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

Status:	Draft Tentative Report for Panel Review
Release Date:	February 16, 2021
Panel Meeting Date:	March 11-12, 2021

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.

1620 L Street, NW, Suite 1200 ♢ Washington, DC 20036-4702 ♢ ph 202.331.0651 ♢ fax 202.331.0088 ♢
cirinfo@cir-safety.org

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Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Monice M. Fiume MMF
Senior Director
Date: February 16, 2021
Subject: Safety Assessment of *Melaleuca alternifolia* (Tea Tree)-Derived Ingredients as Used in Cosmetics

Enclosed is the Draft Tentative Report of the Safety Assessment of *Melaleuca alternifolia* (Tea Tree)-Derived Ingredients as Used in Cosmetics. (It is identified in this report package as *melalt032021rep*.) Upon initial review of the safety of these 8 ingredients at the December 2020 meeting, the Panel noted the report was robust with data for a substance with the generic name tea tree oil, and the Panel considered these data relevant to the 2 oil ingredients in the report (i.e., Melaleuca Alternifolia (Tea Tree) Flower/Leaf/Stem Oil and Melaleuca Alternifolia (Tea Tree) Leaf Oil).

However, it was not clear to the Panel whether those data are also relevant to the 6 non-oil ingredients (i.e. 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, and Melaleuca Alternifolia (Tea Tree) Leaf Water). Accordingly, an Insufficient Data Announcement was issued requesting the following:

- methods of manufacture, composition, and impurity data for the non-oil ingredients (named above); if these are significantly different than that of the oils, then the following are also needed:
    - irritation and sensitization data for Melaleuca Alternifolia (Tea Tree) Extract at the expected maximum concentration of use, and
    - other toxicity endpoints, specifically to include genotoxicity data

VCRP data for 2021 have been received, and the frequency of use data have been updated accordingly. Frequency of use decreased for most of the ingredients, and Melaleuca Alternifolia (Tea Tree) Leaf Powder, which was reported to be used in 3 formulations in 2020, is now, not reported to be used. Most notably, the frequency of use for Melaleuca Alternifolia (Tea Tree) Leaf Oil decreased from 724 reported used in 2020 to 536 reported uses in 2021, with uses reported in leave-on formulations decreasing from 418 to 300, and in formulations with dermal contact decreasing from 557 to 409.

The following unpublished data on Melaleuca Alternifolia (Tea Tree) Leaf Extract (*melalt032021data1*) have recently been submitted by the Council, and are included in the report (as indicated by yellow highlighting):

1. Native Extracts. 2020. Safety Data Sheet: Melaleuca Alternifolia (Tea Tree) Leaf Extract.
2. Southern Cross University. 2020. Certificate of Analysis Fragrance Allergens: Melaleuca Alternifolia (Tea Tree) Leaf Extract.
3. Southern Cross University. 2018. Certificate of Analysis: Melaleuca Alternifolia (Tea Tree) Leaf Extract.
4. Native Extracts. 2020. Manufacturing Concentrate Flowchart.
5. Native Extracts. 2019. Manufacturing Oil Flowchart. [Not included in the report; please indicate if you find the information relevant to safety of these ingredients.]
6. Southern Cross University. 2020. Certificate of Analysis Fragrance Allergens: Vitis Vinifera (Grape) Seed Oil and Melaleuca Alternifolia (Tea Tree) Leaf Extract.
7. Native Extracts. 2018. Safety Data Sheet: Vitis Vinifera (Grape) Seed Oil and Melaleuca Alternifolia (Tea Tree) Leaf Extract.
8. Southern Cross University. 2018. Certificate of Analysis (fatty acids): Vitis Vinifera (Grape) Seed Oil and Melaleuca Alternifolia (Tea Tree) Leaf Extract.

1620 L Street, NW, Suite 1200, Washington, DC 20036
(Main) 202-331-0651 (Fax) 202-331-0088
(email) cirinfo@cir-safety.org (website) https://cir-safety.org

Data obtained from an industry video describing the manufacture of tea tree oil were also added to the report (and indicted by yellow highlighting). A literature review on tea tree oil was submitted by the Australian Tea Tree Industry Association (ATTIA; *melalt032021data_2*), as were comments following the December meeting (*melalt032021comments_ATTIA*). Please note, while the entire literature review that was received is included for your review, the only new data obtained from it were a 4-h semi-occlusive irritation study in rabbits.

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

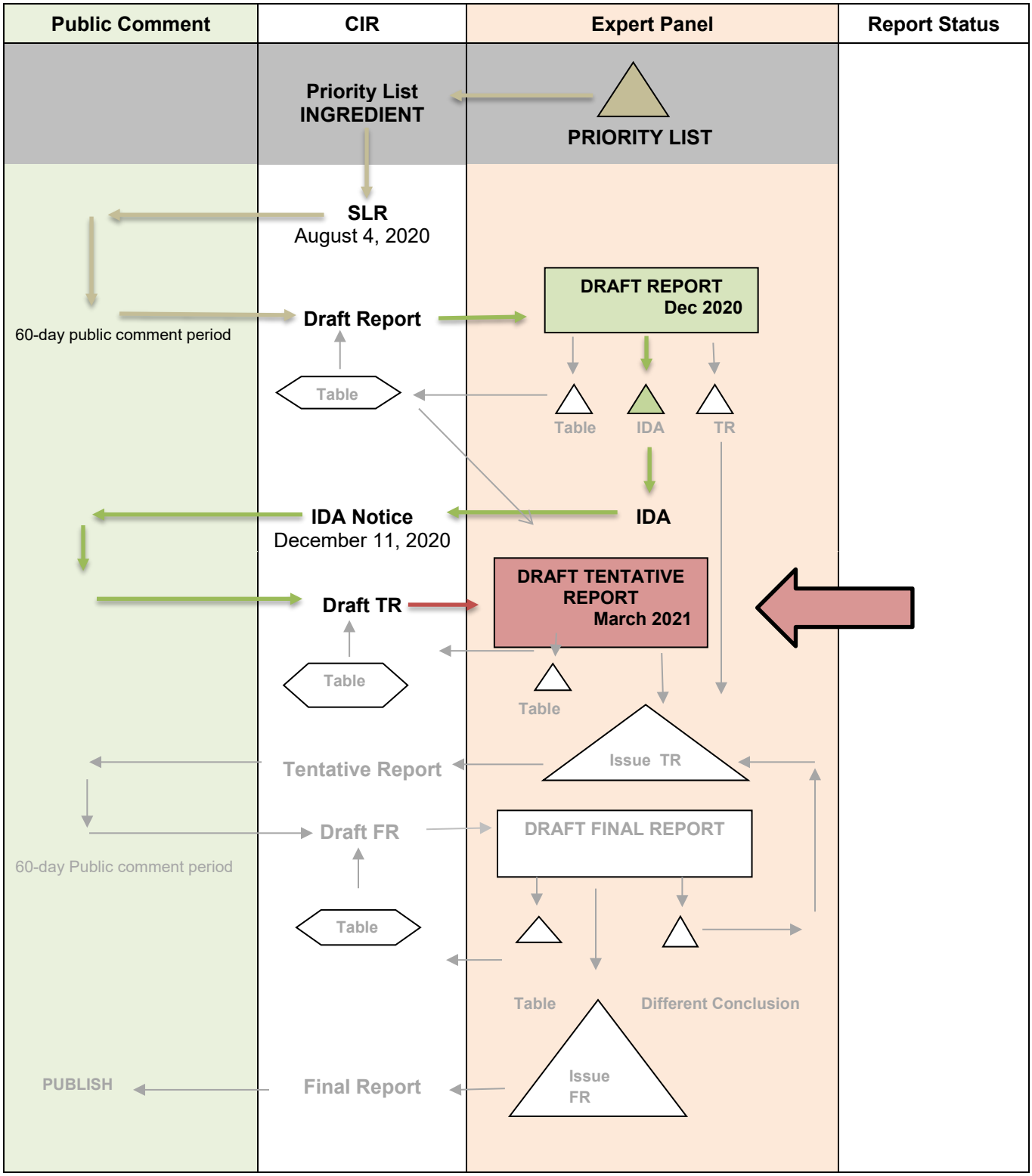
melalt032021flow:	report flowchart
melalt032021hist:	report history
melalt032021prof:	data profile
melalt032021min:	transcripts
melalt032021strat:	search strategy
melalt032021FDA:	2021 VCRP data

Based on the proceedings and comments from the December 2020 meeting, a draft Discussion has been prepared. The Panel should carefully consider and discuss the new data and the draft Abstract and Discussion presented in this report, and issue a Tentative Report with a safe, safe with qualifications, insufficient data, unsafe, or split conclusion.

SAFETY ASSESSMENT FLOW CHART

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

MEETING March 2021



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.

Because it was unclear whether the data on tea tree oil was relevant to the non-oil ingredients, the Panel issued an Insufficient Data Announcement requesting the following:

- methods of manufacture, composition, and impurity data for the non-oil ingredients named above; if these are different than the of the oils, then the following are also needed:
    - irritation and sensitization data for Melaleuca Alternifolia (Tea Tree) Extract at the expected maximum concentration of use, and
    - other toxicity endpoints, specifically to include genotoxicity data

Draft Tentative Report: March 11-12, 2021

The following unpublished data on Melaleuca Alternifolia (Tea Tree) Leaf Extract were received and incorporated:

1. Native Extracts. 2020. Safety Data Sheet: Melaleuca Alternifolia (Tea Tree) Leaf Extract.
2. Southern Cross University. 2020. Certificate of Analysis Fragrance Allergens: Melaleuca Alternifolia (Tea Tree) Leaf Extract.
3. Southern Cross University. 2018. Certificate of Analysis: Melaleuca Alternifolia (Tea Tree) Leaf Extract.
4. Native Extracts. 2020. Manufacturing Concentrate Flowchart.
5. Native Extracts. 2019. Manufacturing Oil Flowchart.
6. Southern Cross University. 2020. Certificate of Analysis Fragrance Allergens: Vitis Vinifera (Grape) Seed Oil and Melaleuca Alternifolia (Tea Tree) Leaf Extract.
7. Native Extracts. 2018. Safety Data Sheet: Vitis Vinifera (Grape) Seed Oil and Melaleuca Alternifolia (Tea Tree) Leaf Extract.
8. Southern Cross University. 2018. Certificate of Analysis (fatty acids): Vitis Vinifera (Grape) Seed Oil and Melaleuca Alternifolia (Tea Tree) Leaf Extract.

Data from an industry video describing the manufacture of tea tree oil were also added to the report.

Melaleuca alternifolia (Tea Tree)-Derived Ingredients * – March 11-12, 2021 – Writer, Monice Fiume																																		
					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		X	X																														
Melaleuca Alternifolia (Tea Tree) Leaf Oil	X			X				X								X								X	X			X					
Melaleuca Alternifolia (Tea Tree) Leaf Powder				X																														
Melaleuca Alternifolia (Tea Tree) Leaf Water	X		X																															
Data on ingredients with general names; it is not known how these compare to cosmetic ingredients – this is for informational purposes only																																		
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	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

DECEMBER 2020 MEETING – INITIAL MEETING/DRAFT REPORT

Belsito Team – December 7, 2020

DR. BELSITO: Okay. Are we done with that? So then we get to move on to *Melaleuca alternifolia* also known as tea tree oil and -- all right. Okay. Now it's letting me save. Okay.

So this is also the first time we're looking at six of these ingredients. And Monice posed a question of the fact that she posted all of the abbreviations up front and what we thought of that. I actually liked it, because it gave me one place to go back and look if I somehow missed the abbreviation, but I don't know what the others thought of this. If it should occur when it's first used as typically done. So I guess that's the first comment for the team.

DR. LIEBLER: I like it as well as long as it's bookmarked, and it is. So I know Wilma indicated she preferred the -- I think I interpreted her saying she preferred the abbreviations being laid out where they first are used.

DR. KLAASSEN: One can do both.

DR. LIEBLER: Yeah. Exactly. A lot of journals do that.

DR. KLAASSEN: Yeah. That's what I prefer. The first time you do spell it out, but you also have it here.

DR. LIEBLER: Yeah. I think that would make everybody happy.

DR. KLAASSEN: Yeah.

DR. LIEBLER: I mean how often do we get a chance to make everyone happy?

DR. KLAASSEN: Not often.

DR. BELSITO: Okay. In the first paragraph of the introduction we don't mention the use of it as a -- we do mention a fragrance ingredient, but we don't mention that that's not in the purview of the panel. We mention that the anti-acne agent is not considered a cosmetic function, but we don't mention that we're not reviewing the fragrance aspect of this. I think that needs to be included, no?

MS. FIUME: So if it's used as a fragrance and has other functions, and it is under the purview of the panel, or if RIFM has not said that it's on their list. So looking at Table 1, I don't think there are any that have only a use as fragrance. It's also used as an antioxidant, which is the reason that that's not pointed out in that introduction.

DR. BELSITO: Okay. But are we looking at its safety as a fragrance? Because normally in other materials, I think like benzyl alcohol, didn't we say something in the introduction that we're not looking at its safety as used in fragrance materials or as a fragrance?

MS. FIUME: In that case it probably had a RIFM -- and I believe it did have a RIFM review.

DR. BELSITO: Yeah. Yes. It did.

MS. FIUME: So I don't know if this one does have a RIFM review. And if it doesn't, then the panel generally looks at it for all uses.

DR. BELSITO: I'm almost certain that RIFM has not looked at this.

DR. LIEBLER: So if it does have a use as an antioxidant and as a fragrance, we're looking at the use -- we're looking at its safety as used. And whether fragrance is listed as a -- we can include a sentence we're not evaluating safety as a fragrance. But if it's used, and it has another use, then we're evaluating it, right?

DR. BELSITO: Right.

DR. LIEBLER: So we're not talking about taking anything out.

DR. BELSITO: Okay. I'm just -- because we mentioned the anti-acne and that's a drug, and we're not reviewing it as a drug. I just thought normally we had a little catch phrase that we're also not reviewing it as a fragrance, but.

DR. LIEBLER: Okay.

DR. BELSITO: I just had another comment maybe it was on the introduction. I really like the last paragraph in the -- or the last sentence in the second paragraph and think it almost should serve as a boilerplate for these natural complex substances. It says, "Naturally occurring combinations rarely demonstrate the same biologic activity as the individual separate components. Potential toxicity is a functional response to exposure of a mixture of different chemical compounds." And I almost think that should be a boilerplate for all of these botanicals.

MS. FIUME: Okay. I will note that for all of us.

DR. BELSITO: Did other people -- that's just my opinion, so I'm bringing it up to the team. Do other people like that statement?

DR. SNYDER: Yes.

DR. BELSITO: Curt, Dan?

DR. KLAASSEN: I have to think about that. I'm not confident. And where in -- repeat where you found that.

DR. BELSITO: So it's PDF Page 11. It's the last sentence in the second paragraph of the introduction.

DR. KLAASSEN: Okay. Say that once more. What page? I got lost.

DR. BELSITO: PDF Page 11.

DR. KLAASSEN: Yeah. Okay.

DR. BELSITO: The second paragraph of the introduction, the last sentence.

