of CAMAG; I have had no input into this section except for the provision of materials (leaf and oil samples) to assist Dr Reich and some very minor editing for continuity.

I do however (and this will likely disappoint Dr Reich) strongly recommend that unless there is a legal or other strong imperative to retain this section that it be removed in its entirety because, in my opinion and that of several other experts in the field of essential oil analysis, the use of HPTLC analysis is severely limiting and in many cases effectively useless for the identification and quantification of compounds in the substance being tested where adulteration (falsification) is suspected.

With over 70% of all EU sourced samples of TTO tested in the early years of the development of chiral purity testing for TTO showing clear evidence of adulteration (see the attachment '**BP**

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Submission - Addition of Chiral Ratios for TTO July 2019' for more detail) the ability to detect adulteration is vital particularly for the safety of consumers. I can now report that the incidence of adulteration detected using GC-FID (in 29 samples from Spain in this instance) has dropped to around 30% in 2019. Even though this is still not good enough it is a significant improvement on 2012 – 2014 before chiral purity testing was developed and introduced.

Flashpoint

The inclusion of a flashpoint is recommended because TTO is classed as a Dangerous Good (Flammable Class III) and there have been instances where the flashpoint has been falsified in the past (eg to 61+ °C) to enable transport under a lower DG class. This fraudulent activity could be extremely detrimental if an accident occurs in the future and emergency responders are provided with incorrect information. The inclusion of a Flashpoint may provide a legal basis for action in instances where fraudulent activity is detected or results in an OH&S or safety breach.

Sampling

Sampling procedures are absolutely vital to ensure representative samples are tested and shipped so this section has been included and is strongly recommended in whatever format and detail the expert committee feels is sufficient. As I have no access to the Ph Eur database I am unsure what is available; I have instead copied almost verbatim the section from the ISO 4730 Standard and included a suggestion to "*Adapt from and insert Ph Eur Reference if available*".

Storage

The current version of BP/Ph Eur only mentions a maximum storage temperature. While this is commendable it is not practical in some instances eg where large (up to 17,100 kg in a 20' container) sea freight consignments are made.

Temperature is important but not as important as the use of appropriate containers, inert gas blanketing and/or sparging and correct sealing of containers to exclude air (oxygen).

I have included a number of recommendations in this area and would be willing to provide more information should the expert committee request it; it is largely based on personal experience, communication from experts in the field and ATTIA's Code of Practice.

I look forward to hearing from you as soon as practically possible in relation to a revision of the Monographs.

Yours sincerely,

Tony Larkman

CEO – ATTIA Ltd

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Australian Standard
K175—1967

OIL OF MELALEUCA ALTERNIFOLIA

STANDARDS ASSOCIATION OF AUSTRALIA
Incorporated by Royal Charter

A committee comprising producers and users of essential oils, together with the Museum of Applied Arts and Sciences, NSW, was entrusted with the preparation of this standard.

This standard, prepared by Committee CH/21, Australian National Committee on Essential Oils, was approved on behalf of the Council of the Standards Association of Australia on 25 July 1967.

In order to keep abreast of progress in the industries concerned, Australian standards are regularly reviewed. Suggestions for improvements, addressed to the Headquarters of the Association, will be welcomed.

The specification is intended to include the technical provisions necessary for the supply of the materials referred to but does not purport to comprise all the necessary provisions of a contract.

PREFACE

This standard for oil of Melaleuca alternifolia is one of a series of Australian standards for essential oils being developed by the Australian National Committee on Essential Oils.

It has been based on proposals submitted by the Museum of Applied Arts and Sciences.

The standard requires reference to a number of Recommendations and Draft Recommendations of the International Organization for Standardization (ISO).

First issued 1967

PUBLISHED BY THE STANDARDS ASSOCIATION OF AUSTRALIA
SCIENCE HOUSE, 157 GLOUCESTER STREET, SYDNEY

5 METHODS OF TEST.

5.1 **Preparation of Test Sample.** See ISO Recommendation ISO/R356—Preparation of Test Sample Applicable to Methods of Test for Essential Oils.

5.2 **Relative Density at 20°/20°C.** See ISO Recommendation ISO/R279—Determination of the Density and Relative Density of Essential Oils.

5.3 **Optical Rotation at 20°C.** See Draft ISO Recommendation No. 89—Determination of the Optical Rotation of Essential Oils.

5.4 **Refractive Index at 20°C.** See ISO Recommendation ISO/R280—Determination of the Refractive Index of Essential Oils.

5.5 **Solubility in Ethanol.** See Draft ISO Recommendation No. 869—Determination of the Solubility of Essential Oils in Ethanol.

How the ISO4730: 2017 Standard Helps Identify Fraudulent Tea Tree Oil

March 2017

Pure Australian Tea Tree Oil (TTO) steam distilled from *Melaleuca alternifolia* should always conform to the International Standard **ISO 4730: 2017 "*Oil of Melaleuca, Terpinen-4-ol type*"**.

Species

There are only two named species in the Standard from which TTO can be steam distilled:

1. *Melaleuca alternifolia* (Maiden et Betche) Cheel
2. *Melaleuca linariifolia* Smith

ISO 4730: 2017 specifies that for *Melaleuca alternifolia,* only the terpinen-4-ol chemotype is to be used; this ensures the terpinen-4-ol level in the TTO exceeds the minimum of 35.00%.

Chromatographic Profile

ISO 4730: 2017 specifies the levels of 15 of the 113+ components of pure Australian TTO:

Component	ISO 4730: 2017 Range	
	Minimum %	Maximum %
α-pinene	1.00	4.00
sabinene	traces [a]	3.50
α-terpinene	6.00	12.00
limonene	0.50	1.50
p-cymene	0.50	8.00
1,8-cineole	traces [a]	10.00
γ-terpinene	14.00	28.00
terpinolene	1.50	5.00
terpinen-4-ol	35.00	48.00
α-terpineol	2.00	5.00
aromadendrene	0.20	3.00
ledene	0.10	3.00
δ-cadinene	0.20	3.00
globulol	traces [a]	1.00
viridiflorol	traces [a]	1.00
[a] traces: <0.01%		

Physical Requirements

ISO 4730: 2017 specifies several physical parameters of pure Australian TTO:

Physical Properties	ISO 4730: 2017 Range	
	Minimum %	Maximum %
Appearance	Clear, mobile liquid	
Colour	Colourless to pale yellow	
Odour	Characteristic	
Relative Density (20°C)	0.885	0.906
Refractive Index (20°C)	1.475	1.482
Optical rotation (20°C)	+ 7.00 °	+ 12.00 °
Miscibility in 85% (v/v) ethanol (20°C)	Less than 2 volumes	

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ISO 4730: 2017 provides a range (maximum and minimum) for all objective parameters to allow for the variation that occurs in natural products such as pure Australian TTO. This natural variability is usually caused by slight differences in growing conditions from year to year, minor genetic variation from plantation to plantation and differing distillation techniques used.

Note: Minor variations can also occur with identical samples. This is usually due to differing conditions (temperature, humidity, atmospheric pressure etc) when an analysis is done, as well as the specified tolerances of the testing equipment. When a sample of TTO is sent to a laboratory for analysis a minimum of 15 ml is usually required; ISO 4730: 2017 specifies a sample size of 50 ml.

Enantiomeric Distribution

In addition to the physical and chromatographic requirements, ISO 4730: 2017 also stipulates a range for the enantiomeric (chiral) ratio of terpinen-4-ol to provide an extra measure of authenticity as shown below:

	ISO 4730: 2017 Enantiomeric Distribution	
terpinen-4-ol	**Minimum %**	**Maximum %**
(R) or (+) or (D)	67.00	71.00
(S) or (-) or (L)	29.00	33.00

Parameters Explained

Chromatographic Profile

Pure TTO contains 113+ compounds which can be identified and quantified using a gas chromatograph (CG). It is impractical to base a Standard on all 113+, so Standards committees look very carefully at the GC profile for a substance and choose a limited number of components based on both their importance and uniqueness. This is similar to a fingerprint search: is not possible to compare the entire print, so reference points are used. If any sample does not contain all 15 listed substances that are within the ranges given, then it cannot be sold, used or described as pure TTO.

The absence of a compound is just as telling as having too much, especially the four compounds where the minimum percentage is 'traces' or less than 0.01%.

Physical Properties

Because cheating (extending, adulterating, etc.) can occur, Standards committees also provide a list of physical properties to help an analyst to determine the likelihood of a sample being pure TTO. Some of these are advisory while others are compulsory. They are a mix of subjective and objective properties.

Note: On very rare occasions, pure Australian TTO steam distilled from Melaleuca alternifolia doesn't fully conform to the Standard (e.g. high p-cymene or low terpinen-4-ol). This is usually due to either the incorrect chemotype being selected and grown or incorrect distillation, storage and handling procedures.

Subjective properties

The **appearance**, **colour** and **odour** are subjective (not measured precisely). The Standard describes these so an analyst can, based on both experience and observation, state that a sample either conforms or fails.

Objective properties

These are measured using calibrated precision instruments. The results are compared to the ranges given in the Standard to either conform or fail.

Relative Density

Relative density (RD) is the ratio of the density (mass of a unit volume) of a substance to the density of a given reference material, usually water. If the RD = 1 then it is equivalent to pure water. If the RD is less than 1.00 it is less dense than water and will float. A good example is ice which has an RD of 0.91.

For pure TTO the RD at 20°C must be between 0.885 and 0.906.

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Refractive Index

Refractive index (RI) is the measurement of how hard it is for light to travel through a medium. The higher the number the harder it is. It is compared to air with an RI of 1.00. Examples of the RI for liquids at 20 °C:

Water	1.333
Ethanol	1.360
Benzene	1.501

For pure TTO the RI at 20°C must be between 1.475 and 1.482.

Optical Rotation

When plane-polarised light is passed through a sample of known chiral (enantiomeric) content, the plane of the polarised light is rotated by a very specific and measurable quantity. This is known as the optical rotation (OR) of a substance. This technique is used to measure the purity and concentration of familiar chemicals:

Sucrose	+66.47°
Cholesterol	−31.5°
Camphor	+44.26°
Penicillin V	+223°

For pure TTO the OR at 20°C must be between +7.00° and +12.00°.

Miscibility in 85% (v/v) ethanol

It should be possible to completely dissolve a measured volume of pure TTO in less than twice that volume of 85% ethanol at 20°C to obtain a clear solution.

Enantiomeric Distribution

In the latest (2017) version of ISO 4730, the ranges for the enantiomeric (chiral) distribution of terpinen-4-ol, the most abundant compound in TTO, is included to provide an extra measure of authenticity for pure TTO.

Some components found in TTO (e.g. terpinen-4-ol, α-terpineol, limonene and α-pinene) exist in two enantiomeric forms designated as (R) and (S), D and L or (+) and (-), respectively, to indicate they rotate plane polarised light either to the right (+) or the left (-).

Many enantiomers have distinctly different properties so their presence in the correct form and ratio is critical. Also 100% pure natural essential oils such as TTO contain these enantiomers in known and characteristic ratios. This is upset by the addition of adulterants which may be industrial waste from normalising other essential oils (eg Eucalyptus, Pine and White Camphor) or compounds synthesised either from other essential oil components (eg terpinen-4-ol synthesised from sabinene) or from fossil fuels.

Hence the measurement of the enantiomeric (chiral) ratio is given in an informative annex (Annex C) of ISO 4730: 2017 to provide an inexpensive but very accurate measure of extra authenticity for 100% pure TTO.

For pure TTO, the enantiomeric distribution for terpinen-4-ol must be within the ranges given:

For the Dextro (D) or (+) enantiomer:	**67.00% to 71.00%**
For the Levo (L) or (-) enantiomer:	**29.00% to 33.00%**

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An Example of a conforming and two non-conforming samples

The following table lists the results of three samples analysed at the Southern Cross University Plant Science's Analytical Research Laboratory. One is 100% pure Australian TTO that has been grown under strict quality assurance while the other two show clear evidence of adulteration:

Component	ISO 4730: 2017 Range		Pure Australian TTO		Adulterated "TTO" #1		Adulterated "TTO" #2	
	Minimum %	Maximum %	Result	Outcome	Result	Outcome	Result	Outcome
α-pinene	1.00	4.00	2.35	conforms	3.25	conforms	3.23	conforms
sabinene	traces	3.50	0.20	conforms	0.14	conforms	0.03	conforms
α-terpinene	6.00	12.00	9.72	conforms	7.92	conforms	9.49	conforms
limonene	0.50	1.50	0.81	conforms	**2.13**	**fail**	**2.66**	**fail**
p-cymene	0.50	8.00	2.20	conforms	2.39	conforms	2.38	conforms
1,8-cineole	traces	10.00	1.91	conforms	3.23	conforms	1.72	conforms
γ-terpinene	14.00	28.00	20.78	conforms	21.35	conforms	21.08	conforms
terpinolene	1.50	5.00	3.50	conforms	2.99	conforms	3.08	conforms
terpinen-4-ol	35.00	48.00	43.76	conforms	40.60	conforms	41.46	conforms
α-terpineol	2.00	5.00	2.94	conforms	4.53	conforms	**5.57**	**fail**
aromadendrene	0.20	3.00	1.02	conforms	1.46	conforms	1.71	conforms
ledene	0.10	3.00	0.89	conforms	0.55	conforms	0.61	conforms
δ-cadinene	0.20	3.00	0.78	conforms	0.39	conforms	**0.07**	**fail**
globulol	traces	1.00	0.12	conforms	0.44	conforms	0.57	conforms
viridiflorol	traces	1.00	0.10	conforms	0.14	conforms	0.11	conforms
Physical Properties	**ISO 4730: 2017 Range**		**Pure Australian TTO**		**Adulterated TTO**		**Adulterated TTO**	
	Minimum %	**Maximum %**	**Result**	**Outcome**	**Result**	**Outcome**	**Result**	**Outcome**
Appearance	Clear, mobile liquid		conforms	conforms	conforms	conforms	conforms	conforms
Colour	Colourless to pale yellow		conforms	conforms	conforms	conforms	conforms	conforms
Odour	Characteristic		conforms	conforms	**Pine like**	**fail**	**Pine like**	**fail**
Relative Density (20°C)	0.885	0.0906	0.897	conforms	0.897	conforms	0.893	conforms
Refractive Index (20°C)	1.475	1.482	1.4788	conforms	1.477	conforms	1.4773	conforms
Optical rotation (20°C)	+ 7.00°	+ 12.00°	+10.32°	conforms	**+6.99°**	**fail**	**+5.80**	**fail**
Terpinen-4-ol (R) or (+)	67.00	71.00	68.30	conforms	**54.94**	**fail**	**45.76**	**fail**
Terpinen-4-ol (s) or (+)	29.00	33.00	31.70	conforms	**45.06**	**fail**	**54.24**	**fail**
Miscibility in 85% (v/v) ethanol (20°C)	< 2 volumes		0.60	conforms	0.50	conforms	**> 2 volumes**	**fail**

The 100% pure Australian TTO conforms to all parameters in the Standard and can therefore be sold, used and described as 100% pure TTO steam distilled from *Melaleuca alternifolia*.

Both adulterated samples failed: **Sample #1** failed 5 of the total 24 parameters while **Sample #2** failed 8 of the total 24 parameters.

Adulterated Sample #1

This is a very cleverly constructed example of adulteration and is an excellent demonstration of how a 'good' laboratory can dilute a 100% pure natural product with only one aim: increasing profitability at the expense of the producer and of course the consumer. This comparative analysis of the sample against the ISO 4730: 2017 Standard clearly demonstrates this.

This sample likely contains a high percentage of TTO. It has however been diluted, probably with terpinen-4-ol to boost it above 40.00%; limonene (which is strongly optically positive) has also been added to readjust the optical rotation.

In fact this sample conforms to the 2002 British Pharmacopeia (BP) Standard. It is worth noting that the BP Standard has not been updated since 2002. After more than 15 years both the BP and the *Ph. Eur.* Standard are urgently in need of updating to reflect advancements in modern analytical techniques; ATTIA Ltd therefore considers both BP and *Ph. Eur.* Standards worthless in their current form.

Note that while sample #1 conforms to most of the parameters for ISO 4730: 2017 it fails the enantiomeric (chiral) distribution for terpinen-4-ol, a clear and indisputable indicator that this has been boosted (likely with terpinen-4-ol derived from pine oil based on the odour detected). Despite having 42%+ more limonene than the maximum allowed this sample still does not quite make the minimum range for the optical rotation (OR) in ISO 4730: 2017.

Most tellingly the enantiomeric ratio for terpinen-4-ol was **18% below** the lower range for the R (+) and **36.5% over** the upper range for the D (-) enantiomers of terpenen-4-ol given for 100% pure TTO in ISO 4730: 2017.

It is likely this started out as a low terpinen-4-ol TTO which was purchased cheaply from a producer with the incorrect chemotype (remember it should be the terpinen-4-ol chemotype) and adulterated to make it fit the BP Standard. This does not make it TTO and it is worrying to note that the following compounds were also detected in the sample, none of which occur naturally in 100% pure TTO steam distilled from *M. alternifolia*:

- ρ-menth-3-ene 0.08%
- ρ-menth-1-ene 0.09%
- *trans*-pinocarveol 0.49%

These compounds are most commonly found in pine oil, indicating that this was the source of the terpinen-4-ol used. It is impossible to know what else is present from batch to batch because the adulteration uses uncontrolled waste from a diverse range of industrial fractionation processes to boost the quality of pine oil. It is entirely possible that pesticides, phthalates or other harmful compounds are present in this or other adulterated batches.

Adulterated Sample #2

It is likely that the product was originally an essential oil of some sort but it would have been of very poor quality (eg low terpinen-4-ol and maybe high ρ-cymene). In an attempt to make the product conform, the perpetrator would again have added terpinen-4-ol (often made from sabinene derived from pine oil), aiming for 40%+ which is what many buyers are after. The terpinen-4-ol added to this product has a different optical rotation to that found naturally in TTO so they would then also add synthetic limonene to balance this out but once again they had to overdo the limonene (77% more than the maximum) to achieve the OR of +5.80; once again this passes the BP Standard but the 2017 version of ISO 4730 requires a minimum optical rotation of +7.00 which they were likely unaware of at the time.

If you look at the results, they got the terpinen-4-ol level right so it conforms; but this attempt to construct 'tea tree oil' failed in many other very obvious ways:

1. The α-terpineol level is too high because the terpinen-4-ol used to get to the desired 40%+ level likely also contained a significant portion of α-terpineol due to poor fractionation control.
2. The limonene level is 77% too high because they needed to add it to the product to try to balance the optical rotation.
3. The δ-cadinene level is below the minimum required. This is precisely why minor components are in the Standard: they are always in a pure, natural sample of TTO in the ranges given in ISO 4730: 2017; if they are not present at these levels, then something is wrong with the purity of the sample.
4. The optical rotation is plus (+) 5.58°. Despite overdoing the limonene they still didn't get the optical rotation right although they tried hard to get it to conform to the BP Standard.
5. Most tellingly the enantiomeric ratio for terpinen-4-ol was **31.7 % below** the lower range for the D (+) and **64% over** the upper range for the L (-) enantiomers of terpenen-4-ol given for 100% pure TTO in ISO 4730: 2017.
6. The product failed to meet the miscibility test – it took more than 2 volumes of 85% ethanol to produce a clear solution.

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It is again likely this started out as a low terpinen-4-ol TTO which was purchased cheaply from a producer with the incorrect chemotype and adulterated to make it fit the BP Standard. This does not make it TTO and, of much greater concern, the following compounds were also detected in the sample, none of which occur naturally in 100% pure TTO steam distilled from *M. alternifolia.* These additional compounds, never found in 100% pure natural TTO indicate that the industrial waste used to adulterate this product was of even poorer 'quality' than that used in sample #1:

- p-menth-3-ene 0.09%
- p-menth-1-ene 0.30%
- trans pinocarveol 0.33%
- plinol-D 0.09%
- cis-β-terpineol 0.26%

Once again pesticides, phthalates or other harmful compounds may be present in this or other adulterated batches. Plinol-D is likely to have been sourced from fractionating white camphor oil.

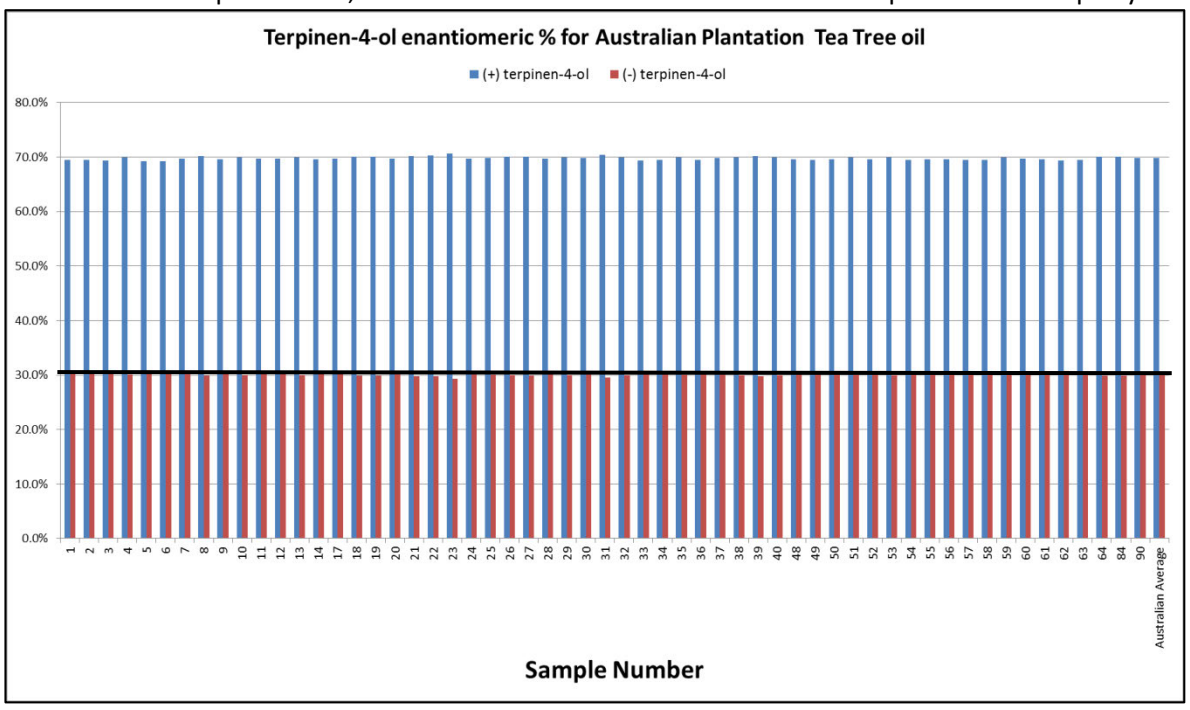
Enantiomeric (chiral) data

While ISO 4730: 2017 only specifies the enantiomeric distribution for terpinen-4-ol, ATTIA routinely requests that chiral data on a further two optically active compounds (limonene and α-terpineol) are also measured as this not only increases the measure of extra authenticity from one to three compounds but also provides a telling 'at a glance' first test for authenticity:

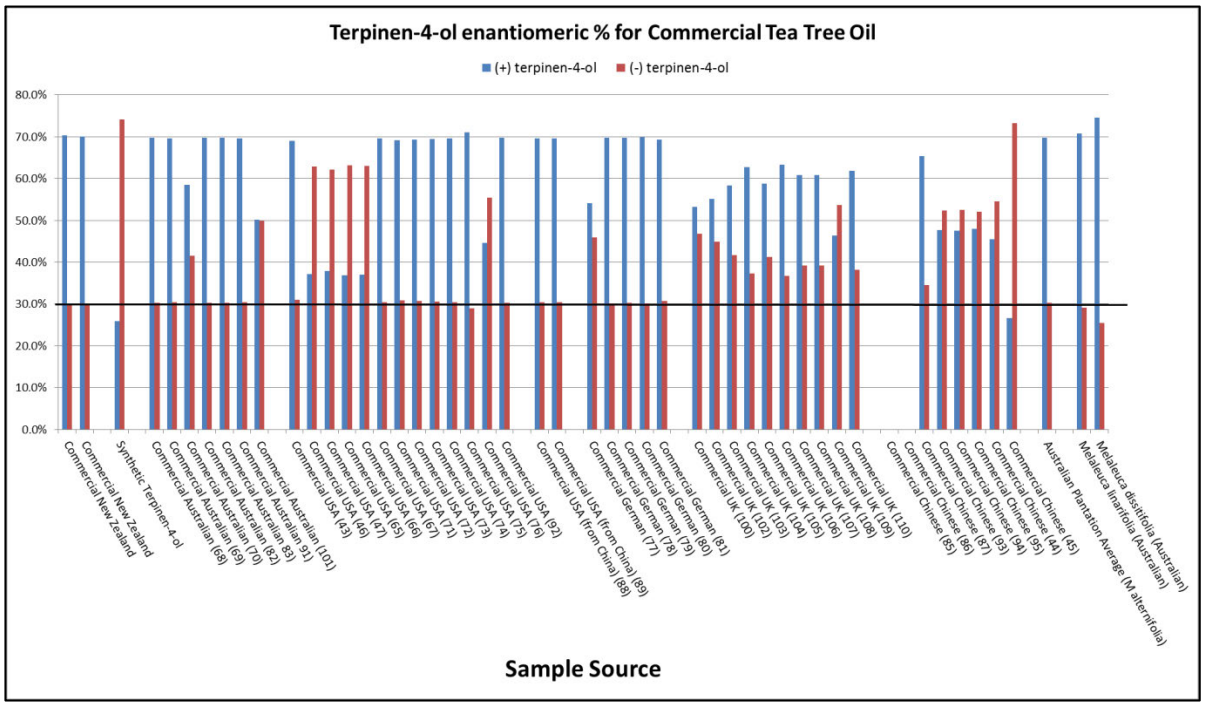
Sample	(-) limonene	(+) limonene	(+) terpinen-4-ol	(-) terpinen-4-ol	(-) α-terpineol	(+) α-terpineol
Pure Australian TTO	37.30	62.70	68.30	31.70	23.70	76.30
Adulterated Sample # 1	**16.85**	**83.15**	**54.94**	**45.06**	**73.73**	**26.28**
Adulterated Sample #2	**4.25**	**95.75**	**45.76**	**54.24**	**84.57**	**15.43**

It is immediately obvious from this that the Pure Australian TTO is as claimed while adulteration is equally obvious for all three compounds measured in the other two samples.

The enantiomeric distribution for terpinen-4-ol, α-terpineol and limonene was accurately measured for 57 samples of 100% pure Australian TTO sourced directly from plantations with samples representing production over a period of five years. The graph for terpinen-4-ol below shows how accurate chiral ratios are in 100% pure TTO; the data for the other two compounds is equally compelling:



When the same was done for a total of 48 commercial samples sourced from around the world a graph immediately and clearly shows which samples are authentic and which have been adulterated:



The following data on 100% pure Australian TTO of known provenance has been collected over the past three years to provide a basis for setting upper and lower ranges (the mean + 3 x the standard deviation (SD) from 131 samples) with a high degree of confidence for these three compounds:

	(-) limonene	(+) limonene	(+) terpinen-4-ol	(-) terpinen-4-ol	(-) α-terpineol	(+) α-terpineol
Average (n=131)	39.81%	60.20%	69.12%	30.89%	25.53%	74.45%
Median (n=131)	39.85%	60.16%	69.47%	30.54%	25.23%	74.74%
Lowest	35.09%	58.19%	66.41%	28.96%	23.23%	68.86%
Highest	41.81%	64.91%	71.04%	33.59%	31.14%	76.77%
Standard Deviation (SD)	0.98%	0.98%	0.86%	0.85%	1.27%	1.28%
3x SD	2.94%	2.95%	2.57%	2.56%	3.80%	3.84%
Lower Range (Average - 3x SD)	**36.87%**	**57.24%**	**66.55%**	**28.33%**	**21.73%**	**70.61%**
Upper Range (Average + 3x SD)	**42.76%**	**63.15%**	**71.68%**	**33.45%**	**29.33%**	**78.29%**

Conclusion

Adulterated material such as those shown in the table on page 4 is often described and offered as 'tea tree oil' or even as '100% pure Australian tea tree oil steam distilled from *Melaleuca alternifolia*' to world markets on a daily basis. It is not TTO, rather a mix of unknown chemicals from unidentified sources that have been put together with a single objective: profit.