DR. KLAASSEN: Page 11 is definition and plant identification?

DR. BELSITO: No. PDF Page 11 is the introduction.

DR. KLAASSEN: Okay.

DR. BELSITO: Second paragraph, last sentence.

DR. KLAASSEN: I guess my question is, is that first half really, really true? And we never test all the separate compounds, so we don't really know. I like the second part of the sentence. I just don't know if the first part of the sentence --

DR. BELSITO: Would it make you happier, Curt, if we say naturally occurring combinations "may not" demonstrate the same biological activity, rather than rarely?

DR. KLAASSEN: I just think that there's so little data to know if this sentence is true or not.

DR. BELSITO: Dan, any comments? Dan, you're muted if you're still with us.

DR. LIEBLER: Sorry. I did mute. I apologize. I agree with Curt that we don't have sufficient data to say rarely. On the other hand, I think our collective experience has been that they may not demonstrate the same biological activity. I agree with substituting "may not" and keeping that sentence there.

DR. KLAASSEN: So you're going to take out "rarely demonstrate" and insert --

DR. BELSITO: May not demonstrate.

DR. LIEBLER: May not demonstrate.

DR. KLAASSEN: Take out "rarely," and "may not." I have no problem with that. I think that probably is getting the same thought across.

DR. LIEBLER: Yes.

DR. BELSITO: Okay. I figured it would be a good boilerplate for all the natural complex substances we're looking at.

DR. LIEBLER: And I do agree with that point, Don.

DR. BELSITO: Okay.

DR. SNYDER: Would it be easier just to say complex mixtures -- "The potential toxicity of complex mixtures is a function of response to exposure," or something, instead of the -- I think we can shrink that down. Maybe we can wordsmith it.

DR. BELSITO: Okay. You want to do that, Paul?

DR. SNYDER: Yeah. I can try to do that.

DR. BELSITO: Okay. And then just maybe post it to all of us?

DR. SNYDER: Yeah.

DR. BELSITO: Okay. So presumably, now that we've been told that the extract is the whole plant, if my understanding is correct, we're looking at roots, bark, the woody portions. Is that correct?

DR. LIEBLER: Yes.

DR. BELSITO: We're looking at the whole (audio skip).

DR. LIEBLER: Right.

DR. BELSITO: Okay. Then I have a question for you, Dan, on the stability. If there's no oxidation of tea tree oil on degradation, how are peroxides formed? This is PDF Page 12, under Stability.

DR. LIEBLER: Okay. I'm scrolling up. Okay. I didn't look at the paper to see how they did this. But they're saying no appreciable oxidation or degradation of tea tree oil, two references cited. And then they say no change in level of terpineol. But then they talk about changes in the level of terpinene, alpha- and gamma-terpinene and an upward trend in paracynene observed and peroxide levels increased.

Now that is change. That is degradation and is oxidation.

DR. KLAASSEN: Right.

DR. LIEBLER: You know, the thing is you could have a one, or two, or five percent loss of a precursor to oxidation, but the amount of peroxide generated could be toxicologically significant. In other words, depending on its measured loss (audio skip) -- depending on how you measure the loss of the precursor, it may appear to be insignificant. But the oxidation product, even if it's only a few percent might be significant toxicologically.

DR. BELSITO: Okay.

DR. LIEBLER: That's the whole idea of an impurity. It's a small percentage of the total, but it still can have an effect. And I think some of these oxidation products could be sensitizing.

DR. BELSITO: Um-hmm. Well, oxidized tea tree oil is a sensitizer.

DR. LIEBLER: Yeah. Right. So I think this stability paragraph doesn't do away with the issue.

DR. BELSITO: I just thought it was weird that it said there's no appreciable oxidation, and then there are peroxides formed.

DR. LIEBLER: I think we have to look at the paper. Because if they're just looking at whether or not the component, the potentially oxidizable components are changing in concentration, that's one way to measure it. But depending on the measurement method, they may not appear to go down much even though a significant amount of oxidation products are indeed being formed. And unless you're directly measuring the oxidation products, you would have no way of knowing that.

So they do say downward trend for a couple of chemicals, upward for another was observed, and the peroxide levels increased. That last bit there, peroxide levels increased, to me is the tell-tale sign that there is indeed oxidation going on.

DR. BELSITO: Okay.

DR. SNYDER: So I had a general comment about the report.

DR. BELSITO: Yeah.

DR. SNYDER: So under Chemical Properties, we define tea tree oil as a volatile essential oil. And then we have Method of Manufacture, tea tree oil is defined by ISO standard. Where is tea tree oil in the ingredients that we're looking at? Where does it fit with regards to tea tree flower, leaf, stem oil or tea tree leaf oil or -- so all the data -- we have a lot of data that's defined as tea tree oil, but it's not an ingredient here. So what is it covering? I guess I'm asking the group, where is that at?

DR. BELSITO: I just assumed it was all the various ways the oil could be derived.

DR. SNYDER: But does it include -- does the tree oil include the flower, the leaf, the stem? And then there's just the leaf oil? So I was confused as to what that leaf tree oil data was covering, in regard to the ingredients.

DR. BELSITO: I don't think we know.

DR. LIEBLER: Yeah. That needs to be clarified. I actually found a YouTube video that I sent to Monice and Lisa Peterson, that described -- it appeared to be from an industry source describing the preparation of tea tree oil. And they basically, you know, lawnmower up these small plants, which is what they use to make this stuff. And then saplings, essentially the entire sapling, so it includes flower, leaf, and stem to make this oil. Basically, it's a steam distillate. And that is the stuff that they refer to as tea tree oil.

DR. SNYDER: Because then on page 12, under the Method of Manufacture, it says as an essential oil obtained by steam of the leaves and terminal branchlets.

DR. LIEBLER: Yeah. So that's leaves, stems -- doesn't say flower there but --

DR. SNYDER: So we can make a reasonable interpretation that it's covering the majority of the ingredients that were under review?

DR. LIEBLER: Yes. That's what I did, Paul. And that's why method of manufacture, even though it's mainly for tea tree oil, I'm inclined to think this represents all the other ingredients since the tea tree oil is the extract of the whole plant.

DR. SNYDER: Okay.

MS. FIUME: I will tell you -- I mean that definition is there. According to the INCI dictionary, it's a technical name for tea tree leaf oil. But I agree the definition that is given in the report for that ingredient, the ISO definition, does seem to involve a little more than the leaf, which is why the generic name has been used throughout the report, because we don't have a one-to-one link to the INCI name.

DR. LIEBLER: Yeah. It seems to me preparation of a product just from the leaves would be a lot more time consuming, separating the leaves from any stem and shoots and so forth.

DR. BELSITO: Flowers.

DR. LIEBLER: Yeah. But I felt that the tea tree oil methods and composition and impurities clear all the ingredients.

DR. BELSITO: Okay. Anything further on this point? Okay, Monice, on PDF page 13, the next to the last line I just have a question about your concentrations there, because it's 1.1 and then 11.7 -- or 1.1, 11,7. I presume that should be 11.7?

MS. FIUME: I'll go back and double check. That's probably a typo.

DR. BELSITO: Yeah. Then I just had a comment on PDF Page 14, about certain components the COLIPA 2002. It says, when formulating tea tree oil in a cosmetic product, companies should consider that the sensitization potential increases when certain constituents of the oil become oxidized. And manufacturers should consider use of antioxidants and/or specific packaging to minimize exposure.

My comment was that this was before the QRA was introduced. And this is also used in deodorants and ancillary products, which is an area that has gotten other materials such as the fragrance, Lyral, into problems and resulted in that fragrance material being banned in Europe. So I think when we get down to sensitization, we need to talk about this. I think this is one of these -- it should be -- we should point out the oxidation issue, but also something to the extent of when formulated to be non-sensitizing as part of our conclusion.

DR. LIEBLER: I completely agree with you, Don. I think the challenge of trying to do a QRA on this is that we don't really have control over the concentration of the oxidation products. It's going to be highly variable. But we know that they could be there. And so I think this can probably be handled in the discussion. It's a very relevant point, and I even agree with the issue of formulated to be non-sensitizing.

DR. BELSITO: So this is a penetration enhancer, so that'll have to be in the discussion?

DR. KLAASSEN: Since you're near Page 13 -- or were -- on page 13 the paragraph that starts out with, "According to the ISO standards," -- about the third paragraph?

DR. BELSITO: Yeah.

DR. KLAASSEN: If you go down to about the sixth line it says, however for cosmetics, according to the EC regulation such and such, the presence of limonene in the cosmetic product must be indicated blah, blah, blah. I wonder if that's still true.

DR. BELSITO: Yeah. So EU, Curt, has 26 ingredients that need to be labeled if their concentrations are above certain levels, and limonene is one of them. So this is an EU labeling regulation.

DR. KLAASSEN: For what reason?

DR. BELSITO: Because they're sensitizers.

DR. KLAASSEN: This is because of a sensitization reaction?

DR. BELSITO: Right. Right. So the EU has identified 26 fragrance materials which they consider to be among the more sensitizing fragrances and require them to be labeled if present in total amount. So that would cover limonene coming from not only tea tree oil but from other botanical sources in the product.

DR. KLAASSEN: Well, maybe I did not realize that. There's been a lot of work on limonene in regard to kidney toxicity and cancer, and that's all been kind of worked out. So that's why I was coming up with that question. But now that you've explained it to me, and we always have the -- I shouldn't say always -- but every once in a while, we have a problem with a chemical that has sensitization. Could we likewise use this kind of a thought process and use labeling rather than almost banning it or -- you see what I'm saying?

DR. BELSITO: Well, in the United States a cosmetic product has to be fully labeled except the fragrances can just be grouped. So basically if you had a company that was manufacturing only for the U.S., and they had limonene in it, they could just put fragrance. But if they want to market it in the EU, and the limonene exceeds those concentrations, they have to list it on the label. So I mean, in the U.S. we have great labeling laws, it's just for fragrance we don't.

DR. KLAASSEN: But how about for a cosmetic?

DR. BELSITO: Yeah. A cosmetic has to be fully labeled as to its ingredients. But the difference is for fragrance material, in the U.S., our regulations are such that you don't have to identify fragrance. But if you're P&G or you're -- well, Unilever's a

British company anyway. But if you're P&G, you're Colgate, you're a U.S. based company and you're manufacturing worldwide, you read their labels, they have the 26 fragrances if their product contains it, labeled.

So the only difference in the labeling laws between the U.S. and Europe, are that there are 26 fragrances that have to be listed if they're contained. Otherwise, U.S. cosmetics are fully labeled as to everything that's in it, except that they don't specify fragrance unless they're a multinational.

So I guess, Dan, my question to you, listening from before, is that for my conclusion I thought that tea tree leaf oil, all of the various oils we were looking at were safe when formulated to be non-sensitizing. But the other constituents that weren't oils needed composition and impurities, and if different from the oil sensitization and irritation, a 28-day dermal. But you feel that we can use the oil to read across to all of these constituents?

DR. LIEBLER: Yeah. I do. I think the -- so the way the oils is prepared is from steam treatment of the plant material. And I think that's going to get most of the same organics that you're going to get from the extracts, which are going to be hydroalcoholic extracts, and the powder in the water. It's going to produce at least as much of these organics, which are the oxidizable components that will give rise to sensitization.

So I think that the tea tree oil, it essentially covers those. It's not a specifically identical process. But the end product of the process is pretty similar. I say the end product of the process is going to be similar with respect to the presence of the oxidizable sensitizing components. So I think handling that in a discussion, dealing with the oxidation issue is the driver of sensitization, and then formulate to be non-sensitizing is the right way to go.

DR. BELSITO: Okay. So then what I have for the discussion is obviously the botanical boilerplate, the aerosol boilerplate, penetration enhancement. I don't know if you want to mention the DART endocrine disruption at very high doses, which aren't physiologic and pertinent to the levels we're looking at. The sensitization potential of oxidized product, and the fact that we feel the data on tea tree oil covers the other ingredients in the material. Is that it?

DR. LIEBLER: Yeah.

DR. BELSITO: Okay. So then safe as used when formulated to be non-sensitizing, using QRA or other appropriate methodologies. Is that where we're going?

DR. LIEBLER: Yes.

DR. BELSITO: Okay. And then the other question that Wilma started at is the large variation in composition depending upon sources like Australia, Vietnam, China. Do we want to say anything about that in the discussion? I think the idea of formulated to be non-sensitizing covers those variations. But because I don't really see any other composition differences that would bother me in terms of other tox endpoints.

DR. LIEBLER: Well, we could always add one sentence, indicating that various cultivars are likely to have varying content of some of the oxidizable constituents that would drive sensitization. So we could put that in if it comes up. Maybe don't bring it up, but we could put it in if it comes up in discussion tomorrow.

DR. BELSITO: Okay. So if it comes up, we can say that the formulation to be non-sensitizing covers those variations.

DR. LIEBLER: Yeah.

DR. BELSITO: Anything else? Okay. So it's 12:09 Eastern. We break for lunch. Is 1:00 sufficient for everyone to have lunch?

DR. LIEBLER: Sure.

MS. FIUME: The thing is -- but Don, can I ask you a question before everyone signs off?

DR. BELSITO: Sure.

MS. FIUME: Dan, you had mentioned earlier that the, I guess, the amount of material of the oxidized material could create a problem for the QRA. Does anything need to be mentioned specific to that in the discussion?

DR. BELSITO: Yeah. So we talked about the sensitization potential of oxidized material. And I think just like COLIPA did back in 2002, a statement that a methodology should be employed to minimize oxidation in final formulation.

DR. LIEBLER: I think, Monice, it would be actually very hard to do a QRA if you don't know what the oxidized product content is. And so that's not practically determinable unless you were operating a big research lab. And so, that won't come into play here. So COLIPA language is the right way to go.

MS. FIUME: I'm sorry, the what language is the right way to go?

DR. LIEBLER: The COLIPA language that Don just mentioned.

MS. FIUME: Okay. Great. Thank you. All right.

DR. LIEBLER: Okey-doke?

MS. FIUME: Yeah. Thank you very much.

DR. BELSITO: Okay.

Cohen Team- December 7, 2020

DR. COHEN: Melaleuca alternifolia. This one is -- Monice has this one. Monice, you're on?

DR. HELDRETH: No, Monice is running the other breakout room, so you're stuck with me on this one.

DR. BERGFELD: Real stuck.

DR. COHEN: Okay. Okay. No. All good. So this is a draft report. It's the first time we are reviewing this. This safety assessment has eight derived ingredients. It's used as a skin conditioning agent. The max use is 0.3 percent in rinse off and 0.63 percent in a cuticle softener, but there's a lot of missing information on concentration of use.

The VCRP data showed the leaf oil doubling in the past few years, and the leaf oil concentration coming down quite a bit from 15 percent a couple of years ago in 2015, in the face and neck, to 0.63 percent. In cuticle softeners, we have method of manufacturing for the leaf water and oil.

DR. PETERSON: No, we don't really have --

DR. COHEN: We don't?

DR. PETERSON: So actually, Dan did some digging and sent a fun YouTube video for the method of manufacturing for the tea tree extract. And it would be probably the same method of manufacturing for the leaf, stem, flower.

Basically, they clip everything off at the ground, put it in a big vat, mash it up, do some steam distillation, and then separate the steam from the -- the water from the oil, and that's how they get the oil. So there is this YouTube video that I can -- Dan forwarded it to Monice. If you guys want to watch it, I can forward it to the group. Anyway, it's about a two-minute video that explains the process.

DR. BERGFELD: Thank you.

DR. PETERSON: So Dan thought it was groundbreaking because it's probably the first time a YouTube video would be a reference for a report. But I do think that we're missing a lot of --

DR. COHEN: We still want it in prose, though, I suppose.

DR. PETERSON: In prose. Yeah, but it's a -- I mean, I'm just saying that it's out there. It's up to -- it's out there. But there is a lot of missing information for the lower use ones, I thought.

DR. SLAGA : Well, there's a lot of data on the oil.

DR. PETERSON: Yeah.

DR. SLAGA : And it's actually GRAS too.

DR. SHANK: And it's safe as used, the oil.

DR. SLAGA : Yeah, safe as used for the oils. I agree, Ron.

DR. SHANK: Okay.

DR. SLAGA : The rest of them, there is not much data to --

DR. SHANK: Right.

DR. SLAGA : So it's the first time, let's ask for what we can get.

DR. SHANK: If we ask for a complete workup of the tea tree extract, maybe that would cover all the other ingredients for systemic toxicity and sensitization.

DR. SLAGA : Yeah. I agree.

DR. COHEN: So can you just articulate for me, for the extract we're looking for what specifically?

DR. SHANK: Okay. The tea tree extract, which is the whole plant --

DR. COHEN: Yes.

DR. SHANK: -- I would ask for 28-dermal toxicity, genotox, developmental and reproductive tox, and skin sensitization. And given that, then we could apply that to all of the other ingredients.

DR. SLAGA : I agree.

DR. COHEN: Okay.

DR. SHANK: I don't really know what this oxidized oil means. It's in the list of ingredients, but no reported uses, and there is very little data on it.

DR. COHEN: For which one?

DR. SHANK: Tea tree oil oxidized.

DR. PETERSON: Well, I thought that that was actually an important issue to talk -- that should be in the discussion or something. Because if you use it fresh, it seems to be used safer than when it's been aged and not stored fresh. So, you know, most of us, when we buy a product and we use it right away, probably it's not going to be a problem. But, if you age it and so -- but that's more on the users end probably than -- but I do think it's worth having in the discussion that this -- it seems like a lot of the issues come --

DR. SHANK: So what is --

DR. PETERSON: -- from the oxidized.

DR. SHANK: What is the problem with oxidized oil?

DR. PETERSON: Well, it's got (audio skip).

DR. COHEN: It says sensitizer.

DR. SHANK: The only information we have on it is animal sensitization.

DR. PETERSON: I thought there was human information.

DR. SHANK: And some clinical studies.

DR. BERGFELD: Well, we have an LLNA as well.

DR. SHANK: Pardon me?

DR. COHEN: Lymph node.

DR. BERGFELD: We have a lymphocyte test.

DR. PETERSON: Plus, the clinical studies were done with the oxidant.

DR. BERGFELD: Lymph node assay.

DR. PETERSON: And there's clinical data that --

DR. COHEN: Yeah, and typically, we're patch testing to oxidized tea tree oil as part of our diagnostic work up. It's five percent oxidized tea tree oil.

So just a question, some help from the group. The oil, particularly oxidized, is a known sensitizer, we see increasing use, but we see decreasing concentration. So, when we're saying safe as used, how does that translate to concentration of use? Is it the lower concentration as of the date of the report that's being used, or does the (inaudible) of the historic concentrations that are much higher?

DR. HELDRETH: So the conclusions for the CIR reports, when they say, safe as used, the conclusion also goes on to say, as described in this report. So you would look for the worst case scenarios that are in our concentration use table, and look at those max use concentrations that are recited there. That's what the conclusion pertains to. If someone were to come up --

DR. BERGFELD: Which is backed up by clinical studies. Bart, I'm sorry. But that information then, the use information, is supported by animal and human studies.

DR. HELDRETH: Okay. Yeah, but that -- I think, if --

DR. BERGFELD: Because sometimes they -- it's used at higher or lower than it's tested.

DR. HELDRETH: Right. But, when the Panel eventually makes a conclusion on this report, if they say, safe as used, they mean it's safe up to the concentration maximums that are recited in the report.

DR. BERGFELD: Yeah. Right.

DR. HELDRETH: So, if someone comes along and makes a product with a much higher concentration, or in a different use category, or they use it in a product and just didn't report that higher concentration, the Panel's safety conclusion just really doesn't apply to that. It's outside of the parameters of what the Panel reviewed and, therefore, their conclusion doesn't cover that situation.

DR. SHANK: Well, why the oxidized oil listed as an ingredient when it's not used? Am I to understand what you're talking about is a product that has the tea tree oil and then it goes rancid? We've never considered that kind of a scenario, just the formulations, not what happens when the product is used in part and then left over and goes rancid. So why is the oxidized oil in here?

DR. COHEN: I didn't look at it like that. I was looking at it in its typical use of being broadcasted on skin or hair, and then being subject to oxidation from routine use.

DR. SHANK: When it's applied to the --

DR. COHEN: But not specific product rancidity.

DR. SHANK: So the oil oxidizes very rapidly, as soon as you apply it to the skin or hair?

DR. COHEN: Lisa, can you comment on that?

DR. BERGFELD: Do we know that?

DR. PETERSON: I know that it oxidizes.

DR. SHANK: I don't underst- --

DR. PETERSON: I don't remember the timeframe of the oxidation, if that's been studied.

DR. BERGFELD: I don't think so.

DR. PETERSON: But we could certainly look for that.

DR. SHANK: Because we've look at a lot of oils, and we've never asked this question.

DR. SLAGA : Right.

DR. SHANK: If the oil becomes oxidized, is it still safe?

DR. BERGFELD: That's correct.

DR. SHANK: I'm not saying we can't do that, but it's just a departure.

DR. BERGFELD: Maybe it's a discussion point rather than a conclusion point.

DR. SHANK: Okay.

DR. COHEN: I think -- yeah. This comes up with other fragrances like limonene and linoleoyl as the oxidation products are more sensitizing.

DR. SHANK: Uh-huh.

DR. BERGFELD: Well, practically, these products are made and formulated, and put in bottles, and are left on shelves for years. So the question is, when does that oxidation process take place? Months later? Years later?

DR. COHEN: So can we ask for further information about that?

DR. BERGFELD: Yeah.

DR. HELDRETH: Yes.

DR. SHANK: It's the first time.

DR. COHEN: Ron, I think it's coming up in the context of the clinical studies and the way that we diagnose patients. We're using oxidized tea tree oil, and we're using some oxidized botanical oils to diagnose contact dermatitis. So that's come up quite a bit lately, and, I think, perhaps that's how it's infiltrating here.

DR. SHANK: Okay.

DR. BERGFELD: Could you explain how you got to that point?

DR. COHEN: I think for discussion also --

DR. SHANK: Pardon me?

DR. BERGFELD: How did the North American Contact Dermatitis Group get to the point that they should use the oxidized rather than the fresh?

DR. COHEN: I can ask Don to comment on that.

DR. BERGFELD: Mm-hmm.

DR. PETERSON: Because this is an -- actually, this is the first time where there's been clinical studies where they talk about use -- at least in my tenure here over the year, and we've done quite a few botanical oils. This is actually the first time I've seen it in the clinical.

So, yeah, I think it is worth finding out how long it takes, and that this is an issue. It seems to me it belongs in the discussion. And it could explain why sometimes you're seeing sensitization and sometimes you're not. So, to me, it's a useful piece of information, but it wouldn't change how you assess the safety of the fresh product, which is a different thing, which I think gets to your point, Ron.

DR. SHANK: Yes. Yes.

DR. SLAGA : Also, wouldn't be a function if there is, like, other oils that you would use in a house too -- in foods. There's usually something to prevent the oxidation, some antioxidant, be it BHA, BHT, something to keep it stable.

DR. PETERSON: So then we would recommend or have in the discussion that this product should be to --

DR. COHEN: I suspect that's to keep it stable in the finished product.

DR. PETERSON: But then we should -- does that mean there's a sort of statement of needs to be formulated such that it doesn't oxidize?

DR. COHEN: Well, (audio skip).

DR. SLAGA: I guess. There's probably other ingredients that help -- that are in the tea tree ingredients that would help prevent it from oxidizing. Oxidants -- antioxidants are pretty common.

DR. BERGFELD: We should ask the industry to define this. Maybe someone is in the audience that is from the companies that produce these products.

DR. SLAGA: Yeah. That would be good.

DR. COHEN: Anyone on now?

DR. HELDRETH: Alex, I see you have a hand up. Do you have something to add, Alex?

MS. KOWCZ: Yeah, I do. I just wanted to add that I think Monice has done this, and I know she's not on the call right now. But there is an Australian Tea Tree Industry Association. And we were hoping that they would be on the call today, but I don't think anyone is there.

DR. PETERSON: Well, there's somebody with their hand raised. This Phillip.

DR. HELDRETH: That's Alex.

MS. KOWCZ: Yeah, I don't know where he's from.

DR. HELDRETH: Yeah, we invited Mr. Larkman, and he accepted the invite, but I don't know if he's on or not. I don't see him in this room at least.

MS. KOWCZ: But the only one thing that we did find out is the routine patch testing, Dave -- so this is very interesting for us -- is usually conducted with a lot of essential oils, but they're not usually with an oxidized form.

And so, this association was very strict in they're trying to develop an ISO standard, and they're doing additional testing. They feel the tests that are done with the oxidized tea tree oil overestimates the sensitization potential of essential oil. So I just wanted to make sure and just give a little bit of more information.

Okay. So we do have someone on the line right now, and I think it's Phillip, correct? So he is calling in from the ATTIA, which is the Australian Tea Tree Industry Association, so I'll let him speak, and I'll get off.

MR. PRATHER: Great. Thank you, Alexandra. Thank you for that discussion. I think the question at hand --

DR. COHEN: Okay.

MR. PRATHER: Can you hear me all right?

DR. SHANK: Yes.

DR. PETERSON: Yes.

MR. PRATHER: Okay. Wonderful.

MS. KOWCZ: Yes, perfectly.

MR. PRATHER: So thank you for the opportunity to speak --

DR. COHEN: Yes.

MR. PRATHER: -- and appreciate the discussion you've had so far. I'm from the Australian Tea Tree Industry Association, the vice president. Also I'm an independent producer/manufacturer of tea tree oil here in Australia.

The topic of the oxidized tea tree oil, it has come up because of some various patch test that have been commercially produced, which intentionally oxidize the tea tree oil. When we inquired as to why they did that in their manufacturing process of their test kits, they responded that it was because it produced a better result.

We have long-term shelf-life tests of tea tree oil, both in a retail format in a neat oil and also in formulated products. And the tea tree oil maintains its integrity within specifications of the ISO standard, well beyond a three-year shelf life in a closed container.

In formulations, obviously, that changes based on the formulation, but, unless you intentionally oxidize the oil, it stays relatively stable in a consumer post-purchase format.

DR. SHANK: Thank you.

MR. PRATHER: Are there any specific questions that anybody would have?

DR. BERGFELD: Thank you. Yeah, is it bottled in a brown bottle?

DR. COHEN: Just a follow-up question.

MR. PRATHER: Typically, it is. There are some companies that do put it in a clear or a blue or a different bottle. My company, in particular, has worked with a U.S. retailer that packages into a clear, glass bottle. We have done the shelf-life test for them and have found that at three years, there is no degradation. This is at 40 degree Celsius and under ultraviolet, accelerated aging conditions.

DR. SHANK: Good.

DR. COHEN: Just a comment and then a question, relative to Wilma's point. The determination of the most appropriate patch test concentration, that takes some time and trial to get to, and similarly with limonene and linoleoyl, I think your comment about produced a better result really was detecting the greatest number of patients (audio skip) to that particular chemical, so the oxidized portion captured more people.

So I understand the issue of the stability of the non-oxidized products for three years. But, under routine use, is there any information about the speed and the quantity of oxidation that occurs with intended use?

MR. PRATHER: I do not have that data available to me, but I'm sure that we would be able to produce that with some of the data that we have generated for some European testing that we have done in the last two years. So that is something we can provide to the Panel.

DR. COHEN: I think that would be really helpful.

MR. PRATHER: Okay.

DR. BERGFELD: So, if I could ask a question. So your feedback, Phillip, for sensitization on the tea tree oil, I gather is low. On your personal feedback, your company's feedback.

MR. PRATHER: It is.

DR. BERGFELD: Is it?

MR. PRATHER: It is low based upon the fresh oil being used in a formulation, or the bottle being used in a post-purchase consumer basis where the bottle is opened, the oil is accessed and then the lid is put back on. It lasts for -- you know, we have to -- I believe we put a one year recommended shelf life on that once it's being open and closed repeatedly by the consumer.

DR. BERGFELD: Okay.

MR. PRATHER: We do have a White Paper, that's produced by ATTIA, on the recommended use and shelf life of tea tree oil. So that's something we can provide to the Panel.

DR. BERGFELD: Please do.

DR. COHEN: Yeah, I think that would be really helpful. I think other points in the discussion might be the co-reactivity in patch test reports with fragrances like fragrance mixed balsam of Peru, colophony and certain essential. That comes up in the literature.

Perhaps in the clinical studies like the North American Group, the Mayo Clinic, all of these are lagging indicators of the commercial use of the product since they're often in time periods that are several years behind the publication dates. And those are patients using products for a year or two or more behind that. So that's the point of the comment I made about the reduced concentration in commercial products today as opposed to a few years ago. So any other comments?

DR. SHANK: No.

MR. PRATHER: Okay.

DR. BERGFELD: Obviously, this has to come into the discussion, this point of oxidation versus fresh, with the documentation as supplied to us.

DR. SHANK: Right.

DR. COHEN: Right. Yes. Agreed. So, for the extract, we would read across the others, but we need 28-day dermal tox, genotox, DART, skin sensitization and irritation.

DR. SHANK: Correct.

DR. PETERSON: Mm-hmm.

DR. COHEN: So that's an insufficient data.

DR. SHANK: Right.

DR. PETERSON: Yep.

DR. COHEN: Any other comments or points? That was a great discussion. Okay.

Full Panel – December 8, 2020

DR. BELSITO: Okay, so Tea Tree oil, we looked at all of this and we felt that oil, which included the flower, leaf and stem oil, were safe when formulated to be non-sensitizing, using reliable data such as QRA or other methodologies.

And, in the discussion just to point out that -- well, there are a lot of discussion points, we can go through those later -- but the conclusion was safe as used when formulated to be non-sensitizing.

DR. BERGFELD: All right, is there a second? This is Tea Tree oil.

DR. COHEN: Second.

DR. BERGFELD: Any further discussion? Any comments for the discussion?

DR. BELSITO: Yes --

DR. COHEN: So --

DR. BELSITO: We had the botanical boilerplate, the aerosol boilerplate, a penetration enhancement. We had the discussion that the DART and endocrine disruption occurred at very high non-physiologic doses that wouldn't be achieved in cosmetic use. That methodology should be employed to minimize oxidation of Tea Tree oil in the final cosmetic products.