No testing for safety and efficacy has ever been done on any of these concoctions.

By claiming this fabrication as TTO the perpetrators of this fraud rely entirely on the decades of research, good-will and the excellent reputation for safety and efficacy that pure Australian TTO enjoys.

August 2018

The Australian Tea Tree Oil (TTO) Industry

Tea Tree (*Melaleuca alternifolia)*

There are nearly 300 species in the myrtle family, *Myrtaceae* which are commonly known as paperbarks, honey-myrtles or tea trees. 'Tea tree' is also used for many *Leptospermum* species. The name tea tree was first recorded by Captain Cook during his voyage aboard the HMS Endeavour when he reached the eastern seaboard of *Terra Australis* in April 1770; he saw indigenous people using *Myrtaceae* to make a tea.

The Narrow Leaved Tea Tree (*Melaleuca alternifolia*) is an Australian native endemic to the eastern seaboard of Australia with a natural range from Newcastle, NSW in the south to Gladstone, QLD in the north and west to the Great Dividing Range. In its natural environment it grows to 8m (26ft) tall and prefers wetlands along river margins and around billabongs.

Figure 1: *Melaleuca alternifolia* in its natural habitat

It has been used by the indigenous Bundjalung people in its native range for many thousands of years to sooth and to promote healing in topical applications. The aborigines used the leaves and twigs to prepare poultices and antiseptic washes.

The species was first classified in 1904 by Maiden & Betch who described it as *Melaleuca linariifolia, var. alternifolia.* In 1924 Edwin Cheel first described *Melaleuca alternifolia* as a separate species writing *'M. linariifolia var. alternifolia is now separated as a species distinct from M. Linariifolia"* in the Journal and Proceedings of the Royal Society of NSW.

These two species (*M. alternifolia* and *M. linariifolia*) are closely related and it is likely that they will cross breed although their natural ranges differ slightly making this an uncommon occurrence in nature. Both species are named as producing oil that complies with the ISO 4730: 2017 Standard for the **Essential oil of Melaleuca, terpinen-4-ol type (Tea Tree oil)**.

In 1924 Arthur Penfold extracted the essential oil from the leaves and twigs of *M. alternifolia* for the first time and went on to record the 'germicidal value' of TTO as 10-13 times higher than phenol which was then commonly used by surgeons in operating theatres. Penfold also noted that TTO was not as caustic and so safer for both patients and staff than phenol. This is when the TTO industry was born.

The ISO 4730 Standard uses 'terpinen-4-ol type' in its title because *M. alternifolia* has 3 distinct cardinal chemotypes; each has a markedly different oil profile. There are also several intermediate chemotypes, probably resulting from cross-breeding of the cardinals in the wild. Only the terpinen-4-ol chemotype is suited for production of 100% pure Australian TTO. The other chemotypes contain much higher levels of 1,8 cineole (eucalyptol) or terpinolene and don't fit the ISO 4730 or any other accepted Standard.

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Production

Since 1924 when Penfold described TTO as an antiseptic product it has been produced in wood-fired 'bush stills' from wild harvested *M. alternifolia* trees and while this practice still occurs in some areas the bulk of production is now from mechanised plantation monocultures.

Figure 2: A bush still being charged prior to firing

With the introduction of modern antibiotics immediately post WWII the use of TTO declined and manufacturing remained a minor cottage industry until 1976 when Eric White founded Thursday Plantation. Then his son-in-law Christopher Dean's toenail changed the TTO industry forever in 1978 as Christopher began his remarkable one man crusade to introduce TTO to the world. Early attempts in the 1970's to establish plantations evolved, over the next 40+ years, into modern plantations which continue to develop to this day as new technology is introduced and adopted.

Modern plantations, some of which are over 750 hectares, are typically monocultures with standard row spacing of 1 metre and in-row spacings of between 30 and 40 cm (depending on soil quality) resulting in between 33,000 to 35,000 trees per hectare. The trees, which grow to between 6 and 14 feet high in just 12 months, are harvested at ground level annually and re-coppice. They are remarkably hardy and very difficult to kill; indeed they are considered a pest species by some graziers.

ATTIA's ongoing breeding program which has run since 1991 has increased yield from ~120 kg/ha to over 450 kg/ha in a good season.

Figure 3: A newly established TTO plantation

The entire biomass, which contains between 1% to 2% oil is finely chopped then fed into sealed stainless steel bins where steam is introduced. A batch usually takes 2 hours to be fully distilled and the steam is passed through a condenser to reduce the temperature to between 35°C and 55°C before passing into a separator where the TTO floats to the top to be siphoned off, allowed to settle, filtered and then stored ready for sale. The by-product, 98-99% of the material harvested, is stockpiled and either spread back onto the paddocks or sold as garden mulch. Another by-product, the condensed steam which has been in intimate contact with the oil, is marketed as TTO hydrosol.

Figure 4: Harvesting tea trees prior to distillation

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Figure 5: Tea tree distillery (L) and tea tree oil in a separator apparatus post-distillation (R)

The life expectancy of a modern plantation is 15 – 20 years because breeding efforts continue to produce better yielding varieties although one small paddock, which was established in 1980, will be harvested for the 38th consecutive year later in 2018 and continues to yield around 200 kg per hectare (kg/ha).

Figure 6: Aerial view (L) and ground level view of a modern tea tree plantation

The seed from *M. alternifolia* is tiny (there are over 60,000 seeds per gram) and germinates poorly so specialised nurseries are needed to produce the seedlings for plantations. This process takes 2-4 months then the seedlings are planted out and grown for between 12 – 18 months before the first cut. Yields are generally lower for the first 2 to 3 harvests with full production potential realised in the 3rd or 4th season.

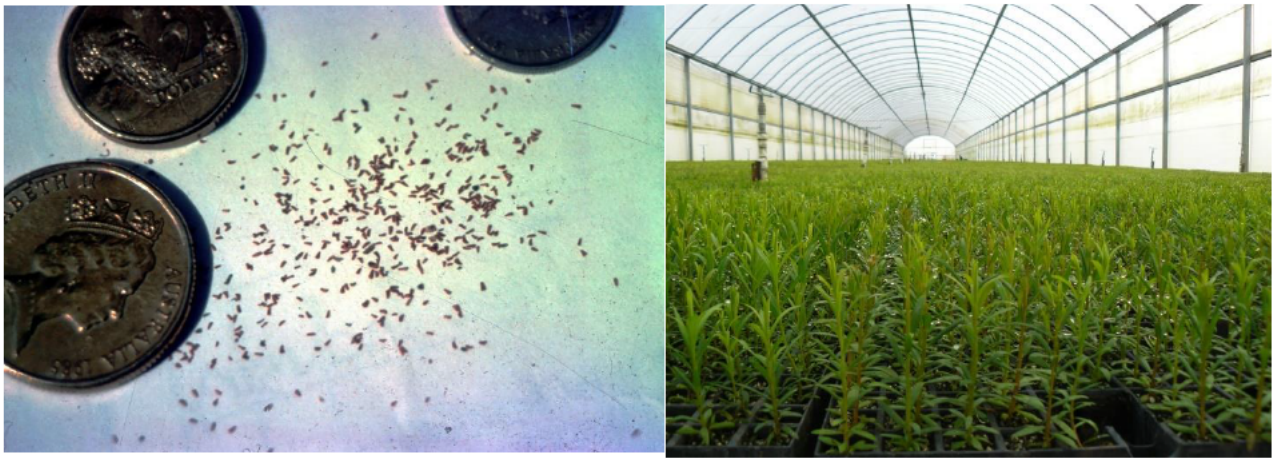

Figure 7: *Melaleuca alternifolia* seed (L) and 10 week old seedlings in a nursery (R)

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In 2017 the estimated area dedicated to plantation production was 4,000 hectares and production from this is estimated to be close to 1 million kg of TTO or 250 kg/ha. The highest yielding new varieties can yield up to 500 kg/ha but this depends on climatic and agronomic conditions; Australia has a high incidence of drought and flood so this is rarely achieved in some localities and almost never in all areas.

Production data is collected and published annually by ATTIA Ltd. Table 1 below summarises these figures for the past decade and it is worth noting that the projected 2018/19 figure, which came from a survey in March 2018, is likely to be very conservative: ATTIA now expects production to exceed 2017/18 and may even get to 1 million kg because despite ongoing drought conditions most production areas have had a better than expected season since the survey was conducted. The most remarkable figure in Table 1 is the dramatic 62% rise in exports in 2017/18 to 945,000 kg with no change in price per unit.

Year	2008/09	2009	2010	2011	2012/13	*2013/14*	*2014/15*	*2015/16*	*2016/17*	*2017/18*	*2018/19 projected*
Reporting Period	1 Apr to 31 Mar	1 Jan to 31 Dec	1 Jan to 31 Dec	1 Jan to 31 Dec	1 Jan to 31 Mar	1 Apr to 31 Mar	1 Apr to 31 Mar	1 Apr to 31 Mar	1 Apr to 31 Mar	1 Apr to 31 Mar	1 Apr to 31 Mar
Opening Stock	17	34	71	156	146	4	13	3	12.5	15.0	16.0
Production	427	427	511	402	407	*551*	*667*	*845*	*714*	*890*	*870*
Available supply	**444**	**461**	**582**	**558**	**553**	***555***	***680***	***848***	***726***	***905***	***886***
Sales (implied) demand	410	390	426.5	411.5	549	541.7	677	836	711	889	876
Demand change (%)	-7%	-5%	9%	-3.5%	33.4%	-1.3%	*25.0%*	*23.4%*	*-14.9%*	*25.0%*	*-1.5%*
Closing Stock	**34**	**71**	**156**	**146**	**4**	***13***	***3***	***12.5***	***15***	***16***	***10***
ABS Export (MT)					N/A	443	582	612	584	945	N/A
Export change (%)					N/A	N/A	32%	5%	-5%	62%	N/A
Domestic demand (implied)					N/A	99	95	90	90	95	95
In transit volume (implied)					N/A	0	0	133	37.93	-116.00	n/a

Table 1: Supply & Demand Data 2008/9 - 2018/19 MT = Metric Tonne

Market

Harvest for TTO commences annually in late April and continues until December so data is collected and collated commencing on 1st April to coincide with lowest available stock levels. Australian Bureau of Statistics (ABS) export data for 2017/18 and 2016/17 are shown in tables 2 and 3 on page 6. North America (USA, Canada, Mexico) is at 55% the largest export destination for 100% pure Australian TTO.

We know from a comprehensive survey of manufacturers and consumers in the European Union (EU) conducted in 2016 that the EU imports around 300,000 kg of TTO per year.

A standout figure from the ABS data is the change in export volume to the EU. In 2016/17 (Table 4 on page 6) the EU imported 189,000 kg or 63% of total demand from Australia and in 2017/18 and this jumped to 280,000 kg or 93% of total demand. The main driver of this is the adoption, effective 1st July 2018, of the ISO 4730: 2017 Standard for TTO by the EU in REACH[1] legislation to replace the outdated British Pharmacopeia (BP) and the identical European Pharmacopeia (*Ph Eur*) Standards.

This is discussed in more detail on pages 7 - 10 but a simple explanation is that EU importers can no longer import industrial waste from China and either sell it as-is or blended with real TTO to consumers masquerading as '100% pure TTO' with impunity because the ISO Standard is far more robust than the other Standards so it probably costs more to adulterate successfully than to just use the real thing.

The Australian TTO market has, like many other emerging industries, had a turbulent past with overproduction the main problem. This led to a large surplus of TTO in Australia and limited demand. In the late 1990's right through to early 2006 prices languished at or below A$ 15.00 per kg, well below the cost of production resulting in the closure of many plantations. By mid-2006 buyers realised that the days of cheap Australian TTO were over and a price war developed with the price spiking to A$ 57.00 in February 2009 (Table 2 on page 5).

[1] REACH: stands for Registration, Evaluation, Authorisation and Restriction of Chemicals (https://echa.europa.eu/regulations/reach/understanding-reach)

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ATTIA stepped in during 2009/10 and employed an industry development officer who was tasked with stabilising the TTO market and introducing Quality Assurance and stable, orderly marketing – not an easy task given the independent nature of most TTO producers. Between 2009 and 2012 prices dropped just as precipitately as they had risen and by late 2012 prices were back below A$ 30.00/kg and grave fears were held that the industry would simply slide back to rock bottom levels before withering and dying.

However, while prices were fluctuating ATTIA identified that adulteration, principally by Chinese and EU based firms, was the main issue facing the industry as a whole and realised that this could only be combatted by raising awareness of the quality of Australian origin 100% pure TTO when compared with industrial waste from China either as-is or blended with Australian (or other) TTO which was being sold with impunity across all markets as "100% pure TTO steam distilled from *Melaleuca alternifolia*".

To achieve this, a grass roots Quality Assurance (QA) scheme was needed and the vast majority of Australian producers needed to be included to ensure uniformity of production and purity remained at the forefront of all producers' minds at every stage in the production process. ATTIA's Code of Practice (COP) was developed and slowly became accepted and implemented as best practice for the industry as a whole.

Once a start had been made on QA, ATTIA's attention turned to investigating all of the accepted international Standards used globally to describe TTO to see why industrial waste was being sold with such impunity by the majority of traders globally. It quickly became obvious that all of the Standards for TTO were woefully out of date and clever traders realised, probably more than 15 years ago, that they could get away with buying Chinese industrial waste from the rectification of other essential oils such as Eucalyptus and Pine which can be converted to terpinen-4-ol and blended with 100% pure TTO to make the waste vaguely resemble TTO and in so doing make an absolute fortune.

Then the Chinese got into the game and the quality of the products being marketed as '100% pure TTO' really took a tumble with anything, literally anything, that vaguely resembled TTO being sold to anyone who wanted to buy solely on price without ensuring quality assurance principles were adhered to. This impacted not only the Australian TTO producers but also consumers who were buying the product in good faith and applying it liberally both as the whole oil and in cosmetics & therapeutic formulations.

Something had to be done and ATTIA, with the support of Federal Australian grants and a voluntary levy on all ATTIA members, was determined to address the issue.