And, Tea Tree oil covers all oil components, (inaudible). And, also, in regard to your comment at the beginning of the meeting, that differences in the composition based upon the area grown, looking at those variations and particularly the materials, there was significant variation and did not give us cause for concern. Therein the conclusion should be formulated to be non-sensitizing.

DR. BERGFELD: Oh, could you just kind of clarify the method and the oxidation statement that you made?

DR. BELSITO: That methodology should be employed to minimize oxidation of Tea Tree oil --

DR. BERGFELD: Minimize.

DR. BELSITO: Minimize oxidation of Tea Tree oil in the final cosmetic product.

DR. BERGFELD: Excellent. Is there a second? David?

DR. COHEN: Second. Second, yeah. So, we were pretty much in line with your team, Don. We came up with insufficient data for the extract. We weren't sure we could read across from the oil to the whole plant extract. And so we were asking for method of manufacturing and constituents. We came to the same conclusion you did about the oil, and we can talk a little bit more about the oxidation.

DR. BELSITO: I'll let Dan discuss that because it was a point of discussion in our group and Dan felt that we could use the oil. Dan, you want to comment?

DR. LIEBLER: Yeah, so, the description of the Tea Tree oil production essentially is a steam illusion of high-temp water soluble components from the whole plant. Which I interpreted as being likely very similar to an aqueous or a hydro-alcoholic extract. So that's the reason I thought that the Tea Tree oil, which is the entire plant, would cover for the extract.

DR. COHEN: So, Lisa, could you comment on that? You're on mute, Lisa.

DR. PETERSON: Thank you for reminding me. You know, I think that the whole extract is clearly from the whole tree. It doesn't include the roots. And, I'm okay with the read-across. I felt like there's such a substantial variation in the plant depending on -- was this the one depending on where it was grown?

DR. BERGFELD: Yes.

DR. PETERSON: This was the one. That you could probably read across. But again I think that there -- I'm trying to remember why we decided in the end insufficient, because we did have a fairly lengthy discussion about this.

DR. COHEN: Well, the question is, are the other extract, the leaf water, or some of the other extracts, going to have the same constituents as the Tea Tree plant oil.

DR. PETERSON: Yeah, I don't -- I think that there're probably components that are in -- I do believe that the whole extract probably represents all of the individual pieces. And so, you know, I'm okay with the whole read-across.

One could argue that you could be removing things that might be protective or revealing things that are -- because this happens with all herbal substances -- -that, you know, you get a fraction of it that has something. But, you know, there's no evidence of real -- but, I think I'm fine with the read-across, complete read-across.

DR. BERGFELD: Okay. So, David, then you're fine with it?

DR. COHEN: Yeah. I think that's okay. Can I ask Ron and Tom if they have any objections to changing it?

DR. SHANK: Yeah. Are you saying that the oil is basically the same thing as the whole plant extract?

DR. LIEBLER: I'm saying that it's so substantially similar, Ron, because essentially it's a steam distillation of the whole plant.

DR. PETERSON: Well, I guess a question is, when it's a steam distillation and then they let the water separate away from the oil. So when it says extract, what are they dealing with, the mixture of the oil and the water, or are they just looking at the oil?

DR. SLAGA: Just oil.

DR. COHEN: What about the aqueous components of the tree? They would not be in the oil, would they?

DR. PETERSON: No.

DR. SLAGA: No. I don't think it can be used as a read-across. I think the oil is different. They're very specific in a way, and the water components are going to have other things in it.

DR. COHEN: Okay, so, we still have some objections to reading across from the oil.

DR. BELSITO: So, Dan, what I hear the Cohen team saying is that the flower, leaf, stem, oil, the leaf oil are fine; but the extract, the leaf stem extract, the leaf, the leaf extract, the leaf powder and the leaf water are insufficient for what? Composition, impurities?

DR. COHEN: Composition, impurities and I suppose methods of manufacturing.

DR. BELSITO: What about --

DR. PETERSON: And then you would want dermal, sensitivity, irritation on it. Because the only --

DR. SLAGA: Right.

DR. PETERSON: -- thing you have is on the oil.

DR. COHEN: Yes. Agreed.

DR. BELSITO: Unless the composition is similar.

DR. SHANK: And have that on the whole plant extract.

DR. BERGFELD: Are you going to need any genotox?

DR. SLAGA: Yeah, genotox too.

DR. BERGFELD: DART?

DR. SHANK: Depends on what you see in the dermal.

DR. BERGFELD: Okay.

DR. LIEBLER: I see your point about the difference between the extract and the Tea Tree oil as defined by the process described to us. And I can guarantee we'll be having this discussion again next time.

DR. BERGFELD: Okay, are you agreeing, though, to pull these out, the extracts, and ask for more data?

DR. LIEBLER: Sure.

DR. BERGFELD: Okay, Don.

DR. PETERSON: No, I think if they --

DR. BERGFELD: I just want to ask Don, then Lisa. Don, how are you standing on this?

DR. BELSITO: Dan is my expert here, Wilma; this is not my area of expertise.

DR. BERGFELD: Okay. Lisa, did you have something to say?

DR. PETERSON: Well, I think if they can clarify, you know, what really is the extract? What are they talking about. Is it oil and water? Or is it just oil, then --

DR. SLAGA: It's everything.

DR. PETERSON: You know, I just think more information would be really helpful.

DR. LIEBLER: Right. So, that's okay. I was essentially doing an extension of the Tea Tree oil -- so I hesitate to use the term read across unless it's a specific chemical to chemical. But, I was doing an extension of the description of the Tea Tree oil prep, which is a steam distillation. I was extending that to similarity to the result of an extract. I get the differences between some extract and the steam distillation process. So we can ask for it. If we get it, wonderful, and if we don't get it we'll have this conversation again.

DR. BERGFELD: So, we're going to go out as an IDA. Is that agreeable? Since, Don, this is your ingredient, are you going to rescind your motion?

DR. BELSITO: So, the oil is safe as used when formulated to be nonirritating, with all the discussion points that I have raised. Everything but the oil we need manufacturing, composition, impurities. If sufficiently different, sensitization, irritation and possibly other tox endpoints.

DR. BERGFELD: Think that's correct.

DR. COHEN: Don?

DR. BERGFELD: And I think we heard genotox from Tom Slaga.

DR. SLAGA: Yeah.

DR. BELSITO: Well, I mean, if sufficiently difference other tox endpoints.

DR. BERGFELD: Okay, other tox endpoints. Okay. David? David Cohen?

DR. COHEN: Don, I think in your initial motion, did you say formulate to not be sensitizing. And in your current motion you said formulate to not be irritating?

DR. BELSITO: No, sensitizing, I'm sorry.

DR. COHEN: Okay.

DR. BERGFELD: Okay. Thank you, for catching that David. All right, any other discussion? Any other points?

DR. SHANK: Yeah, why is the oxidized oil listed as an ingredient when it's not? Why is it in the list of tea tree-derived ingredients?

DR. BERGFELD: Dan, or Lisa?

DR. BELSITO: Where do you see oxidized oil?

DR. BERGFELD: Oh, they have a lot in discussion.

DR. SHANK: It's in the list of ingredients.

DR. PETERSON: It is in -- yeah, I think that needs to be address by --

DR. LIEBLER: What page are you referring to?

DR. BELSITO: The list of ingredient is leaf stem oil, leaf, leaf extract, leaf oil. There's no oxidized oil.

DR. PETERSON: I think he's talking about this summary document on PDF Page 4 or 5.

DR. SHANK: Right.

DR. BELSITO: The summary document on PDF 4?

DR. PETERSON: Page 6. Page 6.

DR. BELSITO: Page 6 is before the introduction.

DR. PETERSON: Right, that's what he's asking about though.

DR. BELSITO: Well, because it's the oxidized as with many of these plant-derived products, such as limonene and linoleoyl, the actual non-oxidized material is not particularly sensitizing. It's the oxidation products which are sensitizing. So that's where the whole discussion of oxidation and controlling the oxidized product in a final commercial product comes in. Oxidized tea tree oil is not a cosmetic ingredient; I can assure you.

DR. SHANK: Right.

DR. BELSITO: It's a patch testing ingredient. We use it to patch test, because of concerns that the material could be oxidized during the course of consumer use. But it's not a cosmetic ingredient.

DR. BERGFELD: If you look at the list in the introduction of all the ingredients, which are, I guess, nine, the oxidized is not in that group.

DR. BELSITO: Right.

DR. SHANK: Okay.

DR. BELSITO: It's not an ingredient.

DR. BERGFELD: Yeah. Okay.

DR. SHANK: Okay.

DR. COHEN: And, so and, Don --

DR. SHANK: In that table it's just there as a --

DR. BERGFELD: Complimentary

DR. SHANK: -- source of info-- -- for our information?

DR. BELSITO: It's for our information to determine sensitization, Ron, because the sensitizer in Tea Tree oil is probably the oxidizing product.

DR. SHANK: Yeah, okay, I get that. But we haven't considered rancid oils. We've done a lot of oils; we've never considered their oxidized forms as a consideration for safety.

DR. BELSITO: Well --

DR. LIEBLER: I think it was their -- oh, go ahead. Monice is going to --

MS. FIUME: So, Ron, it was added --

DR. SHANK: Never mind.

MS. FIUME: Well, I was going to say it was added based on some comments that we received that in most of the multicenter studies, the NACDG group uses the oxidized oil in the patch testing rather than the unoxidized. So, it was requested that we make it clear to the Panel, that a lot of those results that are seen are with the oxidized oil and not the non-oxidized.

DR. SHANK: Okay, that wasn't clear. Thank you.

DR. BERGFELD: I think that can be clarified in the discussion as well. And I think that was one of Don's lists of needs.

DR. BELSITO: Right.

DR. SHANK: Okay. Thank you.

DR. BERGFELD: Well, not needs, but descriptors, it should be oxidized.

DR. COHEN: Well, thank you.

DR. BERGFELD: David?

DR. COHEN: So, yeah, the oxidation conversation was pretty extensive yesterday. And there's a big difference between what we're patch testing to to increase our level of detection of allergic people, which is why we use oxidized limonene and linoleoyl tea tree. And it's different from this.

I appreciate everything that you put forward, Don. One additional question is, do we need, or is it in our purview, to understand the cadence of that oxidation? So, of course, in the bottle it matters, and a representative from the Australian Tea Tree Oil Society joined our call yesterday. And I think there was a comment about the product is unoxidized for about three years in an opaque bottle. But if it's sprayed on or applied, or washed on and off, is there rapid oxidation that changes the sensitization or the response to it?

DR. BERGFELD: So, typically, David, at least in fragrance materials, an antioxidant would be added to the finished product to (audio skip). So that's my point in the discussion.

DR. COHEN: Okay.

DR. BELSITO: That final formulation should be (audio skip) final product should be formulated to minimize oxidation under conditions of use.

DR. BERGFELD: Did you want to put that in the conclusion, or did you put that in the --

DR. BELSITO: No, it's part of the discussion.

DR. SHANK: This -- yeah.

DR. BERGFELD: Yeah. Okay.

DR. COHEN: Okay. I misunderstood; I thought you meant just in the container.

DR. BELSITO: No.

DR. COHEN: But you're talking about in use.

DR. BELSITO: Right.

DR. BERGFELD: All right. I don't see anyone's hand up. Any other discussion?

DR. BELSITO: Yes, so, Wilma, at the beginning of our meeting yesterday, you asked about the abbreviations occurring up front and what we thought about it.

DR. BERGFELD: Yes. Thank you.

DR. BELSITO: Our panel discussed it. I think in general we liked it. Curt, in particular, would also like the abbreviations in the report when it's first used.

DR. BERGFELD: Dr. Cohen, you want to comment on your team, hint?

DR. COHEN: I didn't hear the last part of it.

DR. BELSITO: So, basically, I think overall our team liked having the list of abbreviations right up front. So if you somehow missed it you could go back and look. But that Curt also felt that the abbreviation should be introduced when it's first used in the body of the reports. So, a combination of both.

DR. COHEN: Yeah, I think that's a good idea.

DR. BERGFELD: Okay, any other discussion regarding the abbreviations? Hearing none, well, let's go forward then with Dr. -- is that someone wanting to speak? Monice?

MS. FIUME: Yes, I don't know if Don's going to address this, I just have one more point that came up yesterday in our discussion. I think, Don, you wanted the opinion of the Panel was the sentence about the components in the biological activity of the components that's included in the introduction. I was under the impression that you wanted the full panel's discussion on that, and consensus on using it, in the third paragraph of the discussion -- I mean, of the introduction, I'm sorry.

DR. BERGFELD: Oh, yeah. So, I actually thought that that was a good boilerplate. Thank you, Monice. So, if you look at the introduction -- is this what you're talking about, the naturally occurring combinations?

DR. SNYDER: Don, it's the last sentence that I put an edit in here for that last paragraph, about the naturally occurring combinations. And, so I have some wording in here that I would like to have highlighted in the next iteration of this, out to the Panel for their input as to the new language, making it a little more clear.

DR. BERGFELD: Could you read it?

DR. SNYDER: Sure.

DR. BELSITO: This is in the introduction.

DR. BERGFELD: Yep.

DR. SNYDER: PDF Page 11, under -- sentence that begins with the name of the ingredient "contains over 100 constituents..." The last sentence regarding the "Naturally-occurring combinations..." We discussed this and decided to shorten it to, "potential toxicity from exposure to mixtures of different chemical compounds may not replicate the biological activity of the individual components." So just a little bit more clearly state that and kind of flip that around.

DR. LIEBLER: yeah.

DR. BERGFELD: Okay.

DR. BELSITO: And we like that statement for all these natural complex substances/(audio skip) boilerplate.

DR. BERGFELD: And you wanted to put that, also, into the discussion routinely? Because we always talk about the complexity of these ingredients?

DR. BELSITO: Yeah, it could go back in the discussion as well.

DR. BERGFELD: Yeah, I think it's good to put it in the discussion as well. All right, is it time to call the question on this particular ingredient? Dr. Belsito, please restate where we stand on this.

DR. BELSITO: So, I think what we're saying is that all of the oil ingredients are safe as used when formulated to be non-sensitizing. For non-oil ingredients we need method of manufacture, composition, impurities. If composition and impurities is significantly different, then we would need sensitization and irritation at concentration of use, and other toxicologic endpoints.

DR. BERGFELD: Now, it's my understanding is it would go -- Bart, you'll have to -- we have a safe conclusion on part of it and a split, unsafe, or a data needed, on other. Is this going out as an IDA or is it going out as a tentative final with an IDA?

DR. HELDRETH: Since this is a draft report, the first time the Panel seen this, it means if a request were to be issued as an insufficient data announcement, then the conclusion of safety for the other ingredients would be held in abeyance until the Panel receives the draft tentative report.

DR. BERGFELD: Good. All right. All those in favor of this conclusion -- excuse me -- opposite. All those that are not in favor, oppose this conclusion, please indicate by stating your name. Hearing none, unanimously approval.

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

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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.

1620 L Street, NW, Suite 1200 ♢ Washington, DC 20036-4702 ♢ ph 202.331.0651 ♢ fax 202.331.0088 ♢
cirinfo@cir-safety.org

ABBREVIATIONS

ACC	allergic contact cheilitis
ACD	atopic contact dermatitis
ADR	adriamicin-resistant
aq	aqueous
AR	androgen receptor
BCOP	bovine corneal opacity and permeability
C1orf116	chromosome 1 open reading frame 116
CAP	compound auditory nerve action potential
CGC	capillary gas chromatography
CIR	Cosmetic Ingredient Review
COLIPA	European Cosmetic Toiletry and Perfumery Association
Council	Personal Care Products Council
CTSD	cathepsin D
CYP4F8	cytochrome P450 family 4 subfamily F member 8
DHT	dihydrotestosterone
Dictionary	*International Cosmetic Ingredient Dictionary and Handbook*
DKG	German Contact Dermatitis Research Group
DMSO	dimethyl sulfoxide
E2	17β-estradiol
EC	European Commission
EC3	estimated concentration of a substance expected to produce a stimulation index of 3
ECHA	European Chemicals Agency
EMA	European Medicines Agency
ERα	estrogen receptor-α
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
FID	flame-ionization detection
GC	gas chromatography
GRAS	generally recognized as safe
GREB1	growth regulation by estrogen in breast cancer 1
GSD	geometric standard deviation
HaCaT	normal human keratinocytes
HET-CAM	hen's egg test on the chorioallantoic membrane
HMPC	Committee on Herbal Medicinal Products
HPLC	high-performance liquid chromatography
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
Ig	immunoglobulin
IGFBP3	insulin like growth factor binding protein 3
ISO	International Organization for Standardization
K_p	permeability coefficient
LBD	ligand-binding domain
LC	liquid chromatography
LLNA	local lymph node assay
MMAD	mass median aerodynamic diameter
MMTV	mouse mammary-tumor virus
MOS	margin of safety
MPO	myeloperoxidase
mRNA	messenger RNA
MS	mass spectrometry
MTT	3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide
MYC	a proto-oncogene
NACDG	North American Contract Dermatitis Group
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
RPE	relative proliferative effect
RPMI	Roswell Park Memorial Institute
SCCNFP	Scientific Committee on Cosmetic Products and Non-Food Products
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
SIDAPA	Italian Society of Allergological, Occupational and Environmental Dermatology
SLS	sodium lauryl sulfate
SPF	specific pathogen-free
SPIN	Significance-Prevalence Index Number
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
Vis	visible
WHO	World Health Organization
WT	wild-type

DRAFT ABSTRACT

The Expert Panel for Cosmetic Ingredient Safety (Panel) assessed the safety of 8 *Melaleuca alternifolia* (tea tree)-derived ingredients as used in cosmetic formulations; 5 of these ingredients are reported to function in cosmetics as skin-conditioning agents. Much of the data in the report were on tea tree oil; the Panel deemed these data relevant to the review of the oil ingredients. Because final product formulations may contain multiple botanicals, each containing the same constituents of concern, formulators are advised to be aware of these constituents and to avoid reaching levels that may be hazardous to consumers. Industry should use good manufacturing practices to minimize impurities that could be present in botanical ingredients. The Panel noted that oxidized tea tree oil could be a sensitizer, and stated that industry should employ methods to minimize oxidation of the oil in the final cosmetic product. The Panel considered all the data and concluded that [TBD].

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 *International Cosmetic Ingredient Dictionary and Handbook* (*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 United States (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; potential toxicity from exposures to mixtures of different chemical compounds may not replicate the biological activity of the individual components.

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 Cosmetic Ingredient Review (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 European Commission (EC) Scientific Committee on Consumer Products (SCCP),[6] European Chemicals Agency (ECHA),[7] and European Medicines Agency (EMA)[3,8,9]). 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.[10] *Melaleuca alternifolia* occurs in riparian zones of freshwater and swamps. It is a commercially-grown plant that is indigenous to Australia,[11] and plants with the genetic make-up necessary to produce the oil are native to northern New South Wales.[12] However, *Melaleuca alternifolia* has been introduced and cultivated in China, Indonesia, Kenya, Madagascar, Malaysia, South Africa, Tanzania, Thailand, the US, and Zimbabwe.[13,14]

Melaleuca alternifolia is a tall shrub or small tree that typically grows up to 7 m high, with a bushy crown and papery bark.[15] The total biomass (above-ground growth) of the tea tree can be subdivided into three components: leaves, fines stems, and main stems.[16] 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.[15] 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.[16] 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.[15] 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;[17] Melaleuca Alternifolia (Tea Tree) Leaf Extract is described as non-volatile.[18] The log P_{ow} of Melaleuca Alternifolia (Tea Tree) Leaf Oil is 3.4 – 5.5.[19] Available properties data for Melaleuca Alternifolia (Tea Tree) Oil, tea tree oil, and Melaleuca Alternifolia (Tea Tree) Leaf Extract are provided in Table 2.

Stability

Tea Tree Oil

Because of the possibility for degradation, a supplier of tea tree oil recommends that the use-by date for tea tree oil sold in commercially-available, small (up to 100 ml), dark, glass bottles stored at ambient temperature be set at 12 mo from when first opened, or 24 mo in unopened bottles.[20] They also recommend that, wherever possible, tea tree oil should be stored at or below 25°C. The supplier also stated that when stored correctly, tea tree oil can retain its quality for periods of up to 10 yr.

In a 3-mo trial examining stability in accelerated (40°C) and real-time shelf conditions, including exposure to fluorescent light, no discernible difference was demonstrated in the tea tree oil quality based on constituent values in either amber or clear glass bottles.[20] In a 12-mo study designed to replicate normal consumer use conditions, there was no appreciable oxidation or degradation of tea tree oil.[12,21] No significant change in the level of terpinen-4-ol was reported. A downward trend in α-terpinene and γ-terpinene, and an upward trend in *p*-cymene, were observed, and 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.

A supplier also provided some data on the stability of tea tree oil in formulated products, using solvent extraction and gas chromatography/flame ionization detection(GC/FID).[22] The rates of degradation of the oil varied with the medium. Degradation in a cream was faster than seen in a gel or a solution. For the tea tree cream, solution, and gel, the constituents were extremely stable over a period of 1.5, 3, and 5 yr, respectively.

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 Extract

A supplier submitted information describing production of a concentrate; details were not provided regarding raw material or solvents, however, the data were provided for Melaleuca Alternifolia (Tea Tree) Leaf Extract.[23] The supplier indicated that raw material is packed into the extraction system and sealed, liquid extractant is added to the vessel, which is then closed and sealed, and the raw material is extracted under pressure in the closed system. The resulting extract is reported to be a pure extract of the raw material used (e.g., plant, bark, fruit).

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 International Organization for Standardization (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.;[24] steam distillation is required to conform to ISO standards.[25] 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.[12] 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).

According to a supplier of Australian tea tree oil, *Melaleuca alternifolia* tea trees are harvested and mulched into biomass, from which the oil is extracted using low-temperature pressurized steam distillation.[26] Oil from glands in the leaves is vaporized with the steam, and the steam is then condensed with cold water. The oil is separated out, and cooled for 16 h. Following cooling, the oil is filtered to remove any organic debris, sampled for quality assurance, and then bottled.

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.[27] 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.[25] 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%.[28] 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.[29] 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.[25] 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.[29]

Composition/Impurities

Melaleuca Alternifolia (Tea Tree) Leaf Extract

According to one supplier, Melaleuca Alternifolia (Tea Tree) Leaf Extract is a cellular extraction of the *Melaleuca alternifolia* leaf and is composed of 20 – 50% *Melaleuca alternifolia* leaf, 34 – 55% glycerin, and 14 – 24% water, and it is preserved with ≤ 0.5% sodium benzoate, ≤ 0.4% citric acid, and ≤ 0.3% potassium sorbate.[18] SCCNFP allergens listed in Annex III of the European Union (EU) Cosmetics Directive (2003/15/EC) were not detected in the extract (limit of detection, 0.001%). Additionally, according to certificates of analysis provided by another source, specifications for Melaleuca Alternifolia (Tea Tree) Leaf Extract (≥ 0.001% leave-on and ≥ 0.01 % w/w rinse-off) indicate that none of the 26 potential fragrance allergens, which according to the EC Directive are required to be listed on the label, were detected (limit of detection of 0.001%).[30] High-performance liquid chromatography (HPLC) - mass spectrometry (MS) of a test sample of Melaleuca Alternifolia (Tea Tree) Leaf Extract identified a range of phenolic and flavonoid derivatives, based on available ultraviolet (UV)-visible (Vis) and MS spectra.[31]

Information was also provided for a cellular extraction composed of < 98% *Vitis vinifera* (grape) seed oil, < 1.0 – 5.0% Melaleuca Alternifolia (Tea Tree) Leaf Extract, and < 0.5% mixed tocopherols (low α-type).[32] SCCNFP allergens listed in Annex III of the EU Cosmetics Directive (2003/15/EC) were not detected in this mixture (limit of detection, 0.001%). Additionally, according to certificates of analysis provided by another source, specifications for the mixture (≥ 0.001 leave-on and ≥ 0.01 % w/w rinse-off) indicate that none of the 26 potential fragrance allergens, which according to the EC Directive are required to be listed on the label, were detected (limit of detection of 0.001%).[33] Fatty acid analysis via GC/FID indicated fatty acid content of the mixture ranged from 0.003% magaric acid to 68.11% linoleic acid.[34]

Melaleuca Alternifolia (Tea Tree) Leaf Oil

Methyleugenol is reported as a minor constituent of Melaleuca Alternifolia (Tea Tree) Leaf Oil.[6] 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.[35] Longer distillation times can result in slightly higher amounts; however, amounts did not exceed 0.07% for exhaustive laboratory distillations. According to the European Commission, based on the Scientific Committee on Cosmetic Products and Non-Food Products (SCCNFP) opinion 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.[36] 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]

Tea Tree Oil

There are several varieties, or chemotypes, of *Melaleuca alternifolia*, and each produces oil with a distinct chemical composition.[37] (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.[38]) 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).[25] The terpinen-4-ol chemotype is typically used in commercial tea tree oil production.

Tea tree oil typically contains approximately 100 constituents;[39] 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,[39] 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%).[24] 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).[6,40,41] 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.[37] 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.[42] 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.[43]

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%.[44] The commercial standard for the composition of tea tree oil that conforms to ISO 4730:2017 is identified in Table 4.[24] World Health Organization (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.[45]) 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.[8,14]

Constituent profiles of tea tree oil from several sources are presented in Table 5.[11,27,39,46-48] 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 the natural content of the individual constituents of tea tree oil.[4,6,16,39,49] Incomplete distillation results in enhanced terpinen-4-ol levels and lower levels of sesquiterpenoids. The composition of tea tree oil 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.[50] Using liquid chromatography(LC)/UV and LC/MC/MC spectrometry 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.[39]

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.[6,39] 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.[22]

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%.[3,6,39] The composition of tea tree oil at various stages of oxidation is presented in Table 9.[51]

Oxidation processes also lead to the formation of peroxides, endoperoxides, and epoxides.[6,39] As tea tree oil undergoes oxidation, peroxide values increase from zero to "unacceptable" levels in the early stages of oxidative degradation.[22] 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.[6]

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.[52] A certificate of analysis states that the presence of these heavy metals was < 1.0 ppm.[53] Heavy metal impurities are expected to be low because steam distillation does not concentrate these impurities.[54]

The recommended maximum pesticides residue limits for aldrin and dieldrin in tea tree oil, according to the WHO, are not more than (NMT) 0.05 mg/kg.[11] Possible adulterants of tea tree oil include camphor, eucalyptus, cajuput, broadleaf paperbark, Masson pine, maritime pine, and Chir pine.[13] 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.[55]

USE

Cosmetic

The safety of the cosmetic ingredients addressed in this assessment is evaluated based on data received from the US Food and Drug Administration (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 Voluntary Cosmetic Registration Program (VCRP) database. Use concentration data are submitted by the cosmetic industry in response to a survey, conducted by the Personal Care Products Council (Council), of maximum reported use concentrations by product category.

Collectively, the frequency and concentration of use data indicate that 6 of the 8 ingredients included in this safety assessment are used in cosmetic formulations; however, although all 6 in-use ingredients are listed by the VCRP in 2021,[56] concentration of use data collected in 2019 only reported use for 3 ingredients.[57] According to 2021 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 536 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 and Melaleuca Alternifolia (Tea Tree) Leaf Powder are not reported to be in use.

Melaleuca Alternifolia (Tea Tree) Leaf and Melaleuca Alternifolia (Tea Tree) Leaf Oil are reported to be used in products applied near the eye (concentration of use not reported), and Melaleuca Alternifolia (Tea Tree) Flower/Leaf/Stem Extract and Melaleuca Alternifolia (Tea Tree) Leaf Oil in products that can result in incidental ingestion (e.g., at up to 0.02% of the oil 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 is reported to be used in baby products; concentration of use data were not reported for this category.

Additionally, some of the *Melaleuca alternifolia* (tea tree)-derived ingredients are used in cosmetic sprays and powders 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,[57] and 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.[56] 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.[58,59] 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.[60,61] 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.[60] 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. 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.[62-64]

In 2002, the European Cosmetic Toiletry and Perfumery Association (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.[6] When formulating Tea Tree Oil in a cosmetic product, companies should consider that the sensitisation potential increases if certain constituents of the oil become oxidised. To reduce the formation of these oxidation products, manufacturers should consider the use of antioxidants and/or specific packaging to minimise exposure to light."

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.[65] 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.[40] 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).[12] Use in mouthwash at a typical concentration of 0.2% is also indicated.

Non-Cosmetic

Tea tree oil is listed as a generally recognized as safe (GRAS) flavoring substance by Flavor and Extract Manufacturer's Association (FEMA).[66,67]

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.[28,68] 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;[8] the Committee on Herbal Medicinal Products (HMPC) concluded that, on the basis of its long-standing use, tea tree oil preparations can be used for these uses.[3,9]

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.[11] 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.[4,12]

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 over-the-counter (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.[69] Drug products containing these ineligible active ingredients will require approval under a new drug application or abbreviated new drug application 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.[54] 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.[70] 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.[22]

In Vitro

The dermal penetration potential of tea tree oil was estimated in numerous in vitro studies (using both pig ear skin[71,72] and human skin[41,73-76]), 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.[77] 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.[71] All markers were retained to some extent by the whole skin.