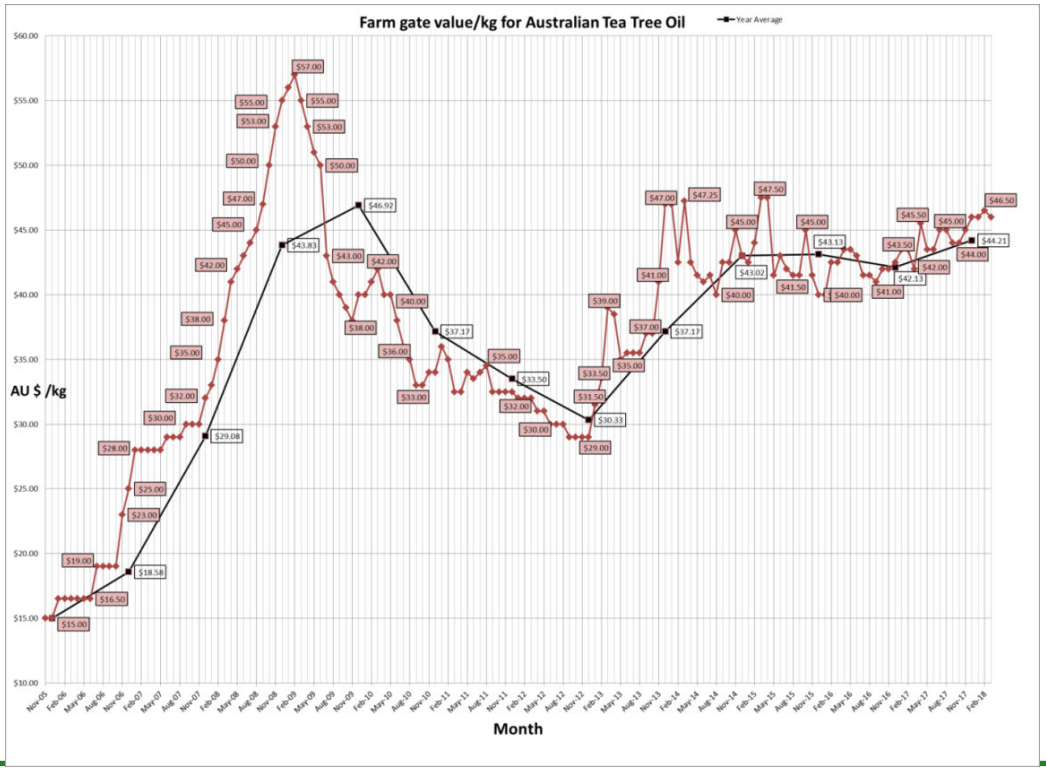

Table 2: TTO prices Nov 2005 - Feb 2018

FOB Australia	2017/18	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Totals
Asia	Total Sum of quantity (kg)	4,637.00	4,395.00	2,328.00	4,955.00	12,466.00	11,580.00	28,518.00	14,152.00	4,604.00	13,029.00	24,269.00	5,165.00	130,098.00
Africa/Mid East	Total Sum of quantity (kg)	0.00	3,662.00	0.00	0.00	0.00	0.00	100.00	480.00	260.00	0.00	833.00	4,255.00	9,590.00
Europe	Total Sum of quantity (kg)	6,893.00	40,320.00	4,545.00	24,383.00	24,956.00	7,465.00	14,860.00	37,416.00	39,030.00	12,300.00	48,178.00	19,349.00	279,695.00
North America	Total Sum of quantity (kg)	41,902.00	24,929.00	10,247.00	41,793.00	35,555.00	51,894.00	56,260.00	40,632.00	79,086.00	39,759.00	46,475.00	55,484.00	524,016.00
South America	Total Sum of quantity (kg)	100.00	0.00	430.00	200.00	0.00	0.00	0.00	0.00	0.00	430.00	0.00	950.00	2,110.00
TOTAL	Total Sum of quantity (kg)	53,532.00	73,306.00	17,550.00	71,331.00	72,977.00	70,939.00	99,738.00	92,680.00	122,980.00	65,518.00	119,755.00	85,203.00	945,509.00
	% per month	5.66%	7.75%	1 86%	7.54%	7.72%	7.50%	10.55%	9.80%	13.01%	6 93%	12.67%	9.01%	100.00%

Table 3: FOB export volume by month and region for harvest year 2017/18 by month

FOB: Free on board

FOB Australia	2016/17	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Totals
Asia	Total Sum of quantity (kg)	4,207.00	3,446.00	2,654.00	7,639.00	5,295.00	3,381.00	6,319.00	5,209.00	3,311.00	11,284.00	1,493.00	12,112.00	66,350.00
Africa/Mid East	Total Sum of quantity (kg)	325.00	400.00	0.00	1,490.00	55.00	0.00	3,620.00	0.00	0.00	870.00	1,140.00	0.00	7,900.00
Europe	Total Sum of quantity (kg)	23,397.00	6,364.00	16,714.00	7,266.00	9,135.00	15,955.00	37,999.00	6,105.00	20,967.00	15,287.00	13,163.00	17,205.00	189,557.00
North America	Total Sum of quantity (kg)	31,342.00	13,688.00	29,730.00	11,220.00	4,739.00	32,745.00	65,526.00	14,770.00	34,461.00	42,371.00	8,603.00	28,485.00	317,680.00
South America	Total Sum of quantity (kg)	540.00	0.00	76.00	0.00	0.00	0.00	0.00	0.00	551.00	0.00	848.00	0.00	2,015.00
TOTAL	Total Sum of quantity (kg)	59,811.00	23,898.00	49,174.00	27,615.00	19,224.00	52,081.00	113,464.00	26,084.00	59,290.00	69,812.00	25,247.00	57,802.00	583,502.00
	% per month	10.25%	4.10%	8.43%	4.73%	3 29%	8.93%	19.45%	4.47%	10.16%	11.96%	4.33%	9 91%	100.00%

Table 4: FOB export volume by month and region for harvest year 2016/17 by month

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Standards

There are four internationally accepted Standards for TTO:

- International Standards Organisation (ISO) 4730
- Australian Standards (AS) 2783
- British Pharmacopeia (BP)
- European Pharmacopoeia (*Ph Eur*)

It is worth noting that there is no United States Pharmacopeia (USP) monograph for TTO although ATTIA hopes that this will change in the next few years once the BP/*Ph Eur* is revised.

The ISO and AS Standards are always identical because they are revised automatically in lock-step. The same happens with the *Ph Eur* and BP Standards although no one knows for sure if Brexit will impact on this arrangement. So in reality there are two sets of Standards that need to be monitored: ISO and *Ph Eur*.

The first known Standard (aka monograph) for TTO was issued by BP in 1949. The next meaningful issue was a new Australian Standard **AS K175** issued in 1967. This was revised in 1985 and renamed **AS 2782**. The first ISO Standard, **ISO 4730**, was issued in 1996 with a concurrent revision to AS 2782 in 1997 to mirror ISO 4730. The BP (and *Ph Eur*) Standard was revised to mirror the ISO 4730 - 1996 Standard in 1997 and neither BP nor *Ph Eur* has been revised since then – 22 years! The ISO/AS Standards were revised in 2004 and again in 2017; it is this Standard that ATTIA uses exclusively.

However, the *Ph Eur*/BP Standard is the most commonly used Standard for trading TTO globally; it is used in Australia by the Therapeutic Goods Administration (TGA) and by almost all trading houses in Europe and North America. *Ph Eur*/BP was last revised in 1996 and since 2012 the consistent, repeated efforts by ATTIA and some EU traders to have these Standards revised has failed. This changed only a few weeks ago when ATTIA finally persuaded the TGA, after repeated effort since 2014, to write to BP requesting an update. There are two likely drivers for this sudden about-face by the TGA and *Ph Eur*/BP:

1. The EU's REACH legislation enshrined the use the ISO 4730: 2017 Standard in preference to the *Ph Eur* Standard because it is more robust.
2. An Australian Federal Senator recently questioned the TGA in a Senate Estimates hearing about the use of a 22 year old Standard which allowed adulterated material to be sold as '100% pure TTO' in Australia with impunity and if they intended to switch to the ISO Standard as had happened in the EU. The TGA immediately wrote to the BP asking for a review.

ATTIA consistently uses the ISO/AS Standards and has done so since 1996; it will continue to be the preferred Standard for the foreseeable future because it is by far the more robust as can be seen in Table 5 on page 8.

It is immediately obvious that the BP/*Ph Eur* Standard is based on the 1996 version of ISO 4730, note that they failed to include 4 of the 15 compounds used in the ISO Standard. It is equally obvious that the *Ph Eur* Standard has not been revised since 1996 while the ISO Standard has undergone 2 major revisions.

The most important revision to the latest ISO 4730: 2017 Standard is the inclusion, for the first time, of the enantiomeric (chiral) ratio for terpinen-4-ol with a defined range. It is this inclusion more than the other minor though important changes to the ISO Standard that has been a game changer for adulteration in TTO.

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Chemical Components	BP and Ph Eur 1996		BP and Ph Eur 2018		ISO 4730: 1996		ISO 4730: 2004		ISO 4730: 2017	
	Min %	Max %	Min %	Max %	Min %	Max %	Min %	Max %	Min %	Max %
α-pinene	1.00	6.00	1.00	6.00	1.00	6 00	1 00	6.00	1.00	4.00
sabinene	-	3.50	-	3.50	traces	3 50	trace	3.50	traces	3.50
α-terpinene	5.00	13 00	5.00	13.00	5.00	13.00	5 00	13.00	6.00	12.00
limonene	0.50	4.00	0.50	4.00	0.50	4 00	0 50	1.50	0.50	1.50
p-cymene	0.50	12 00	0.50	12.00	0.50	12.00	0 50	8.00	0.50	8.00
1,8-cineole	-	15 00	-	15.00	-	15.00	trace	15.00	trace	10.00
γ-terpinene	10 00	28 00	10.00	28.00	10 00	28.00	10.00	28.00	14.00	28.00
terpinolene	1.50	5.00	1.50	5.00	1.50	5 00	1 50	5.00	1.50	5.00
terpinen-4-ol	30 00	-	30.00	-	30 00	-	30.00	48.00	35.00	48.00
α-terpineol	1.50	8.00	1.50	8.00	1.50	8 00	1 50	8.00	2.00	5.00
aromadendrene	-	7.00	-	7.00	traces	7 00	trace	3.00	0.20	3.00
ledene	N/A	N/A	N/A	N/A	N/A	N/A	trace	3.00	0.10	3.00
δ-cadinene	N/A	N/A	N/A	N/A	traces	8 00	trace	3.00	0.20	3.00
globulol	N/A	N/A	N/A	N/A	traces	3 00	trace	1.00	traces	1.00
viridiflorol	N/A	N/A	N/A	N/A	traces	1 50	trace	1.00	traces	1.00
Enantiomeric ratio for (+) terpinen-4-ol	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	67.00	71.00
Enantiomeric ratio for (-) terpinen-4-ol	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	29.00	33.00
Physical Parameters	**BP and Ph Eur 1996**		**BP and Ph Eur 1996**		**ISO 4730: 1996**		**ISO 4730: 2004**		**ISO 4730: 2017**	
Flash point (closed cup) - mean value	+59.00°	+59 00°	+59 00°	+59.00°	+59.00°	+59.00°	+59.00°	+59.00°	+59.00°	+59.00°
Apperrance	Clear, mobile liquid		Clear, mobile liquid		Clear, mobile liquid		Clear, mobile liquid		Clear, mobile liquid	
Colour	Colourless to pale yellow		Colourless to pale yellow		Colourless to pale yellow		Colourless to pale yellow		Colourless to pale yellow	
Odour	Characteristic		Characteristic		Characteristic		Characteristic		Characteristic	
Relative Density (20° C)	0 885	0.906	0.885	0.906	0.885	0.906	0.885	0.906	0.885	0.906
Refractive Index (20° C)	1.475	1.482	1.475	1.482	1.475	1.482	1.475	1.482	1.475	1.482
Optical Rotation	+5 00	+15.00	+5.00	+15.00	+5 00	+15.00	+5.00	+15.00	+7.00	+12 00
Misc. in ethanol (20° C)	less than 2 volumes		less than 2 volumes		less than 2 volumes		less than 2 volumes		less than 2 volumes	
N/A = Not Applicable	Changes from ISO 4730: 2004 to ISO 4730: 2017 highlighted in red									

Table 5: Revisions to BP/*Ph Eur* and ISO Standards

Enantiomeric (Chiral) ratios

Some molecules exist naturally in two forms: a left and right version - the same way your hands are mirror images of each other (image right). This is known as chirality and when the % of each form is measured using a Gas Chromatograph (GC) it can be expressed either as the enantiomeric (chiral) ratio or as a percentage. They are expressed this way because a chiral molecule is optically active: it bends plane polarised light either left (-) or right (+).

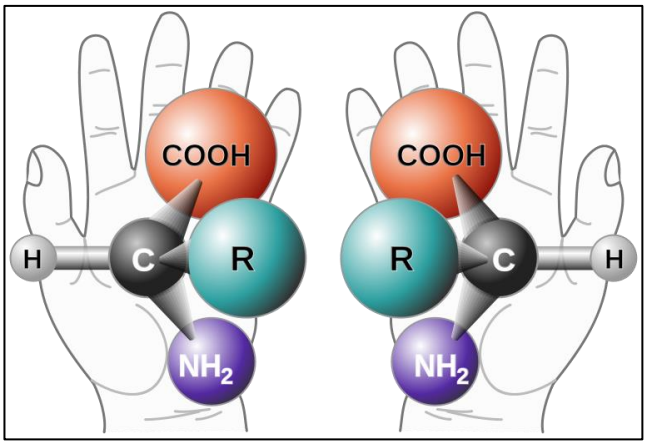

Figure 8: Left (-) and Right (+) Chirality

Note: Optical Rotation (OR) is another, cruder, way of measuring chirality because each enantiomer bends polarised light either to the left (-) or L or to the right (+) or R. The OR of TTO has been included in the various Standards since 1985 as +5° to +15° (see Table 5 above). This was tightened in ISO 4730: 2017 to +7 to +12 to make it harder to adulterate using limonene; this is explained later.

In 2010 ATTIA started accumulating both known origin 100% pure Australian TTO samples directly from COP accredited plantations and also commercially available samples from the EU, the US and Asia. These were analysed using the current ISO 4730 Standard.

ATTIA quickly realised that up to 70% of all TTO sold in the EU and 50% in the USA did not even closely resemble TTO; it was pure industrial waste with an alarming number of adulterants some of which are genuinely harmful as they have been classified as carcinogens or dermal irritants. A new approach was needed and it was decided that a new test must be devised aimed at making it at least A$10/kg more expensive to adulterate TTO and that this test must be included in a revision of all Standards. Another target was to achieve uniformity in all internationally accepted Standards for TTO to make life easier for all involved in the trade.

Experts from several Australian research institutes were consulted and a Leach et al paper from 1993 titled "Enantiomeric composition of the principal components of the oil of *Melaleuca alternifolia*" provided the inspiration needed to investigate the chirality of optically active compounds in TTO.

One well known example of a chiral molecule is Thalidomide which was given to pregnant women in the 1950's and was found to cause deformity in children born to mothers who took the drug. It was withdrawn during the early 1960s. This compound has two forms – it is a chiral molecule as you can see in Figure 9 so it has a **Right** or (+) form and a **Left** or (-) form.

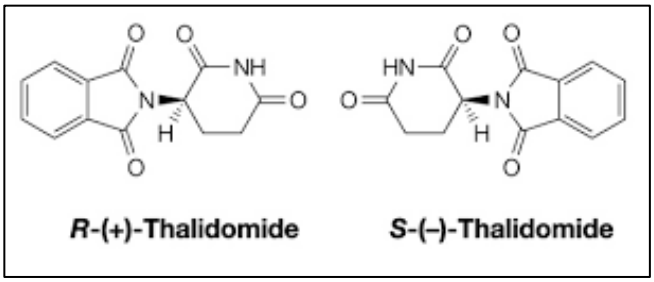

Figure 9: Chiral forms of Thalidomide

Unfortunately for the tens of thousands of parents and babies born after exposure to this drug it was discovered that the two different forms (also known as enantiomers or optical isomers) act slightly differently. The (**+**) form causes birth defects while the (**-**) form acts as originally intended as a sedative to combat morning sickness, a remarkable difference in activity *in vivo* for what could be considered the same molecule.

There are many other examples of chiral molecules in nature and another example is limonene which is most commonly found in citrus oils (orange, lemon, bergamot etc), the (+) enantiomer is highly optically active so a small addition to a batch significantly alters the optical rotation of the whole batch.

Tea Tree oil contains at least 4 chiral compounds:

- ✓ Terpinen-4-ol
- ✓ Limonene
- ✓ α-terpineol
- ✓ α-pinene

ATTIA collaborated with three Australian and one US based university to investigate the occurrence of three of these compounds in samples of TTO collected directly from the distilleries of producer members to avoid the chance of inadvertently collecting adulterated samples. They repeatedly and accurately measured the percentage of each of the chiral forms in more than 50 of these pure Australian Tea Tree oil samples collected over 5 years of production to see if this ratio would provide a unique, easy to measure and relatively fool-proof 'fingerprint' for Tea Tree oil steam distilled from *M. alternifolia*. Figure 10 on page 10 shows how measuring the chiral ratio of terpinen-4-ol (which at ~40% of the whole oil is the principle compound in TTO) can be used to quickly and relatively cheaply determine if a product is adulterated.

100% pure TTO is consistently 70% (-) or L and 30% (+) or R or put another way *M. alternifolia* is predominantly left handed when it synthesises terpinen-4-ol.

Fortuitously the principal source of adulterants for terpinen-4-ol, which are usually derived from rectifying Pine and Eucalyptus oils when TTO is blended, contains a predominance of the (+) or R enantiomer for terpinen-4-ol.

This means that when the percentage of each enantiomer is accurately measured in a sample the data can be shown graphically (Figure 10 on page 10) where it is immediately obvious from the bars which are adulterated and which are pure. The bars to the right on the graph shows only a few of the 50 samples of known provenance 100% pure TTO used, the samples on the right are identified by source country and as you can see the majority are adulterated, some very crudely. This allows this test to be consistently applied with a high degree of certainty.

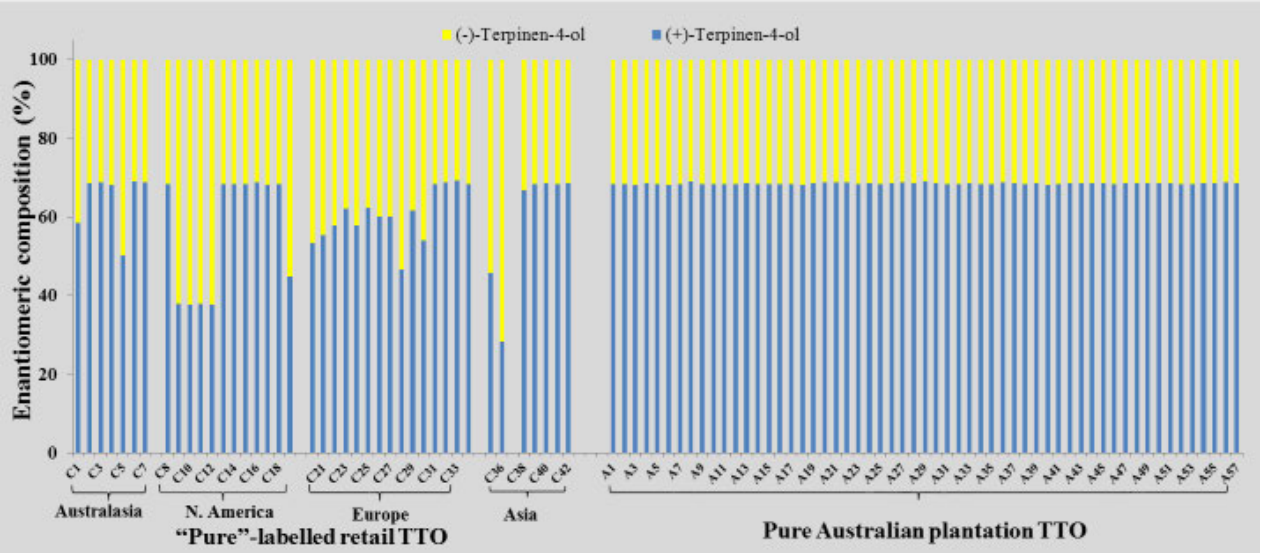

Figure 10: Chiral ratios for terpinen-4-ol

Data for limonene and α-terpineol are just as telling though these are not included here. Data for α-pinene is not included, it is harder to detect reliably.

The latest version of the ISO Standard was released in February 2017 and includes an advisory range for the enantiomeric abundances of terpinen-4-ol (Table 5 on page 8). This allows a discerning user to employ chirality successfully because a reliable and repeatable method has now been incorporated in the latest version of the ISO Standard.

Adulteration

When TTO is adulterated it can, as discussed previously, be 100% industrial waste or a blend of real TTO (often low quality and usually oxidised) and industrial waste. A clever chemist can relatively easily make up a brew that conforms to the BP or *Ph Eur* Standard and this is equally true for the ISO 4730: 2004 Standard.

Much of the source material for adulterating TTO is derived from Eucalyptus and Pine species. Crudely rectified terpinen-4-ol from these (any many other) sources is the most commonly added to oxidised or poor quality material which is usually derived from oil distilled from the incorrect (usually 1,8 cineole) chemotype to generate batches of adulterated material which is then sold as so-called pure TTO. As discussed earlier, Eucalyptus and Pine species synthesise predominantly the (+) or R enantiomer so any addition from these sources significantly alters the chiral ratio which can be measured. The optical rotation (OR) of the product is also altered so perpetrators often (though not always) add pure limonene from citrus to readjust the OR to the *Ph Eur*/BP Standard. Limonene sourced from citrus is also predominantly the (+) or R enantiomer and again the chiral ratio can be measured.

The most recent set of tests in early 2018 were a random set of 14 samples of TTO purchased from a major on-line platform because it was becoming increasingly clear that many retailers on this platform are selling material too cheaply to be credible; a prime flag that adulterated material is being passed off as "100% pure TTO" when it is not.

The anonymised data from these 14 are summarised in Table 6 on page 11 and when they are sorted (as shown in the lower section) by the (-) limonene from low to high an almost complete range of examples of current adulteration practices is revealed:

- The worst of these are samples 10, 4 and 14 which are appalling and very crude attempts to mimic TTO. They have almost no **(-)** limonene indicating that a large quantity of the limonene is sourced from citrus in an attempt to meet the OR specified in *Ph Eur*/BP. The chiral data is almost identical for all 3 indicating these adulterated products are all purchased from the same (Chinese) source. The terpinen-4-ol and α-terpineol data is also significantly skewed.

- The next 2 samples (13 & 7) are an example of 'intermediate' adulteration where Chinese material has been blended with TTO, possibly where the TTO is derived from the wrong (1.8 cineole) chemotype.
- Samples 3, 8 and 9 are 'better' attempts at adulteration; they have either only slightly diluted the original TTO or they have started using highly purified terpinen-4-ol. Of these the trace adulterant p-menth-1-ene, which is never present in 100% pure TTO, was detected in samples 3 and 8 while in sample 9 there is no way to be absolutely sure if the material is adulterated or an outlier and is indeed 100% pure; ATTIA has concluded that on balance of probabilities it is slightly adulterated.
- Samples 11, 5, 2, 1, 12 and 6 are 100% pure TTO.

ATTIA has written to each manufacturer advising them of the results. Of these two who were previously selling adulterated material have now committed to only selling COP accredited 100% pure Australian TTO and of these one has applied successfully to use the ATTIA logo on their products.

Data sorted bylaboratory reference, red denotes adulterated:												
Sample No	Origin	(-) limonene	(+) limonene	(+) terpinen-4-ol	(-) terpinen-4-ol	(-) α-terpineol	(+) α-terpineol	Lab	Date	Source	Type	Notes
ARL180666	Sample 1	39.55%	60.45%	68.50%	31.50%	24.31%	75.69%	SCU	07-Feb-18		Oil	Pure
ARL180667	Sample 2	39.39%	60.61%	68.28%	31.72%	24.47%	75.53%	SCU	07-Feb-18		Oil	Adulterated
ARL180668	Sample 3	33.76%	66.24%	59.62%	40.38%	45.51%	54.49%	SCU	07-Feb-18		Oil	Adulterated
ARL180669	Sample 4	4.69%	95.31%	45.66%	54.34%	15.27%	84.73%	SCU	07-Feb-18		Oil	Adulterated
ARL180670	Sample 5	39.30%	60.70%	68.51%	31.49%	24.78%	75.22%	SCU	07-Feb-18		Oil	Pure
ARL180671	Sample 6	40.67%	59.33%	68.45%	31.55%	27.70%	72.30%	SCU	07-Feb-18		Oil	Pure
ARL180672	Sample 7	22.30%	77.70%	64.67%	35.33%	19.40%	80.60%	SCU	07-Feb-18		Oil	Adulterated
ARL180673	Sample 8	37.51%	62.49%	65.44%	34.56%	21.35%	78.65%	SCU	07-Feb-18		Oil	Adulterated
ARL180674	Sample 9	36.68%	63.32%	68.28%	31.72%	24.62%	75.38%	SCU	07-Feb-18		Oil	Marginal
ARL180675	Sample 10	4.17%	95.83%	46.12%	53.88%	15.65%	84.35%	SCU	07-Feb-18		Oil	Adulterated
ARL180676	Sample 11	39.16%	60.84%	68.61%	31.39%	24.51%	75.49%	SCU	07-Feb-18		Oil	Pure
ARL180677	Sample 12	40.51%	59.49%	68.69%	31.31%	26.94%	73.06%	SCU	07-Feb-18		Oil	Pure
ARL180678	Sample 13	17.89%	82.11%	62.74%	37.26%	19.14%	80.86%	SCU	07-Feb-18		Oil	Adulterated
ARL180679	Sample 14	4.81%	95.19%	45.70%	54.30%	15.19%	84.81%	SCU	07-Feb-18		Oil	Adulterated
Data sorted by (-) linonene levels, red denotes adulterated:												
Sample No	Origin	(-) limonene	(+) limonene	(+) terpinen-4-ol	(-) terpinen-4-ol	(-) α-terpineol	(+) α-terpineol	Lab	Date	Source	Type	Notes
ARL180675	Sample 10	4.17%	95.83%	46.12%	53.88%	15.65%	84.35%	SCU	07-Feb-18		Oil	Adulterated
ARL180669	Sample 4	4.69%	95.31%	45.66%	54.34%	15.27%	84.73%	SCU	07-Feb-18		Oil	Adulterated
ARL180679	Sample 14	4.81%	95.19%	45.70%	54.30%	15.19%	84.81%	SCU	07-Feb-18		Oil	Adulterated
ARL180678	Sample 13	17.89%	82.11%	62.74%	37.26%	19.14%	80.86%	SCU	07-Feb-18		Oil	Adulterated
ARL180672	Sample 7	22.30%	77.70%	64.67%	35.33%	19.40%	80.60%	SCU	07-Feb-18		Oil	Adulterated
ARL180668	Sample 3	33.76%	66.24%	59.62%	40.38%	45.51%	54.49%	SCU	07-Feb-18		Oil	Adulterated
ARL180674	Sample 9	36.68%	63.32%	68.28%	31.72%	24.62%	75.38%	SCU	07-Feb-18		Oil	Marginal
ARL180673	Sample 8	37.51%	62.49%	65.44%	34.56%	21.35%	78.65%	SCU	07-Feb-18		Oil	Adulterated
ARL180676	Sample 11	39.16%	60.84%	68.61%	31.39%	24.51%	75.49%	SCU	07-Feb-18		Oil	Pure
ARL180670	Sample 5	39.30%	60.70%	68.51%	31.49%	24.78%	75.22%	SCU	07-Feb-18		Oil	Pure
ARL180667	Sample 2	39.39%	60.61%	68.28%	31.72%	24.47%	75.53%	SCU	07-Feb-18		Oil	Pure
ARL180666	Sample 1	39.55%	60.45%	68.50%	31.50%	24.31%	75.69%	SCU	07-Feb-18		Oil	Pure
ARL180677	Sample 12	40.51%	59.49%	68.69%	31.31%	26.94%	73.06%	SCU	07-Feb-18		Oil	Pure
ARL180671	Sample 6	40.67%	59.33%	68.45%	31.55%	27.70%	72.30%	SCU	07-Feb-18		Oil	Pure

Table 6: Chiral ratios from 14 samples sourced from an on-line platform

For reference purposes Table 7 below lists the chiral ratios for 160 known origin 100% pure TTO samples.