It was also demonstrated that the formulation vehicle affects absorption.[72] 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.[76] 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.[71] 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.[72] 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%.[41] 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.[78]

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.[79] 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.[80] 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 permeability coefficient (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 phosphate-buffered saline (PBS) was placed in the receptor chamber.[81] 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 polyethylene glycol (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).[80] 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.[7] Oral, dermal, and inhalation absorption was reported as 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.[82] In another study, tea tree oil had a dermal LD_{50} > 2 g/kg in rabbits.[6,7] Dermal applications of "very high concentrations" of tea tree oil have been reported to cause tea tree oil toxicosis in dogs and cats.[83,84]

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.[6] 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.[82] For tea tree oil, the LD_{50} was > 2 g/kg (in PEG 400) in female mice[7] and calculated as 2.3 g/kg bw and ~1.7 g/kg bw (in peanut oil) in specific pathogen-free (SPF) and non-SPF Sprague-Dawley rats, respectively.[7]

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.[7] 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.[6]

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.[27] (Additional details, including whether or not collars were used or if the test site was covered, were not provided.) Serum glutamine-oxaloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (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 Organisation for Economic Co-operation and Development (OECD) test guideline (TG) 407.[7] 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 no-observable-adverse-effect-level (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.[7] 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.[85] 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,[7,86,87] in chromosomal assays using Chinese hamster lung fibroblasts (V79) cells (≤ 58.6 µg/ml)[7] or human lymphocytes (≤ 365µg/ml),[88] 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),[7] or in a Comet assay using normal human keratinocytes (HaCaT) cells(≤ 0.064%).[89] 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.[6] 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,[90] it impaired the growth of human M14 melanoma cells,[91] and it induced apoptosis in human malignant melanoma (A-375) and squamous cell carcinoma (Hep-2) cells.[92] 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.[93] 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.[94] 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).[95] The IC_{50} in several other cancer cell lines ranged from 12.5 µg/ml (24 h) in human HT29 colon cancer cells,[96] to 2800 µg/ml (4 h) in epithelioid carcinomic (HeLa), hepatocellular carcinomic (Hep G2), and human chronic myelogenous leukemia (K-562) cells.[97] In immunocompetent C57BL/6 mice, tea tree oil inhibited the growth of subcutaneous tumors; effectiveness was carrier-dependent.[98] 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 estrogen receptor-α (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 estrogen response element (ERE)-dependent luciferase activity was stimulated in a dose-dependent manner (maximum activity observed at 0.025%).[99,100] 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 17β-estradiol (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 relative proliferative effect (RPE) by 10%.[78] 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.[101] 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).[99] 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 ligand-binding domain (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 dihydrotestosterone (DHT) on androgenic activity was evaluated in MDA-kb2 breast cancer cells transfected with an androgen- and glucocorticoid-inducible mouse mammary-tumor virus (MMTV)-luciferase reporter plasmid.[100] 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 androgen receptor (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%.[99] 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.[6] 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,[100] 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.[102] 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 compound auditory nerve action potential (CAP) to tone bursts before and after instillation of the oil into the middle ear.[103] 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 tumor necrosis factor-α-induced adherence reaction of human peripheral neutrophils.[104] Tea tree oil was diluted to concentrations of 0.025 – 0.2% using dimethyl sulfoxide (DMSO) and Roswell Park Memorial Institute (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% 2,4,6-trinitrochlorobenzene (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. [105] 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 mid-wavelength irradiation (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 a minimal erythema dose 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 myeloperoxidase (MPO) activity was examined using groups of 3 - 4 ICR mice.[106] 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 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.[107] (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.[107] 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 hypothalamic-pituitary-adrenal axis mediated the anti-inflammatory effect of tea tree oil administered to the same strain of mice.[108]

Human

Dermal

The effect of tea tree oil on a histamine-induced wheal and flare reaction was examined.[109] 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.[110] 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 peripheral blood mononuclear cells (PBMC); toxicity ranged from 9% (not significant), with 0.004% tea tree oil, to 69% (significant), with 0.016% tea tree oil.[111] In an 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide (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,[112] and after a single 24-h occlusive application[82,113] 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.[27] In rabbits, concentrations of up to 75% were, at most, slightly irritating;[6] with undiluted tea tree oil, a 4-h semi-occlusive application[114] and application for 72 h to intact and abraded skin produced severe irritation.[6,7] In 22 human subjects, a 48-h occlusive patch with 1% Melaleuca Alternifolia (Tea Tree) Leaf Oil in petrolatum (pet) produced no irritation.[113,115] 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).[16] Two dermal irritation studies were performed with 25% tea tree oil; in one study, no irritation was reported (details were not provided).[16] 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.[116-118] 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%.[6,12]

In the local lymph node assay (LLNA), tea tree oil was predicted to be a weak or moderate sensitizer at a concentration up to 50%,[3,6,7] and a moderate sensitizer when tested undiluted.[6,7] In guinea pig studies, tea tree oil was not sensitizing (30% at challenge)[3,7] or had a low sensitizing capacity (tested "pure");[119] 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.[3,120] 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.[119] In clinical studies, Melaleuca Alternifolia (Tea Tree) Leaf Oil at 1% in pet (22 subjects; maximization test)[113,115] and 10% in caprylic/ capric triglycerides (102 subjects; modified human repeated insult patch test (HRIPT)),[121] 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.[122] 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.[116-118]

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.[113,123] Thirty min after application, the skin was treated with a combination of psoralen and long-wave ultraviolet radiation 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.[11] Tea tree oil can cross react with colophony.[40]

OCULAR IRRITATION

In Vitro

Tea Tree Oil

In a hen's egg test on the chorioallantoic membrane (HET-CAM) assay, 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.[6] 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 bovine corneal opacity and permeability (BCOP) test was performed in accordance with OECD TG 437 to evaluate the irritation potential of undiluted tea tree oil.[7] 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.[6] 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).[6] 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 New Zealand white (NZW) rabbits.[7] 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 North American Contract Dermatitis Group (NACDG) screening series since 2003.[124] 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.[125] 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%).[126] 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).[124,127-131] 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.[132] 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 markers fragrance mix and *Myroxilon pereirae*.[133] 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.[134] Testing at the Northwestern Medicine patch-testing clinic found no difference in positive results between patients with or without atopic dermatitis.[135]

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.[136] 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%).[137] Three of 60 patients (5%) with lip allergic contact cheilitis (ACC) (2001 - 2004) had positive reactions to oxidized tea tree oil.[138] 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.[139] 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).[140] 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.[141]

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;[142] in June – August 2003, 5/160 subjects had irritant reactions to lotions containing 5% tea tree oil.[142] In Sweden (prior to 2004), 2.7% of 1075 patients tested had a positive reaction to 5% tea tree oil in alcohol.[143] 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),[4,6,144] and testing at 5% (oxidized) in pet (1998 - 2003) produced positive results in 0.9%-1.0% of the patients tested.[145] 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.[146] 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.[147] 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.[148,149] 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.[150] 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,[6,151] and in Spain (prior to 2015), 0.4% of patients had positive reactions to testing with 5% tea tree oil in pet.[152] In the United Kingdom (UK) (1996 - 1997), 7 of 29 patients thought to have a cosmetic dermatitis had positive patch test reactions to tea tree oil, applied neat,[153] 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,[154,155] 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.[125]

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);[156] 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.[157] Additionally, positive reaction rates of up to 4.8% have been reported with 10% tea tree oil.[156]

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.[3,12] 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 vulvar pruritus were patch-tested with a battery of allergens, including tea tree oil (undiluted) and common OTC topical vulvar treatments.[158] 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 there was a possibility of an allergic group reaction caused by contamination of the colophony with the volatile fractions of turpentine.[6,151] 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.[144] However, in another study, no correlation was reported between positive reactions to tea tree oil and to colophony.[143] 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.[159] In several case reports of reactions to tea tree oil (described later in this report), reactions were also noted with eucalyptol,[49] colophony,[160,161] and ascaridole.[162]

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,[49,119,120,152,162-164] other direct skin applications,[119,160-162,165-173] and from use of hand wash or shampoos[119,174,175] 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.[176] Positive reactions were also reported in a patient with hand eczema following inhalation of tea tree oil vapors.[177]

Oral ingestion can be poisonous; serious symptoms, such as confusion and ataxia, can occur.[68] 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.[178] In Australia, a retrospective study of essential oil exposure was conducted by analyzing calls to the New South Wales Poisons Information Centre (NSWPIC) during July 2014 – June 2018; NSWPIC takes about half of all calls to poisons information centers in Australia.[179] 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 a margin of safety (MOS) had not been calculated, and the safety of tea tree oil could not be assessed.[6] 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).[6] 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. Systemic exposure dose (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.[40] 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 2021 US FDA VCRP data and Council survey results, 6 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 536 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 of tea tree oil was reported to be 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 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, concentrations of up to 75% were, at most, slightly irritating; with undiluted tea tree oil, a 4-h semi-occlusive application and application for 72 h to intact and abraded skin produced severe irritation. 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.[143] 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.

DRAFT DISCUSSION

[**Note**: This Discussion is in draft form, and changes may be made following the Panel meeting.]

This assessment reviews the safety of 8 *Melaleuca alternifolia* (tea tree)-derived ingredients as used in cosmetic formulations. The majority of the data included in the report are on tea tree oil. Although this name is not an International Nomenclature Cosmetic Ingredient (INCI) name, the Panel considered these data relevant for evaluating the safety of the oil ingredient named in this report, i.e., Melaleuca Alternifolia (Tea Tree) Flower/Leaf/Stem Oil and Melaleuca Alternifolia (Tea Tree) Leaf Oil)

The Panel noted that oxidized tea tree oil has the potential to be a sensitizer, and stated that methods should be employed to minimize oxidation of the oil in the final cosmetic formulation. For example, to reduce the formation of oxidation products, manufacturers should consider the use of antioxidants, as well as specific packaging to minimize exposure to light.

Also, because final product formulations may contain multiple botanicals, each possibly containing the same constituents of concern, formulators are advised to be aware of these constituents and to avoid reaching levels that may be hazardous to consumers. For *Melaleuca alternifolia* (tea tree)-derived ingredients, an example of the constituents the Panel was concerned about included 1,8-cineole (also known as eucalyptol), a possible allergen, and terpinolene, α-terpinene, α-phellandrene, and limonene, possible sensitizers. Additionally, the Panel was aware that variances in the composition of tea tree oil based on a geographical or geological difference in growth have been reported, which could also affect the potential for sensitization. Therefore, when formulating products, manufacturers should avoid reaching levels of plant constituents that may cause sensitization or other adverse health effects.

The Panel expressed concern about pesticide residues, heavy metals, and other plant species that may be present in botanical ingredients. They stressed that the cosmetics industry should continue to use current good manufacturing practices (cGMPs) to limit impurities.

Adverse effects that were reported in developmental and reproductive toxicity studies, as well as in studies examining effects on endocrine activity, were noted by the Panel. Because the adverse results were observed at concentrations that were much higher than those used in cosmetic formulations concern, concern for these effects with use in cosmetics was mitigated.

The Panel recognized that tea tree oil can enhance the penetration of other ingredients through the skin. The Panel cautioned that care should be taken in formulating cosmetic products that may contain these ingredients in combination with any ingredients whose safety was based on their lack of dermal absorption data, or when dermal absorption was a concern.

Finally, some of the *Melaleuca alternifolia* (tea tree)-derived ingredients are used in cosmetic sprays or powders, and could possibly be incidentally inhaled during customary use. Therefore, the Panel discussed the issue of potential inhalation toxicity. Little inhalation toxicity data (i.e., acute studies rats) were available. However, the Panel noted that in aerosol products, 95% – 99% of droplets/particles would not be respirable to any appreciable amount. Furthermore, droplets/ particles deposited in the nasopharyngeal or bronchial regions of the respiratory tract present no toxicological concerns based on the chemical and biological properties of these ingredients. Coupled with the small actual exposure in the breathing zone and the concentrations at which the ingredient is used, the available information indicates that incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects. A detailed discussion and summary of the Panel's approach to evaluating incidental inhalation exposures to ingredients in cosmetic products is available at https://www.cir-safety.org/cir-findings.

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	19
solubility		
in water (mg/l at 25°)	insoluble in water	19
	332.1 (estimated)	180
other	1 part miscible with 2 parts ethanol (85% v/v) at 20°C	19
	soluble in alcohol, fixed oil, paraffin oil; insoluble in glycerin	180
	miscible in non-polar solvents	37
freezing point (°C)	-22	19
boiling point (°C)	97 - 220	19
relative density	0.885 – 0.906	19
refractive index (at 20°)	1.475 – 1.482	180
optical rotation	+7° to +12°	19
	+5º to + 15º	180
log P_{ow}	3.4 – 5.5	19
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	24
	colorless to pale yellow liquid, with a myristic odor	11
	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	7
solubility	insoluble in water; soluble in 2 volumes of 85% ethanol (20ºC)	6
	sparingly soluble in water; miscible with non-polar solvents	
freezing point (°C)	-22	7
boiling point (°C)	97 - 220	7
relative density (at 20ºC)	0.885-0.906	24
	0.89	7
refractive index	1.475 - 1.482	6
	1.465 - 1.495	53
vapor pressure (Pa at 25°C)	2100	6
optical rotation	+ 7° to + 12°	24
log P_{ow} of constituents	2.82 – 6.64	6
$\log_{10} P_{ow}$ of constituents	3.4 - 5.5	7
α-terpineol	3.4	
terpinen-4-ol	3.5	
α-terpinene	5.2	
γ-terpinene	5.3	
	Melaleuca Alternifolia (Tea Tree) Leaf Extract	
physical characteristics	translucent yellow to brown mobile liquid with a characteristic odor	18
solubility	soluble in water	18
specific gravity (at 20°)	1.130 – 1.280	18
refractive index (at 20°)	1.370 – 1.550	18

Table 3. Composition of the 6 *Melaleuca alternifolia* chemotypes measured by headspace GC[25]

	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)[24]	European Pharmacopoeia[3]	WHO Specifications[11] (Melaleuca Alternifolia (Tea Tree) Leaf Oil)
α-pinene	1-4%	1-6%	not specified (NS)
sabinene	trace – 3.5%	NMT 3.5%	not less than (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)[11]	Supplier Information (GC)[46] (Melaleuca Alternifolia (Tea Tree) Leaf Oil)	Test Samples (steam-distilled; (GC or GC/MS)[39]	Test Sample (GC/MS)[47]	Test Sample (steam-distilled from leaves; GC/MS)[27]	Essential Oil (from leaves)[48]
α-pinene	1-5%	1-6%	2.6%	2.52%	2.0%	2.4%
sabinene	none reported (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 tea tree oil at different collection times during distillation[39]

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 [39]

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%	NR	NR	NR	NR	NR
p-cymene	2.9%	8.0%	35.3%	21.7%	32%	4.3%
1,8-cineole	5.1%	NR	NR	NR	NR	NR
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]	NR	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
NR – not reported

Table 9. Composition of tea tree oil at various stages of oxidation[51]

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 (2021)[56] and concentration of use (2019)[57] 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*	**43**	**NR**	**17**	**0.001-0.01**	**13**	**NR**
Duration of Use						
Leave-On	*29*	*NR*	*13*	*0.01*	*10*	*NR*
Rinse-Off	*13*	*NR*	*4*	*0.001*	*3*	*NR*
Diluted for (Bath) Use	*1*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type						
Eye Area	NR	NR	NR	NR	1	NR
Incidental Ingestion	NR	NR	1	NR	NR	NR
Incidental Inhalation-Spray	10[a]; 14[b]	NR	3[a]; 8[b]	NR	2; 3[b]	NR
Incidental Inhalation-Powder	4[b]	NR	8[b]	NR	3[b]	NR
Dermal Contact	43	NR	14	0.001-0.01	12	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	2	NR	NR	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	1	NR
Mucous Membrane	9	NR	1	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR

	Melaleuca Alternifolia (Tea Tree) Leaf Extract		**Melaleuca Alternifolia (Tea Tree) Leaf Oil**		**Melaleuca Alternifolia (Tea Tree) Leaf Powder**	
Totals*	**23**	**0.0001-0.001**	**536**	**0.003-0.63**	**NR**	**NR**
Duration of Use						
Leave-On	*18*	*0.0001*	*300*	*0.003-0.63*	*NR*	*NR*
Rinse-Off	*5*	*0.001*	*221*	*0.0003-0.3*	*NR*	*NR*
Diluted for (Bath) Use	*NR*	*NR*	*15*	*NR*	*NR*	*NR*
Exposure Type						
Eye Area	NR	NR	8	NR	NR	NR
Incidental Ingestion	NR	NR	13	0.0003-0.02	NR	NR
Incidental Inhalation-Spray	3[a]; 14[b]	NR	18; 89[a]; 84[b]	0.01-0.3[a]; 0.03[b]	NR	NR
Incidental Inhalation-Powder	14[b]	NR	4; 84[b]; 3[c]	0.03[b]	NR	NR
Dermal Contact	22	0.0001-0.001	409	0.0003-0.5	NR	NR
Deodorant (underarm)	NR	NR	20[a]	not spray: 0.2; spray: 0.5	NR	NR
Hair - Non-Coloring	1	NR	106	0.0072-0.3	NR	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR
Nail	NR	NR	7	0.005-0.63	NR	NR
Mucous Membrane	2	NR	96	0.0003-0.3	NR	NR
Baby Products	NR	NR	6	NR	NR	NR

	Melaleuca Alternifolia (Tea Tree) Leaf Water					
Totals*	**10**	**NR**				
Duration of Use						
Leave-On	*9*	*NR*				
Rinse-Off	*1*	*NR*				
Diluted for (Bath) Use	*NR*	*NR*				
Exposure Type						
Eye Area	NR	NR				
Incidental Ingestion	NR	NR				
Incidental Inhalation-Spray	4[a]; 3[b]	NR				
Incidental Inhalation-Powder	2; 3[b]	NR				
Dermal Contact	9	NR				
Deodorant (underarm)	NR	NR				
Hair - Non-Coloring	NR	NR				
Hair-Coloring	1	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 headspace solid-phase microextraction (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 multiple headspace extraction 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 limit of detection. 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	[71]
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)*** <u>Amount Released (% of the total amount initially present in the formulations)</u> 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%)	[72]

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
						Amount Permeated 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)*** Amount Released 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%) Amount Permeated 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)*** Amount Released 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%) Amount Permeated 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; stratum corneum and epidermis (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 capillary gas chromatography (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	[73]
tea tree oil	100%	static Franz diffusion cells	Caucasian female abdominal skin; heat-separated epidermis (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 apparent permeability constant (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)	[74]
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% bovine serum albumin	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%	[41]
	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	[75]

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; 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 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.	**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 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	[76]

Table 12. Acute toxicity studies

Ingredient	Animals	No./Group	Vehicle	Concentration/Dose	Protocol	LD_{50} or LC_{50}/Results	Reference
				DERMAL			
tea tree 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	82
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)	6,7
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	83,84
				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	6
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 polychromatic erythrocytes (PCE) and PCE + normochromatic erythrocytes 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	6
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	82
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	7
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	7

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 mass median aerodynamic diameter (MMAD), geometric standard deviation (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	[7]
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	[6]

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	[7]
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	[86]
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	[87]
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	V79 cells	chromosomal aberration assay, with and without metabolic activation in accordance with OECD TG 473; solvent and positive controls	not clastogenic	[7]
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	[88]
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	[88]
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	[7]
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	[89]
			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 9,10-dimethyl-1,2-benz-anthracene, 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	[6]

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.	[90]
tea tree oil	0.005 – 0.03%	human melanoma M14 wild-type (WT) and adriamicin-resistant (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	[91]
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/ propidium iodide staining was measured for apoptosis detection, cell cycle analysis was monitored using flow cytometry, and messenger RNA (mRNA) expression levels of the apoptosis-regulatory genes *P53*, *BAX*, and *BCL-2* were determined by real-time polymerase chain reaction (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	[92]

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	[93]
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	[95]
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	[94]
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	[96]
tea tree oil	0.0001% - 100%, in ethanol	human Hep G2 hepatocellular carcinomic human cell line	[(3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2H-tetrazolium) 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)	[97]
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)	[97]
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)	[97]
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)	[97]
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)	[97]

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.	[98]
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 Dulbecco's modified Eagle's medium); 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.	[94]

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 (growth regulation by estrogen in breast cancer 1(*GREB1*), progesterone receptor (*PGR*), and cathepsin D (*CTSD*)) were measured	All 3 genes showed significant induction when treated with tea tree oil; induction was blocked by co-treatment with fulvestrant	[99]
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 a proto-oncogene (*MYC*), *CTSD*, and insulin like growth factor binding protein 3 (*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.	[100]
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.	[78]
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%.	[101]

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	99
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 steroid receptor coactivator (SRC)-2- nuclear receptor (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.	99
			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 cytochrome P450 family 4 subfamily F member 8(*CYP4F8*), chromosome 1 open reading frame 116 (*C1orf116*), UDP glucuronosyltransferase family 2 member B28(*UGT2B28*), and SEC14-like lipid binding 2 (*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.	100
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%	99
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	99

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	112
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)	82,113
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	27
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	6
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	6
tea tree oil	undiluted; 0.5 ml	3 female NZW rabbits	OECD TG 404; 4 h semi-occlusive application; 4 cm^2 patch	after 60 min: mild; at 24 and 48 h: severe irritant at 72 h: a moderate irritant; 7 and 14 d: mild irritant reversible within 21 d	114
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	6,7
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	113,115
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	16
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')	116-118
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%	6,12

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 5-bromo-2'-deoxy-uridine 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% α-hexylcinnamaldehyde was used as the positive control	estimated concentration of a substance expected to produce a stimulation index of 3 (EC3) value of 8.3% (categorized as weak[7] or moderate[6] sensitization potential) Sensitizing response at 25 and 50% (stimulation index (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)	3,6,7
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	6
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	6
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[7] or moderate[6] 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[6])	6,7
tea tree oil	induction, intradermal: 5% in paraffin oil B.P. and 1:1:1 mixture of the oil, saline, and Freund's complete adjuvant (FCA); epidermal: 100% challenge: 30% in pet	albino guinea pigs, 20/group	guinea pig maximization test; 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	3,7
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	3,120
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 International Contact Dermatitis Research Group (ICDRG)	mean response: 0.4 (24 h); 0.5 (48 h) low sensitizing capacity	119
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	mean response: 0.8 (24 h); 1.0 (48 h)	
			challenge material: oil stored for 2 mo in a brown flask	mean response: 0.55 (24 h); 1.1 (48 h)	
			challenge material: oil stored for 2 mo in a closed flask	mean response: 0.62 (24 h); 0.65 (48 h)	
			challenge material: oil stored for 2 mo in an open flask	mean response: 1.0 (24 h); 1.58 (48 h)	
		10 guinea pigs	challenge material: monoterpene fraction	mean response: 0.85 (24 h); 0.9 (48 h)	

Table 16. Dermal irritation and sensitization studies

Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
			challenge material: sesquiterpene fraction challenge material: thujene/pinene-free fraction	mean response: 0.2 (24 h); 0.18 (48 h) mean response: 1.3 (24 h); 1.7 (48 h)	
		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. sodium lauryl sulfate (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	113,115
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	121
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	122

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	116-118
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%)	126
2003 - 2004; NACDG	oxidized, 5% pet	5137	45 (0.9%)	not stated		124
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)	132
2003 - 2007; NACDG	oxidized, 5% pet	11,649 (ages 19 – 64)	35 (0.3%)	22 (0.2%)		139
2005 - 2006; NACDG	oxidized, 5% pet	4435	1.4%	definite - 8.2% probable - 27.9% possible - 36.1%		127
2007 - 2008; NACDG	oxidized, 5% pet**	5078	1.4%	definite – 5.7% probable – 31.4% possible – 40.0% past – 5.7%	Significance-Prevalence Index Number (SPIN) - 55	128
2009 - 2010; NACDG	oxidized, 5% pet	4299	1.0%	definite - 14.3% probable - 35.7% possible - 21.4%	SPIN – 45 (rank 36)	129
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)	130

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)	[131]
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	[133]
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	[134]
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		[135]
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%)	[136]
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 atopic contact dermatitis (ACD); parent group n = 5148	[137]
		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	[138]
age specific - children						
2003 - 2007; NACDG***	oxidized, 5% pet	1007 ≤18 yr	4 (0.4%)	4 (0.4%)		[139]
2003 – 2004, NACDG***	oxidized, 5% pet	age 0 – 5 y (n not specified)	14.3%	14.3%		[140]
		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%		141
		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%)		139
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)	142
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	142
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	143
1999-2000; Germany and Austria (11 labs); German Contact Dermatitis Research Group (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	4,6,144
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	145
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	145

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	146
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 European Society of Contact Dermatitis guidelines	147
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	148
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	149
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	150
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	6,151
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	152
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	153
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	154
2016, UK	5% pet	1019	0.29%	0.29%		155
2016-2017, UK/Ireland	oxidized, 5% pet	4224	0.45%			125
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	156
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	156
2001-2010; Skin and Cancer Foundation	oxidized, 5% pet**	794	28 (3.5%)	43%		157
	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[124]

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	6,151
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		144
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		143
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	159
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 (+/+++)		49
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		160
tea tree oil		patient with pruritic ery-thematous rash	positive reactions to tea tree oil and colophony		161
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	162
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	162

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	[49]
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.	[119]
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	[120]
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	[152]
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 d 3 and 7 with oxidized tea tree oil	[162]
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 enzyme-linked immunosorbent assay for specific immunoglobulin (Ig) G 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	[163]
tea tree oil	used to treat psoriasis vulgaris		subject became sensitized within 3 mo; also reacted to fragrance mix, balsam of Peru, and turpentine	[119]
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	[164]
other direct skin or nail 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% aqueous (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%	[165]
tea tree oil	patient treated warts on his hands		became sensitized in 3 mo	[119]

Table 19. Case reports with tea tree oil

Test Substance	Subject(s)/Symptoms	Testing	Results/Comments	Reference
pure tea tree oil	patient developed an acute erythematoedematous perioral reaction 9 d after topical use of to treat angular cheilitis	patient was patch-tested with the Italian standard SIDAPA (Italian Society of Allergological, Occupational and Environmental Dermatology), an integrative cheilitis series, a 5% patch of oxidized tea tree oil, and the diluted used product (50% pet), on Van der Bend chambers. Patch tests were applied under occlusion on the back for 2 d; readings were performed on d 2 and d 4.	The patient showed positive reactions to the test product (50% pet; ++ on d 2 and d 4) and to the patch with 5% oxidized tea tree oil (+d 2/++d 4), as well as nickel (++ d 2 and d 4)	166
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	167
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 d 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	160
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	168
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 d 2 (+) and d 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.)	169
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	170
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	119
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	161
tea tree oil	used to treat sunburn		no reactions at site of application, but reacted to tea tree oil at patch testing	119

Table 19. Case reports with tea tree oil

Test Substance	Subject(s)/Symptoms	Testing	Results/Comments	Reference
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. .	168
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	119
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.	171
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 d 2 and 4 (++) with tea tree oil; also with lavender gel (++) and quaernium-15 (+)	172
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	162
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 d 3 Also had positive reaction to the fragrance mix, some oils from the perfume series, and 17 of 20 essential oils that were tested	173
pure tea tree oil	3 wk after application of the oil for suspected onychomycosis, the patient presented with acute periungual eczema on the first toe and on the medial surface of the second toe	Testing was performed using the Italian standard SIDAPA series, the product as used, and diluted to 2% and 5%.	Positive results were obtained with the pure test article (tea tree oil; (++ d 2/+++ d 4), was well as when tested at 2% (++ d 2/++ d 4) and 5% (++ d 2/+++ d 4), as well as for fragrance mix I (++ d 2/++ d 4).	166
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	174

Table 19. Case reports with tea tree oil

Test Substance	Subject(s)/Symptoms	Testing	Results/Comments	Reference
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)	[119]
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	[119]
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	[175]
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)	[176]
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	[177]

Table 20. SED of tea tree oil, assuming 3% absorption [6]

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 [40]

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)	not stated	not stated	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)

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Melaleuca Alternifolia (Tea Tree) Extract	Bath Oils, Tablets, and Salts	1
Melaleuca Alternifolia (Tea Tree) Extract	Bath Soaps and Detergents	6
Melaleuca Alternifolia (Tea Tree) Extract	Other Personal Cleanliness Products	2
Melaleuca Alternifolia (Tea Tree) Extract	Cleansing	3
Melaleuca Alternifolia (Tea Tree) Extract	Face and Neck (exc shave)	12
Melaleuca Alternifolia (Tea Tree) Extract	Body and Hand (exc shave)	2
Melaleuca Alternifolia (Tea Tree) Extract	Moisturizing	10
Melaleuca Alternifolia (Tea Tree) Extract	Paste Masks (mud packs)	2
Melaleuca Alternifolia (Tea Tree) Extract	Other Skin Care Preps	5

Melaleuca Alternifolia (Tea Tree) Flower/Leaf/Stem Extract	Other Hair Preparations	2
Melaleuca Alternifolia (Tea Tree) Flower/Leaf/Stem Extract	Dentifrices	1
Melaleuca Alternifolia (Tea Tree) Flower/Leaf/Stem Extract	Cleansing	2
Melaleuca Alternifolia (Tea Tree) Flower/Leaf/Stem Extract	Face and Neck (exc shave)	8
Melaleuca Alternifolia (Tea Tree) Flower/Leaf/Stem Extract	Moisturizing	3
Melaleuca Alternifolia (Tea Tree) Flower/Leaf/Stem Extract	Paste Masks (mud packs)	1

Melaleuca Alternifolia (Tea Tree) Leaf	Other Eye Makeup Preparations	1
Melaleuca Alternifolia (Tea Tree) Leaf	Foundations	2
Melaleuca Alternifolia (Tea Tree) Leaf	Other Manicuring Preparations	1
Melaleuca Alternifolia (Tea Tree) Leaf	Cleansing	3
Melaleuca Alternifolia (Tea Tree) Leaf	Face and Neck (exc shave)	3
Melaleuca Alternifolia (Tea Tree) Leaf	Moisturizing	2
Melaleuca Alternifolia (Tea Tree) Leaf	Other Skin Care Preps	1