Summary data for chiral ratios from 160 authentic samples:							
		(-) limonene	(+) limonene	(+) terpinen-4-ol	(-) terpinen-4-ol	(-) α-terpineol	(+) α-terpineol
	Average (n=160)	39.45%	60.55%	68.96%	31.04%	25.42%	74.58%
	Median (n=160)	39.66%	60.34%	68.85%	31.15%	25.20%	74.80%
	Lowest	36.00%	58.24%	66.84%	29.36%	23.23%	68.86%
	Highest	41.76%	64.00%	70.64%	33.16%	31.14%	76.77%
	STD Deviation	1.16%	1.16%	0.80%	0.80%	1.16%	1.16%
	3x Std Dev	3.49%	3.49%	2.41%	2.41%	3.47%	3.47%
	Mean - 3x Std Dev (Lower Range)	35.96%	57.06%	66.55%	28.63%	21.95%	71.11%
	Mean + 3x Std Dev (Upper Range)	42.94%	64.04%	71.37%	33.45%	28.89%	78.05%
	3x SD Spread for ranges	6.98%	6.98%	4.83%	4.83%	6.95%	6.95%

Table 7: Summary of chiral data from 160 authentic TTO samples

Education

ATTIA started global campaign in 2013 to educate manufacturers about adulteration by buying retail products claiming to be 100% pure TTO steam distilled from *M. alternifolia* and testing the chiral ratios for terpinen-4-ol, limonene and α-terpineol. If any anomaly was noted the sample would also tested for conformance to the ISO 4730 Standard.

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Where the product was indeed 100% pure TTO a letter would be sent to inform the manufacturer and to thank them sincerely for selling pure TTO.

Where adulteration is identified ATTIA's first approach to the manufacturer assumes the company is unaware of the quality issue with their product. A non-accusatory letter is prepared in the first instance and sent, along with copies of the Certificates of Analysis, to the manufacturer informing them of the outcome of these tests and advising them of steps they should take to prevent this occurring again. One of four responses is typical:

1. The letter is completely ignored
2. The manufacturer expresses shock and, after contacting their supplier, dispute the findings with a range of excuses
3. The manufacturer accepts the findings and seeks alternative suppliers
4. The manufacturer arranges for a threatening letter to be sent by a law firm disputing ATTIA's findings and demands a retraction or includes a cease and desist notice.

The excuses given by major suppliers vary from fatuous and patently incorrect statements such as "our TTO is grown in another country and the chiral ratios differ" to outright lies and attempts to belittle ATTIA's work. Anyone sending a legal letter is automatically flagged as an active and deliberate participant in the procurement and sale of adulterated material masquerading as 100% pure TTO.

In the early days the most common response was to ignore ATTIA's letter but this is gradually changing as more and more people become aware of the widespread adulteration of many essential oils, not just TTO. The use of social media, a successful prosecution of an Australian manufacturer by the consumer watchdog the Australian Competition and Consumer Commission (ACCC) in 2016 and a pending Class Action in the USA has significantly raised awareness of the issue. Consumer interest in this is gaining considerable momentum which is in turn applying pressure on manufacturers to ensure their TTO (and other essential oils) are pure as the label claims and they are also demanding proof of this.

Most importantly, ATTIA has also concentrated on making contact with independent laboratories where TTO is certified to inform them of chiral testing and, in the same non-accusatory manner, advising them that they may be inadvertently certifying adulterated material as 100% pure. This has made a significant difference in some instances but there are still laboratories, one in Toronto, where chiral testing is still not being routinely done for TTO – they claim they are 'too busy'.

Where a manufacturer who has either ignored ATTIA or denied their product has been adulterated is caught out a second time ATTIA writes a repeat warning letter. If no action is taken by the manufacturer ATTIA then collaborates wherever possible with other groups (eg the Class Action in the USA) to try to force them to stop selling adulterated TTO. In some instances the sales platform utilised has also been informed.

Consumer Impact

The thalidomide case clearly shows that the chirality of any compound can have a significant impact and it is well known that D-limonene (the (+) or R enantiomer of limonene) can cause photosensitivity of the skin in some individuals. There is 1% limonene in 100% pure TTO and 60% or 0.6% of this is the (+) enantiomer which is not significant for most people. Studies into the incidence of skin sensitivity with TTO found zero incidence in formulations of up to 10% TTO. It is also reasonable to assume that the enantiomers of compounds present in TTO are not harmful as many of tons of TTO are used annually by consumers with no adverse reports.

We know that TTO can cause allergic reactions (dermatitis) in some people. Just like lactose, peanuts and gluten there are some people who are intolerant to TTO; the incidence is low – estimates range from 0.3 to 0.5% of the population but for the vast majority of the population TTO is well tolerated and has demonstrated effectiveness as an antibacterial, antifungal, antiviral and anti-inflammatory product.

Since 2012 ATTIA has identified a list of over 20 adulterants that are routinely found in varying concentrations in adulterated TTO. These compounds are never found in 100% pure TTO. They are

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contaminants that are in the industrial waste used to adulterate TTO and some are likely to be skin irritants or potential carcinogens.

1. Trans-pinocarveol
2. p-menth-1-ene
3. p-menth-3-ene
4. Methyl chavicol
5. γ-terpineol
6. camphene
7. δ-3-carene
8. terpinen-1-ol
9. borneol
10. myrcene
11. α-phellandrene
12. , cis-β-terpineol
13. iso-borneol
14. trans-β-borneol
15. caryophyllene
16. humulene
17. trans-α-bisabolene
18. linalool (caution: sometimes present in tiny traces in some strains of *M. alternifolia*)
19. ϒ-terpineol
20. α-copaene
21. α-terpinyl acetate

If industrial waste includes some or all of these compounds then there are also likely to be traces of other contaminants (pesticides, parabens, phthalates etc) which can also be harmful to a consumer.

Some TTO brands that use 'certified organic' TTO either in the whole oil or in cosmetic formulations have also been found to contain adulterated material bearing the logo of either "Soil Association Organic" or "Australian Certified Organic".

In recent years the incidence of TTO being named as the cause of dermal irritation or contact dermatitis has risen steeply, particularly in Europe where the largest percentage of adulterated material masquerading as TTO occurs. Is this a coincidence or as a direct result of adulterants? No one knows but it a fact that reported incidences of dermatitis caused by TTO are almost non-existent in Australia where nearly all TTO sold is 100% pure. TTO has gained an unfair reputation as a skin irritant and ATTIA hypothesises that this is largely being caused by the sale of adulterated material.

ATTIA's aim is to promote the use of COP accredited 100% pure Australian TTO to minimise the risk to consumers from a plethora of unknown contaminants that are routinely found in adulterated material which is sold as "100% pure TTO steam distilled from *M. alternifolia*" when it patently is not.

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From: Tony Larkman
To: Bart Heldreth
Cc: Monice Fiume
Subject: RE: Cosmetic ingredient review of tea tree oil
Date: Monday, June 15, 2020 8:08:57 PM

Attachments: JCEM Submission ver 3 (jc.2020-00290) clean 9 Feb 2020.pdf
CTM(2020) 49 102888.pdf

Thanks Bart,
Another topic that may be of interest to you (and you may already have these details) is the issue of endocrine disruptors and essential oils.
ATTIA part sponsored Dr Jesse Hawkins of the **Franklin School of Integrative Health Science** for a study (https://fsihs.org/fiw-researching-claims-regarding-essential-oils-as-endocrine-disruptors/) of this phenomenon.
Dr Hawkins first publication in Complementary Therapies in Medicine (copy attached) exonerates TTO and largely exonerates LO; the final results which are being delayed by COVID-19 will be interesting.
I have written a letter of rebuttal to the paper published by Ramsay et al (https://pubmed.ncbi.nlm.nih.gov/31393563/) which was accepted in Feb 2020 but not yet published – copy attached.

I will pass the dermal study over to you as soon as I have received and assessed it, it is intended as the final study before ATTIA prepares and submits a new dossier to the EU's SCCS requesting a review of their 2008 Opinion on TTO after we have addressed all of their concerns in the Opinion.
Regards,
Tony Larkman
CEO - ATTIA Ltd
[redacted]

Letter to the Editor: Lavender products associated with premature thelarche and prepubertal gynecomastia: Case reports and EDC activities

Tony Larkman, BSc

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Author Disclosure

T. Larkman is CEO of the Australian Tea Tree Industry Association (ATTIA Ltd), an Australian based not-for-profit organisation formed in 1986 as the peak body to promote and represent the interests of the Australian tea tree industry.

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No funding was provided for the preparation/submission of this article.

I am concerned by the conclusions in this paper by Ramsay et al (1) and in particular the statement "*…that physicians are aware that LO and TTO possess EDC activities that should be considered in the evaluation of premature breast development in girls and gynecomastia in boys and adult men*" when there remains reasonable doubt that it is indeed the essential oils that are causing the EDC activity observed. An alternative hypothesis postulated by Carson et al (2) was cited and discussed however the experimental work described to address this may be insufficient to dismiss the hypothesis.

The authors investigated "*…whether the properties of an oil in general could dissolve BPA analogues from the plastic assay plates…*" In their comparative analysis of essential oils versus fatty oils (corn and soy) they did not appear to appreciate these oils' compositional differences. The possibility that compounds in fatty plant oils have different chemical properties to those found in EO's needs to be considered.

We know that both TTO and LO extract styrene trimers from polystyrene labware at concentrations used in this study and that plastic leachates can affect estrogenic responses of MCF-7 cells Ishikawa *et al* (3). This leaching effect is observably higher in the presence of DMEM which was used in this study as well as the referenced Henley *et al* study (4).

The authors implicated two commercial 'Aguas' (Crusellas and Mi Tesoro) as having "*…the presence of linalool and linalyl acetate as well as unidentified substances.*" Samples of both products as well as three other Aguas based on rankings in popular on-line platforms (Augustin Reyes, PMB Agua de Violetas and AFFA Violetas Francescas) were obtained and analysed by an independent accredited laboratory.

No linalyl acetate was reliably detected in any of these products while linalool was only detected (2.28%) in the Augustin Reyes product, indicating that LO is not present or in such minute

quantities to be virtually undetectable. An alternative hypothesis is that a synthetic analogue of LO was used.

The fact that there are, in some instances, significant quantities of compounds with known ED properties (5) (3.61% diethyl phthalate in the Mi Tesoro product) was not taken into account and the authors appear not to have considered the possibility that this substance, which was recorded as '…*unidentified substances*' may have been a causative factor. Another suspected (6) ED compound (3-(4-(tert-Butyl) phenyl)-2-methylpropanal [Lilial]) was detected in one product (Crusellas).

A baby shampoo was also implicated. Again, linalyl acetate was not reliably detected indicating that LO is either not present or if present in such minute quantities as to be virtually undetectable.

We hypothesize synthetic LO analogues were used in all products studied, but the authors did not consider or test for this possibility.

Finally a new systematic review of the literature (7) was recently published investigating the relationship between TTO, LO and paediatric endocrine disorders. This paper exonerates TTO and largely exonerates LO as well concluding "*This systematic review finds that tea tree essential oil is not related to documented cases of endocrine disruption in children, and that there is little to no evidence to substantiate the proposed link between lavender essential oil and endocrine disruption in children.*"

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From: Tony Larkman
To: Bart Heldreth; Monice Fiume
Subject: Cosmetic ingredient review of tea tree oil
Date: Sunday, June 14, 2020 11:36:41 PM
Attachments: Wilkinson TTO Patch Testing Jan 2018.pdf
Occupational Dermatology Research and Education Centre 6 Mar 2020.pdf
RE Tea tree oil as an included product in the Cutaneous Allergy Facial Series.msg

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Dear Drs Heldreth and Fiume,

During the past 6 – 12 months I have maintained irregular contact with Dr Carol Eisenmann in relation to a proposed CIR for tea tree oil and I have, where possible, provided her with some background material to help with the review.
She has suggested I contact you both directly in relation to some additional material that has caught my attention in the past few months:

This issue that may be of interest to the CIR is in relation to patch testing of TTO: all patch testing is done using **oxidised** TTO rather than fresh TTO which I believe to be a **strong and unjustified bias against TTO** resulting in misreporting of both sensitisation and cutaneous reaction to TTO. This is especially pertinent in relation to **quantitative risk assessment for sensitization developed by the fragrance industry**.

The best way I can communicate this is to refer you to the attached email string to the corresponding author (Dr Sophie Rolls) of a recent article in BJD titled "*What's in? What's out? Updating the British Society for Cutaneous Allergy Facial Series*" available at https://onlinelibrary.wiley.com/doi/abs/10.1111/bjd.19127. I have attached the two letters I referred to in my initiating email to Dr Rolls for completeness; these are in relation to other recent publications in the same arena.

Dr Rolls response to my question was interesting: She acknowledged that TTO testing is conducted using oxidised material which she tried to justify. This led me to ask, amongst others, this question:

> "*Based on your response '…**we see patients who do not always follow advice labels with respect to correct storage of their products and who often ignore sell-by-dates. Patients may therefore be exposed to oxidised fragrance chemicals and develop allergy. As our aim is to identify the underlying cause of a patient's dermatitis it is the oxidised TTO which is tested.**' would it not be reasonable to have all of these products oxidised prior to patch testing as there is little doubt in my mind that patients who fail to follow advice labels for TTO-containing products are just as likely to do the same for Peppermint, Lavender, Jasmine or Ylang-Ylang containing products to name just a few?*"

Dr Rolls is now on maternity leave until mid-2021 and I have not received a response to my follow-up questions either from her or the other authors who I subsequently emailed the identical questions and comments to.

On another matter I have still not received the dermal penetration data for TTO I promised Dr Eisenmann by the end of June 2020, there have been issues with some of the skin samples (freezer failure – yuck!) as well as inadequate baseline data - they inadvertently used old standard samples

for some of the terpene constituents and have had to repeat some of the baseline analysis work. I have now been promised the report by the end of July 2020 and am not holding my breath – COVID-19 is not helping.
Regards,

Tony Larkman
CEO - ATTIA Ltd
☎: 02 4017 1336
: 0434 263 664

Email: tlarkman@attia.org.au
Web: www.attia.org.au

5 January 2018

Dr Mark Wilkinson,
Department of Dermatology,
Leeds Teaching Hospitals NHS Trust,
Chapel Allerton Hospital,
Harehills Lane,
Leeds LS7 4SA,
UK

By email: mark.wilkinson15@nhs.net

Dear Dr Wilkinson,

I am writing on behalf of the Australian Tea Tree Industry Association (ATTIA Ltd) in relation to your recent paper published in Contact Dermatitis titled '*A proposal to create an extension to the European baseline series*' available at http://onlinelibrary.wiley.com/doi/10.1111/cod.12918/abstract.

In this paper you have proposed (Table 3) a change to the baseline series and have tea tree oil (TTO) derived from *Melaleuca alternifolia* included at 5% and I note that since TTO is in bold in the table this means that in your opinion there is 'a prevalence that is insufficient to warrant further inclusion'.

There are two points I would like to raise in relation to this paper:

1) While ATTIA has no objection *per se* to the inclusion of TTO at up to 5% pet in an extended series **we object in the strongest possible terms to the discriminatory approach to TTO where it is the only essential oil that is patch tested with deliberately oxidised TTO**.

A recent paper titled '*Positive Patch-Test Reactions to Essential Oils in Consecutive Patients from North America and Central Europe*' clearly demonstrates this bias and there are many other examples of this. Warshaw et al 2017 (https://www.ncbi.nlm.nih.gov/pubmed/28614106 states (bold is my emphasis) :

> "This study used a retrospective analysis of patch-test results and relevant demographic/clinical data collected electronically by the networks, obtained with *Santalum album* 10% petrolatum (pet) (IVDK only); *Cananga odorata* 2% (NACDG) and 10% (IVDK) pet; *Jasminum species* 2% (NACDG) and 5% (IVDK) pet; *Mentha piperita* 2% pet; ***Melaleuca alternifolia*, oxidized (tea tree oil), 5% pet**; and *Lavandula angustifolia* 2% pet (latter 3 NACDG only)."

Another indicator of the bias towards TTO is shown in a paper titled '*Allergic contact dermatitis to plant extracts in patients with cosmetic dermatitis*' by Thomson et al (2000) available from https://www.ncbi.nlm.nih.gov/pubmed/10651699 where the authors describe patch test reactions to TTO without declaring anywhere if the material used was oxidised or not. It is reasonable to assume that the patches used were, as is usual, from Chemotechnique Diagnostics, Vellinge, Sweden who only supply oxidised TTO in their patch series (personal communication Bo Niklasson, CEO & President Chemotechnique Diagnostics).

More recently a paper published in 2013 by Christoffers et al titled '*Co-sensitization to ascaridole and tea tree oil*' (http://onlinelibrary.wiley.com/doi/10.1111/cod.12086/abstract) clearly implicates TTO as a sensitiser and once again the material used was *"…oxidized tea tree oil 5% (Chemotechnique Diagnostics, Vellinge, Sweden)*" yet this is not stated in the title. This again demonstrates the bias researchers have towards the use of oxidised material instead of material as supplied to the world by the TTO producers of Australia which is produced to our stringent QA protocols (ATTIA's Code of Practice) to ensure it is well stored at all times.

T**here are many other examples of this bias in the literature**.

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ATTIA Ltd is extremely disturbed that TTO is being discriminated against in this manner. No other essential oil is tested in its oxidised state and we are unable to reconcile why this discrimination continues to be perpetuated by researchers globally.

There is clear and incontrovertible evidence of the stability of TTO when correctly stored available from this link: https://agrifutures.infoservices.com.au/items/06-026 in a paper titled '*Quality assurance for tea tree oil safety investigative samples*' by Southwell et al (2006) and another paper available from this link: https://agrifutures.infoservices.com.au/items/06-112 titled '*p-Cymene and organic peroxides, indicators of oxidation in tea tree oil'* by Southwell (2006). These papers demonstrate that if TTO is manufactured, stored and transported using stringent quality assurance controls oxidative degradation is minimised or even eliminated for lengthy periods.

These papers have been summarised by ATTIA in a paper titled "*Stability of pure Australian Tea Tree*" published in 2012 and available from this link: http://www.attia.org.au/mce_doc.php?id=18.

Further the SCCS published an opinion on TTO in 2008 (SCCP/1155/08) endorsing ATTIA's Code of Practice (http://www.teatree.org.au/teatree_about_quality.php) in which they state:

> *'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.'*

And

> *'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.'*

A search of the literature on patch testing with TTO has shown that where the source is declared it is almost universally from Chemotechnique Diagnostics, Vellinge, Sweden which means that the material used has been deliberately oxidised prior to use yet the published papers almost universally state that the patch testing is conducted with 'TTO' and not 'oxidised TTO'. In most, if not all, instances the fact that the material is oxidised is buried in the body of the paper. Is it necessary to obscure the fact that only TTO is tested using oxidised material?

This brings me to my second point: **Adulteration in TTO**.

Adulteration of TTO in the past decade has been clearly demonstrated to be widespread and prevalent in the European Union (~70%) and the USA (~50%) using a novel testing regime that was developed by ATTIA Ltd a few years ago where the enantiomeric abundances of stereoisomers of some compounds in TTO can be used to quickly and relatively cheaply determine if the material being used is indeed 100% pure TTO steam distilled from *Melaleuca alternifolia*. There have been three papers published on this work which can be accessed from the links below:

1. **Enantiomeric distribution of selected terpenes for authenticity assessment of Australian *Melaleuca alternifolia* oil**: http://www.sciencedirect.com/science/article/pii/S0926669015000680
2. **Evaluation of fast enantioselective multidimensional gas chromatography methods for monoterpenic compounds: Authenticity control of Australian tea tree oil:** http://www.ncbi.nlm.nih.gov/pubmed/26138602

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3. **Quality Evaluation of Terpinen-4-ol Type Australian Tea Tree Oils and Commercial Products: An Integrated Approach Using Conventional and Chiral GC/MS Combined with Chemometrics:** http://pubs.acs.org/doi/abs/10.1021/acs.jafc.5b00147

This method was, in February 2017, adopted by the International Standards Organisation in their Standard ISO 4730: 2017 (https://www.iso.org/standard/69082.html) where in Annex C (informative) the Standard states:

> *'Some essential oil components can exist in two enantiomeric forms designated as (R) or (S), D or L or (+) or (-) isomers. Many enantiomers have distinctly different properties and hence their presence in the right form is critical. Also, pure natural essential oils contain enantiomers in characteristic ratios. This ratio is upset by the addition of adulterants including synthetic major components of different enantiomeric ratios.*
>
> *Consequently, the measurement of enantiomeric excess or enantiomeric ratio as per ISO 22972 in an informative annex of appropriate isolates in International Standards provides an extra measure of essential oil authenticity.*
>
> *The enantiomeric distribution for terpinen-4-ol is (+) 67 % - 71 % and (-) 29 % - 33 %.'*

ATTIA's main concerns in this area are:

1) Manufacturers such as Chemotechnique may inadvertently be using adulterated material instead of 100% pure TTO to manufacture the oxidised patches that are universally used in laboratories around the world. In November 2014 ATTIA Ltd wrote to Chemotechnique describing to them this new testing regime and eventually, after much prompting, received a brief note from Bo Niklasson the CEO & President of Chemotechnique stating: "*I am so sorry that we have not yet responded to you. The method sounds interesting and I would like to know more to be able to see if this fits our needs. Please send more info and your proposal for cooperation. With kind regards Bo*". ATTIA responded to Bo and have not heard back from him since so it is impossible to confirm that Chemotechnique are in fact using 100% pure TTO for the preparation of their oxidised TTO patches.
2) Adulteration of TTO by unscrupulous manufacturers is generally done for two purposes: 1) to increase profit and 2) to mask out-of-specification material that is often highly oxidised which increases the risk of adverse reaction if used by a consumer. In addition to this risk the adulterants used appear to be industrial waste derived from 'normalising' other essential oils, predominantly (though not exclusively) Pine, Eucalyptus and White Camphor oils. ATTIA has detected an alarming number of adulterants in this material, some of which are known sensitisers.

ATTIA would be grateful if you would consider amending any extension to the European baseline series to ensure that only 100% pure TTO is used and that the material is not oxidised prior to use as we are confident that if these suggestions are followed the incidence of adverse reactions will be far lower than has been reported in the literature to date driven by the bias towards the use of oxidised material.

Yours Sincerely,

Tony Larkman

CEO – ATTIA ltd

PO Box 903, Casino NSW 2470 Tel: 02 4017 1336 Fax: 07 5604 1629 Email: tlarkman@attia.org.au

Occupational Dermatology Research and Education Centre
Attn: Hiromi Mizutani and Rosemary L. Nixon
Skin Health Institute
80 Drummond St,
Carlton VIC 3053

6 March 2020

By email to: hiromim@koto.kpu-m.ac.jp and rnixon@occderm.asn.au

Dear Drs Mizutani and Nixon,

I am writing on behalf of the Australian Tea Tree Industry Association (ATTIA Ltd) in relation to your recently published chapter titled "The Australian Baseline Series" in Contact Dermatitis available at https://link.springer.com/referenceworkentry/10.1007%2F978-3-319-72451-5_68-1.

In **section 2.1.2 Limonene and Linalool** you state (**bold** highlight is mine):

> *Recently, the inclusion of hydroperoxides of limonene and hydroperoxides of linalool has been suggested because of the high contact allergy rate of these allergens. In an international multicentre study, limonene hydroperoxide 0.33% pet. and linalool hydroperoxide 1% pet. registered contact allergy rates of 5.2% and 6.9%, respectively, in patients attending patch testing clinics [15]. In a study from the United Kingdom, 5.0% had a positive patch test to hydroperoxides of limonene 0.3% pet. and 5.9% to hydroperoxides of linalool 1.0% pet [16].* ***In both studies, testing with non-oxidized terpenes appeared to be less useful****.*

The usefulness of including oxidised tea tree oil (TTO) in this (or any other) Baseline Series is questionable. Testing with oxidised terpenes may be useful if searching for a positive control-type substance, however it serves little function when testing for reaction to non-oxidised TTO & well formulated products containing TTO.

The use of oxidised TTO has the potential to mislead the reader with regard to the potential allergic nature of TTO - perfectly illustrated in section **2.5 Tea Tree Oil** which states:

> *Tea tree oil (TTO) oxidized 5% pet. was recognized as a contact allergen when first reported from Australia in 1991 [44]. Since then, a number of cases have been reported, and TTO is now thought to cause the most allergic reactions of all essential oils [45]. It is obtained from the Australian native tea tree Melaleuca alternifolia.*
>
> *It has been used widely as "natural" remedy for acne, wounds, and eczema, especially in Australia [46]. The rates of positive reactions to TTO vary from 0.1% to 3.5%. In the NACDG studies from the United States and Canada, rates of reactions ranged from 0.9% to 1.4% [26, 47–51]. The highest rates were observed in Australian studies: 1.8% [46], 2.5% [4, 52], and 3.5% [4]. TTO 5% has been included in NACDG since 2003. The sensitizers in TTO appear to be ascaridole, terpinolene, α-terpinene, 1,2,4-trihydroxymenthane, α-phellandrene, and limonene [45].*

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Your first sentence correctly states "*Tea tree oil (TTO) oxidized 5% pet. was recognized…*" and I have no objection whatsoever to this statement as it is a verified fact that oxidised TTO is indeed a skin sensitiser.

In your final sentence you have identified some of the more common components of TTO which are known sensitisers. I believe you should also consider including ρ-cymene and any one of a number of organic peroxides that are oxidative derivatives of TTO constituents.

However, throughout section 2.5, with the single exception of the initial mention, you refer to the material being tested as "TTO" and not "**oxidised TTO**", a far more accurate description.

The use of "TTO" instead of "oxidised TTO" is misleading and may unfairly harm the reputation of 100% pure Australian TTO. Producers of 100% pure Australian TTO are audited and certified to an industry Code of Practice that ensures oil is grown, harvested and produced in conditions that deliver unoxidised oil to the end-user.

The simple omission of "oxidised" may automatically, and understandably, lead a reader to assume that TTO is a nasty substance and skin contact should be avoided. This is certainly not the case from both personal experience and from available literature.

There is little doubt in my mind that proactive steps must be taken to help researchers and medical practitioners understand the 'oxidised vs fresh' misconception for TTO in the Australian Baseline (and other) Series.

This is not the first time I have raised this; I have attached a copy of a letter sent in January 2018 to Dr Mark Wilkinson, Department of Dermatology at Leeds Teaching Hospitals NHS Trust on the same topic.

Unfortunately, based on your recent chapter, it is now a published statement that "*TTO is now thought to cause the most allergic reactions of all essential oils [45]*". I accept that you have quoted directly from de Groot et al (2016) [reference 45] however these authors somewhat moderated their statement by also stating "*Fresh TTO is a weak to moderate sensitizer, but oxidation increases its allergenic potency…*" and more importantly "*Patch testing may be performed with 5% oxidized TTO.*"

While researching this topic further I also noticed that a paper by Rutherford et al (2007) is titled '*Allergy to tea tree oil: retrospective review of 41 cases with positive patch tests over 4.5 years*' (https://www.ncbi.nlm.nih.gov/pubmed/17535193) which states:

> "*Tea tree oil 10% is included in our extended European standard series and the 5% commercial allergen, provided by Chemotechnique Diagnostics® (Malmö, Sweden), was also used for patch testing in some patients. The 10% patch test was prepared by a pharmacist by diluting neat tea tree oil (purchased from a pharmacy) that had first been allowed to oxidize by standing the open bottle on a window ledge for several days, with white soft paraffin.*"

Deliberately oxidised TTO is deployed by Rutherford et al at both 5% and 10% while references to the patch testing results, including in this instance in the title, refer only to "TTO" and not "oxidised TTO". The opening sentence in the **Results** section states "*In total we identified 42 positive reactions to tea tree oil…*" Again, only oxidised TTO was used, perpetuating this false rate of positivity for properly stored and handled TTO.

However de Groot et al (2016) concludes that TTO is a '*weak to moderate sensitiser*'. Rutherford et al (2007) conclude in their abstract "*Given tea tree oil from freshly opened tea tree oil products*

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elicits no or weak reactions, oxidized tea tree oil should be used for patch testing." This supports the position that fresh TTO is not a skin sensitiser.

Would you consider conducting a trial where both oxidised and fresh TTO was used on the same cohort and under the same conditions? Publication of these data would go some way towards helping both researchers and medical practitioners understand the 'oxidised vs fresh' issue.

Should you continue to include oxidised TTO in a Baseline Series (though I would strongly prefer that it is not) please forego the use of 'TTO' and instead consistently use 'oxidised TTO' to avoid misleading readers and practitioners alike.

Yours Sincerely,

Tony Larkman
CEO – ATTIA Ltd

PO Box 903, Casino NSW 2470 Tel: 02 4017 1336 Email: ceo@attia.org.au

From: Tony Larkman
To: "ROLLS, Sophie (BARTS HEALTH NHS TRUST)"
Subject: RE: Tea tree oil as an included product in the Cutaneous Allergy Facial Series
Attachments: Chemotechnique 2020-001 - Patch Test Order Form - By Series.pdf

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Hi Sophie,
Thanks for responding and I am delighted that there will be some clarity in any future papers on the status of the TTO being used.
If you don't mind I would like to have a couple of questions answered:

1. Would it be possible to design and run a study where oxidised and fresh TTO was used in patch tests in a statistically significant cohort of volunteers to compare and publish the results and if this is possible can you please advise how much this might cost? I have access to limited R&D funding through an Australian Federal Government scheme that could be used for this.
2. Before I wrote to you I had a look at the BSCA website and tried to access the PILS for TTO (https://cutaneousallergy.org/resources/patient-information-leaflets-pils/) as well as the Recommended Series (specifically for TTO of course) https://cutaneousallergy.org/resources/recommended-series/ but as I am not a member I was unable to access these documents. I would really like to see how the BSCA describes TTO in the Recommended Series as well as in the PILS. Would it be possible to share these documents with me?
3. Finally I had a look through a recent (2020) **Chemotechnique Patch Test Products Order Form - By Series** from Crawford Healthcare Ltd (copy attached) and note that throughout the entire series available there are **only two products that are offered as oxidised** (*Tea tree oil oxidized 5% pet* and *Turpentine oil oxidised 0.4% pet*). On closer inspection there are a number of products of natural plant origin (see list below for a few taken at random) and I wonder why only TTO and Turpentine oil have been singled out in this manner?
   Based on your response '*…we see patients who do not always follow advice labels with respect to correct storage of their products and who often ignore sell-by-dates. Patients may therefore be exposed to oxidised fragrance chemicals and develop allergy. As our aim is to identify the underlying cause of a patient's dermatitis it is the oxidised TTO which is tested.*' would it not be reasonable to have all of these products oxidised prior to patch testing as there is little doubt in my mind that patients who fail to follow advice labels for TTO-containing products are just as likely to do the same for Peppermint, Lavender, Jasmine or Ylang-Ylang containing products to name just a few?

Cosmetic Series
Oakmoss absolute 2% pet
Narcissus poeticus (Narcissus absolute) 2% pet
Jasmine synthetic 2% pet
Lavender absolute (Lavandula angustifolia oil) 2% pet
Cananga oil 2% pet
Rose absolute (Rosa damascena extract, Rose oil) 2% pet
Ylang-Ylang oil (Cananga odorata oil) 2% pet
Geranium oil (Geranium oil Bourbon) 2% pet
Jasmine absolute 2% pet
Sandalwood oil (Santalum album oil) 2% pet
Treemoss absolute (Evernia furfuracea) 1% pet
Tea tree oil oxidized 5% pet
Peppermint Oil (Mentha piperita oil) 2% pet
Turpentine oil oxidised 0.4% pet

International Comprehensive Baseline Series
Peru balsam (Myroxylon pereirae resin) 25%
Tea tree oil oxidized 5% pet
Ylang-Ylang oil (Cananga odorata oil) 2% pet

International Standard Series
Peru balsam (Myroxylon pereirae resin) 25% pet

Plant Series
Anthemis Nobilis extract (Chamomilla Romana) 1% pet
Arnica Montana extract 0.5% pet
Taraxacum officinale extract (Dandelion) 2.5% pet
Achillea Millefolium extract (Yarrow) 1% pet
Chrysanthemum Cinerariaefolium extract (Pyrethrum) 1% pet
Tanacetum vulgare extract (Tansy) 1% pet
Chamomilla Recutita extract (German Chamomile) 1% pet

Thanks & regards,
Tony Larkman
CEO - ATTIA Ltd
[redacted]

From: ROLLS, Sophie (BARTS HEALTH NHS TRUST) [mailto:sophie.rolls@nhs.net]
Sent: Thursday, 30 April 2020 2:09 AM

To: tlarkman@attia.org.au
Subject: Re: Tea tree oil as an included product in the Cutaneous Allergy Facial Series

Dear Mr Larkman,

Thank you for your interest in our paper. We can confirm that it is oxidised tea tree that we are patch testing with as has been recommended on the British Society for Cutaneous Allergy facial series.

We agree that there seem to be many fewer problems of allergy to non-oxidised chemicals such as limonene, linalool and tea tree, compared to oxidised samples.

In everyday practice we see patients who do not always follow advice labels with respect to correct storage of their products and who often ignore sell-by-dates. Patients may therefore be exposed to oxidised fragrance chemicals and develop allergy. As our aim is to identify the underlying cause of a patient's dermatitis it is the oxidised TTO which is tested.

We will ensure in future if we write further papers that it is made clear that it is oxidised TTO which is being tested.

Yours

Sophie

Dr Sophie Rolls
Dermatology, ST4
University Hospital Wales, Cardiff

From: Tony Larkman <tlarkman@attia.org.au>
Sent: 18 April 2020 04:58
To: ROLLS, Sophie (BARTS HEALTH NHS TRUST)
Subject: Tea tree oil as an included product in the Cutaneous Allergy Facial Series

Dear Dr Rolls,
I read your recent article in BJD titled "*What's in? What's out? Updating the British Society for Cutaneous Allergy Facial Series*" available at https://onlinelibrary.wiley.com/doi/abs/10.1111/bjd.19127 with interest because I am fascinated by the apparent bias in reporting the role of tea tree oil as a dermal irritant by researchers. I note that you have mentioned "*Melaleuca alternifolia* (tea tree) 5.0% pet." as one of the 14 of 26 allergens in the current BSCA facial series with a positive patch test rate of greater than 0.3% as well as listing tea tree oil (TTO) in Table 1 as *Melaleuca alternifolia* (tea tree) 5.00% pet. With 4224 patients tested and a positive patch test rate of 0.45% (CI 0.29-0.70)

I have no reason to dispute these data at this time but I am interested in knowing more about the mode of inclusion of TTO in this (and other) Baseline Series.
Can you please advise if the 5% Pet. TTO patches cited are indeed using 100% pure TTO or if the patches use **oxidised TTO**?
It is a simple fact that many, if not all, of these "TTO patches" are sourced from Chemotechnique Diagnostics, Vellinge, Sweden who only supply oxidised TTO in their patch series (personal communication Bo Niklasson, CEO & President Chemotechnique Diagnostics).

If your article proposes including TTO in petrolatum would you and your colleagues please consider specifying that the TTO is unoxidised?
If this is not possible for any reason (and I really cannot see why this would be the case) then I think it is vitally important to ensure that all reporting specifies that oxidised TTO is being used instead of fresh material.
ATTIA members go to great lengths to ensure their TTO is fresh and unoxidised and this is reinforced through use by dates on packaging.

The risk of bias in the way patch test responses are reported is potentially highly significant with many published papers referring to allergic reaction to "TTO" when in fact the testing is done using 'oxidised TTO' – a completely different product and not something I would recommend anyone use on their skin. Your article does not clearly state if the material proposed in Table 1 is oxidised or not – a potentially serious omission.

To provide you with more information on this topic I have attached two letters, sent in Jan 2018 and more recently in March 2020, that attempt to address the concerns of the Australian TTO industry in relation to patch testing (no response received for either).
I particularly want to draw your attention to two statements:

1. By Mizutani et al in https://link.springer.com/referenceworkentry/10.1007%2F978-3-319-72451-5_68-1 where they state in part "***In both studies, testing with non-oxidized terpenes appeared to be less useful***."
2. Rutherford et al in https://www.ncbi.nlm.nih.gov/pubmed/17535193 which states "***Tea tree oil 10% is included in our extended European standard series and the 5% commercial allergen, provided by Chemotechnique Diagnostics® (Malmö, Sweden), was also used for patch testing in some patients. The 10% patch test was prepared by a pharmacist by diluting neat tea tree oil (purchased from a pharmacy) that had first been allowed to oxidize by standing the open bottle on a window ledge for several days, with white soft paraffin***."
   I then wrote "Deliberately oxidised TTO is deployed by Rutherford et al at both 5% and 10% while references to the patch testing results, including in this instance in the title, refer only to "TTO" and not "oxidised TTO". The opening sentence in the **Results** section states "*In total we identified 42 positive reactions to tea tree oil…*" Again, only oxidised TTO was used, perpetuating this false rate of positivity for properly stored and handled TTO."

Yours sincerely,
Tony Larkman
CEO - ATTIA Ltd
☎: 02 4017 1336

: 0434 263 664

Email: tlarkman@attia.org.au
Web: www.attia.org.au

[redacted]

****

[redacted]

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review (CIR)

FROM: Alexandra Kowcz, MS, MBA
Industry Liaison to the CIR Expert Panel

DATE: September 18, 2020

SUBJECT: Scientific Literature Review: Safety Assessment of *Melaleuca alternifolia*-Derived Ingredients as Used in Cosmetics (release date: August 4, 2020)

The Personal Care Products Council respectfully submits the following comments on the scientific literature review, Safety Assessment of *Melaleuca alternifolia*-Derived Ingredients as Used in Cosmetics.

Key Issues

Composition/Impurities, Melaleuca Alternifolia (Tea Tree) Leaf Oil - Regarding the limonene entry in Europe's Cosmetic Regulation, the following limitation is missing from the CIR report: "Peroxide value less than 20 mmoles/L". It is this limitation that "applies to the substance and not to the finished cosmetic product", not the requirement to include limonene on the product label if it exceeds 0.001% in leave-on products and 0.01% in rinse-off products, as stated in the CIR report.

Cosmetic Use; Exposure Estimations and Margin of Safety; Table 11 - The Cosmetic Use section correctly states that the 2008 SCCP opinion does not include calculations of margins of safety, nor does it include calculations of systemic exposure dose (SED). Therefore, it is not clear why the Exposure Estimations and Margin of Safety section and Table 11 include SED values cited to the 2008 SCCP opinion (reference 8). What is the correct reference for these SED estimates?

Sensitization, Table 18 - Did any of the sensitization studies provide enough information to estimate a $\mu g/cm^2$ dose? In Table 18, it would be helpful to state the area of a Finn chamber (50 mm^2).

Additional Considerations

Cosmetic Use - Please state the source/endpoint for the NOAEL of 117 mg/kg/day used in the risk assessment by Germany.

Exposure Estimates and Margin of Safety - In the first sentence, please correct: "was calculated was calculated".

Please indicate which version of the SCCP Notes of Guidance was used to calculate the SED values.

Dermal Penetration/Absorption - If the following refers to 5% tea tree oil in an oil/water emulsion, it should be revised to be clearer: "a 5% oil/water emulsion of tea tree oil".

Effect on Skin Integrity - What was the vehicle used in the studies from reference 66 and 67?

DART - Please indicate if the "adverse effect on mean fetal weights" was a decrease or an increase.

Effect on Spermatozoa - Are the units, ml, associated with 0.4 correct? This is a volume, while it is described as a concentration.

Immunologic Effects, Inhalation; Summary - It should be made clear that the first paragraph that is being cited to reference 94, is actually from what appears to be a review[1] in Polish. Reference 94 does state that the mice were exposed for "multiple sessions." Without additional details, perhaps this information should be deleted from the CIR report.

In the last line of this section, please correct "same species of mice" (should likely be "same strain of mice").

Retrospective and Multicenter Studies - Throughout this section, it should be stated if the subjects were tested with oxidized tea tree oil.

Please correct: "identified as being allergic to herbal medicines and/or botanical ingredients. identified as being allergic to herbal medicines and/or botanical ingredients."

Summary - Rather than stating "More than half of the 8..." why not be specific and state "Five of 8..."

Please correct: "SED values for ranged from 0.030 mg/kg/d..."

Please state the basis of the 117 mg/kg/day NOAEL.

Please correct: "not suppress welling in mice" (should be "swelling")

Please correct: "Emulsions of tea tree oil in were cytotoxic to adherent PBMCs."

Table 12 - What was the SED for neat tea tree oil used on the nails? Please include the NOAEL and source of the NOAEL used to calculate the MOS values as a footnote to this table.

Table 13 - Please review the column headings of the first two columns. The first column heading is currently Test Article/Vehicle, but the vehicle is often stated in the second column with the heading Concentration.

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[1]Skopinska-Rozewska et al. 1997 Antimicrobial and immunotropic action of essential oils. In: Xenobiotics influence on the immune system, iss. IRS, Olsztyn, Poland, 127-136, (in Polish).

The description of the results of reference 59 indicates headspace measurements, but the Procedure column does not give any description of the "headspace". What was the volume of the headspace? Was the Franz cell kept in another chamber?

In the description of the results for reference 60, were the hydrocarbons present in the receptor fluid or in the skin (or both)?

The first set of results for reference 62 indicates that the study was done with HSE. Were the rest of the studies done with HSE or Isopore® membrane?

The concentration and vehicle columns for reference 64 should also indicate that tea tree oil was tested with various excipients (at 1:1 - 50% concentration).

Table 19 - To be consistent with the text, the footnote to Table 19 should indicate that "oxidized" tea tree oil was added to the NACDG test tray in 2003.