Melaleuca Alternifolia (Tea Tree) Leaf Extract	Tonics, Dressings, and Other Hair Grooming Aids	1
Melaleuca Alternifolia (Tea Tree) Leaf Extract	Bath Soaps and Detergents	1
Melaleuca Alternifolia (Tea Tree) Leaf Extract	Other Personal Cleanliness Products	1
Melaleuca Alternifolia (Tea Tree) Leaf Extract	Cleansing	2
Melaleuca Alternifolia (Tea Tree) Leaf Extract	Face and Neck (exc shave)	13
Melaleuca Alternifolia (Tea Tree) Leaf Extract	Body and Hand (exc shave)	1
Melaleuca Alternifolia (Tea Tree) Leaf Extract	Moisturizing	2
Melaleuca Alternifolia (Tea Tree) Leaf Extract	Paste Masks (mud packs)	1
Melaleuca Alternifolia (Tea Tree) Leaf Extract	Other Skin Care Preps	1

Melaleuca Alternifolia (Tea Tree) Leaf Oil	Baby Shampoos	1
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Baby Lotions, Oils, Powders, and Creams	3
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Other Baby Products	2
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Bath Oils, Tablets, and Salts	8
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Bubble Baths	2
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Other Bath Preparations	5
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Eye Lotion	5
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Eye Makeup Remover	2
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Other Eye Makeup Preparations	1

Melaleuca Alternifolia (Tea Tree) Leaf Oil	Perfumes	4
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Other Fragrance Preparation	13
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Hair Conditioner	23
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Hair Spray (aerosol fixatives)	1
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Rinses (non-coloring)	1
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Shampoos (non-coloring)	43
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Tonics, Dressings, and Other Hair Grooming Aids	24
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Other Hair Preparations	13
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Face Powders	4
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Other Makeup Preparations	1
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Basecoats and Undercoats	2
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Cuticle Softeners	2
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Nail Polish and Enamel	1
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Other Manicuring Preparations	2
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Dentifrices	9
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Mouthwashes and Breath Fresheners	2
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Other Oral Hygiene Products	2
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Bath Soaps and Detergents	56
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Deodorants (underarm)	20
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Douches	1
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Feminine Deodorants	1
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Other Personal Cleanliness Products	10
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Aftershave Lotion	2
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Beard Softeners	11
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Preshave Lotions (all types)	3
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Shaving Cream	1
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Shaving Soap	1
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Other Shaving Preparation Products	3
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Cleansing	52
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Depilatories	1
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Face and Neck (exc shave)	63
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Body and Hand (exc shave)	17
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Foot Powders and Sprays	3
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Moisturizing	59
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Night	1
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Paste Masks (mud packs)	10
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Skin Fresheners	2
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Other Skin Care Preps	42
Melaleuca Alternifolia (Tea Tree) Leaf Oil	Suntan Gels, Creams, and Liquids	1

Melaleuca Alternifolia (Tea Tree) Leaf Water	Shampoos (non-coloring)	1
Melaleuca Alternifolia (Tea Tree) Leaf Water	Face Powders	2
Melaleuca Alternifolia (Tea Tree) Leaf Water	Face and Neck (exc shave)	3
Melaleuca Alternifolia (Tea Tree) Leaf Water	Moisturizing	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: January 13, 2021

SUBJECT: Melaleuca Alternifolia (Tea Tree) Leaf Extract

Native Extracts. 2020. Safety Data Sheet: Melaleuca Alternifolia (Tea Tree) Leaf Extract.

Southern Cross University. 2020. Certificate of Analysis Fragrance Allergens: Melaleuca Alternifolia (Tea Tree) Leaf Extract.

Southern Cross University. 2018. Certificate of Analysis: Melaleuca Alternifolia (Tea Tree) Leaf Extract.

Native Extracts. 2020. Manufacturing Concentrate Flowchart.

Native Extracts. 2019. Manufacturing Oil Flowchart.

Southern Cross University. 2020. Certificate of Analysis Fragrance Allergens: Vitis Vinifera (Grape) Seed Oil and Melaleuca Alternifolia (Tea Tree) Leaf Extract.

Native Extracts. 2018. Safety Data Sheet: Vitis Vinifera (Grape) Seed Oil and Melaleuca Alternifolia (Tea Tree) Leaf Extract.

Southern Cross University. 2018. Certificate of Analysis (fatty acids): Vitis Vinifera (Grape) Seed Oil and Melaleuca Alternifolia (Tea Tree) Leaf Extract.

SECTION 1. IDENTIFICATION OF THE SUBSTANCE AND SUPPLIER

PRODUCT IDENTIFER	
Product Name:	**NE Native Snowflower Extract Concentrate SB P3**
Botanical Name:	Melaleuca alternifolia
Product Code:	**ANE0073SB**
INCI Name:	Melaleuca alternifolia Leaf Extract
CAS:	85085-48-9
EC:	285-377-1
Organic Status:	Non-Organic
REACH Restriction:	Exempt from registration ex Annex V
UN Number:	Not required
HS Code:	1302.19.90
RECOMMENDED USE OF THE CHEMICAL AND RESTRICTIONS OF USE	
Relevant identified uses:	Cosmetic ingredient; Topical application; Not to be ingested
Usage:	0.5 - <1.0%
SUPPLIER DETAILS	
Name:	NATIVE EXTRACTS Pty Ltd
Address:	24 Kays Lane ALSTONVILLE NSW 2477 AUSTRALIA
Telephone:	+61 2 6686 5725
Email:	enquiries@nativeextracts.com
Website:	www.nativeextracts.com
EMERGENCY TELEPHONE NUMBERS [24/H/24H] – INTERNATIONAL CENTRES WITHIN YOUR COUNTRY	
AUSTRALIA:	Poisons Information Centre 13 11 26
USA:	Poison Control Centre 1-800-222-1222
GERMANY	Federal Institute for Risk Assessment
ITALY:	National Institute of Health
UNITED KINGDOM:	National Poison Information Services
OTHER COUNTRIES:	Please contact relevant government services

SECTION 2. HAZARDS IDENTIFIED

CLASSIFICATION OF THE SUBSTANCE OR MIXTURE		
POISONS SCHEDULE:	Unscheduled	

HAZARDOUS CHEMICAL – NON-DANGEROUS GOODS:
According to the WHS Regulations and the ADG Code; Globally Harmonized System of Classification and Labelling of Chemicals [GHS]; Regulation (EC) No 1223/2009 of the European Parliament and the Council of 30 November 2009 on cosmetic products (the "Cosmetics Regulation" or the "Regulation"), Governing the composition, labelling and packaging of finished cosmetic products.

CLASSIFICATION:	Skin Corrosion/Irritant	Category 2
	Serious Eye Damage/Eye Irritation	Category 2A
	Specific Target Organ Toxicity Single Exposure	Category 3

LABEL ELEMENTS	
GHS LABEL ELEMENTS:	
SIGNAL WORD:	WARNING
HAZARD STATEMENT[S]	
H315	Causes skin irritation
H319	Causes serious eye irritation
H335	May cause respiratory irritation

PRECAUTIONARY STATEMENT[S]	
PREVENTION:	
P101	If medical advice is needed, have product container or label at hand.
P103	Read label before use.
P271	Use only outdoors or in a well-ventilated area.
P261	Avoid breathing mist/vapour/spray.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
RESPONSE:	
P302+P352	IF ON SKIN: Wash with plenty of soap and water.
P332+P313	If skin irritation occurs: Get medical advice/attention.
P362	Take off contaminated clothing and wash before reuse.
P305+P351+P388	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337+P313	If eye irritation persists: Get medical advice/attention.
P304+P340	IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
P312	Call a POISON CENTRE or doctor/physician if you feel unwell.
STORAGE:	
P403+P233	Store in a well-ventilated place. Keep container tightly closed.
P405	Store locked up.
DISPOSAL:	
P501	Dispose of contents/container in accordance with local/national/international regulations.

SECTION 3: COMPOSTIONAL INFORMATION ON INGREDIENTS

SUBSTANCE CHEMICAL NAME	CAS No	EC	[%w/w]
Melaleuca alternifolia Leaf Extract	**85085-48-9**	**285-377-1**	
Glycerine	56-81-5	200-289-5	34-55%
Melaleuca alternifolia Leaf	85085-48-9	285-377-1	20-50%
Water/Aqua	7732-18-5	231-791-2	14-24%
Sodium Benzoate	532-32-1	208-534-8	≤0.5%
Citric Acid	77-92-9	201-069-1	≤0.4%
Potassium Sorbate	24634-61-5	246-376-1	≤0.3%

Cellular Extraction of Melaleuca alternifolia Leaf. Natural extract preserved with Sodium Benzoate; Citric Acid; Potassium Sorbate

SECTION 4: FIRST AID MEASURES

DESCRIPTION OF FIRST AID MEASURES

EYE CONTACT: If this product comes into contact with the eye:
- Wash out immediately with fresh running water;
- Ensure complete irrigation of the eye by keeping eyelids apart and away from eye and moving the eyelids by occasionally lifting the upper and lower lids;
- Seek medical attention without delay; if pain persists or recurs seek medical attention;
- Removal of contact lenses after an eye injury should only be undertaken by skilled personnel.

SKIN CONTACT: If skin contact occurs:
- Immediately remove all contaminated clothing, including footwear;
- Flush skin and hair with running water (and soap if available);
- Seek medical attention in event of irritation.

INHALATION:
- If fumes or combustion products are inhaled remove from contaminated area;
- Lay patient down. Keep warm and rested;
- Prostheses such as false teeth, which may block airway, should be removed, where possible, prior to initiating first aid procedures;
- Apply artificial respiration if not breathing, preferably with a demand value resuscitator, bag-valve mask device, or pocket mask as trained. Perform CPR if necessary;
- Transport to hospital, or doctor, without delay.

SWALLOWED:
- Immediately give a glass of water;
- First aid is not generally required. If in doubt, contact a Poisons Information Centre or doctor.

INDICATION OF ANY IMMEDIATE MEDICAL ATTENTION AND SPECIAL TREATMENT NEEDED: Treat symptomatically.

SECTION 5: FIRE FIGHTING MEASURES

EXTINGUISHING MEDIA

Water spray or fog; Foam; Dry chemical powder; BCF (where regulations permit).

SPECIAL HAZARDS ARISING FROM THE SUBSTANCE

FIRE INCOMPATIBILITY:
Avoid contamination with oxidising agents i.e. nitrates, oxidising acids, chlorine bleaches, pool chlorine etc. as ignition may result.

ADVICE FOR FIRE FIGHTERS

FIRE FIGHTING:
- Alert Fire Brigade and tell them location and nature of hazard;
- Wear full body protective clothing with breathing apparatus;
- Prevent, by any means available, spillage from entering drains or watercourse;
- Use water delivered as a fine spray to control fore and cool adjacent area.

FIRE/EXPLOSION HAZARD:
- Combustible;
- Slight fire hazard when exposed to heat or flame;
- Heating may cause expansion or decomposition leading to violent rupture of CONTAINERS;
- On combustion, may emit toxic fumes or carbon monoxide (CO);
- Combustion products include; carbon dioxide (CO_2) acrolein, other pyrolysis products typical of burning organic material. May emit poisonous fumes. May emit corrosive fumes.

HAZCHEM: Not applicable.

SECTION 6: ACCIDENTIAL RELEASE MEASURES

PERSONAL PRECAUTIONS, PROTECTIVE EQUIPMENT AND EMERGENCY PROCEDURES

See Section 8.

ENVIRONMENTAL PRECAUTIONS

See Section 12.

METHODS OF MATERIAL FOR CONTAMINATION AND CLEAN UP

MINOR SPILLS:
- Remove all ignition sources;
- Clean up all spills immediately;
- Avoid breathing vapours and contact with skin and eyes;
- Control personal contact with the substance, by using protective equipment.

MAJOR SPILLS:
- MODERATE HAZARD: Clear area of personnel and move upwind;
- Alert Fire Brigade and tell them location and nature of hazard;
- Wear breathing apparatus plus protective gloves.

SECTION 7: HANDLING AND STORAGE

PRECAUTIONS FOR SAFE HANDLING

SAFE HANDLING:
- Avoid all personal contact, including inhalation;
- Wear protective clothing when risk of exposure occurs;
- Prevent concentration in hollows and sumps;
- DO NOT allow clothing wet with substance to stay in contact with the skin.

OTHER INFORMATION:
- Store in original containers;
- Keep containers securely sealed;
- No smoking, naked lights or ignition sources;
- Store in a cool, dry, well-ventilated area.

CONDITIONS FOR SAFE STORAGE, INCLUDING AND INCOMPATIBILITES

SUITABLE CONTAINERS:
Packaging as recommended by manufacturer;
Check all containers are clearly labelled and free from leaks.

STORAGE INCOMPATIBILITY: Avoid reaction with oxidising agents

x: Must not be stored together; **O:** May be stored together with specific preventions; **+:** May be stored together

Flammable	Explosive	Poison	Oxidising	Respiratory	Warning	Corrosive
+	X	O	O	+	+	+

24 Kays Lane ALSTONVILLE NSW 2477 AUSTRALIA **P +61 2 6686 5725**

SECTION 8: EXPOSURE CONTROLS/PERSONAL PROTECTION

CONTROL PARAMETERS

The product is not classified. No control parameters are to be mentioned.

EXPOSURE CONTROLS

APPROPRIATE ENGINEERING CONTROLS:

- Engineering controls are used to remove a hazard or place a barrier between the worker and the hazard. Well-designed engineering controls can be highly effective in protecting workers and will typically be independent of worker interactions to provide third high level of protection;
- The basic types of engineering controls are; Process controls which involve changing the way a job activity or process is done to reduce the risk;
- Enclosure and/or isolation of emission source which keeps a selected hazard 'physically' away from the worker and ventilation that strategically 'adds' and removes' air in the work environment.

PERSONAL PORTECTION:

EYE AND FACE PROTECTION:

- Safety glasses with side shield;
- Chemical goggles;
- Contact lenses may pose a special hazard; soft contact lenses may absorb and concentrate irritants. A written policy document, describing the wearing of lenses or restrictions on use, should be created for each workplace or task.

SKIN PROTECTION: See Hand Protection below.

HAND/FEET PROTECTION:

- Wear chemical protective gloves, e.g. PVC;
- Wear safety footwear or safety gumboots, e.g. Rubber;
- The selection of suitable gloves does not only depend on the material, but also on further marks of quality, which vary from manufacturer to manufacturer;
- Where the chemical is a preparation of several substances, the resistance of the glove material cannot be calculated in advance and has therefore to be checked prior to the application;
- The exact break through time for substances has to be obtained from the manufacturer of the protective gloves and has to be observed when making a final choice;
- Personal hygiene is a key element of effective hand care.

BODY PROTECTION: See Other Protection below.

OTHER: Overalls; PVC Apron; Barrier Cream.

STANDARDS: The following Australian Standards will provide general advice regarding safety clothing and equipment:

AS/NZS 1715:	Respiratory Equipment
AS 1161:	Protective Gloves
AS2919:	Industrial Clothing
AS1336/AS/NZS 1337:	Industrial Eye Protection
AS/NZS2210:	Occupational Protective Footwear

THERMAL HAZARDS: Not available

SECTION 9: PHYSICAL AND CHEMICAL PROPERTIES

PHYSICAL/CHEMICAL PROPERTIES	RESULT	PHYSICAL/CHEMICAL PROPERTIES	RESULT
APPEARANCE:	Mobile liquid	BOILING POINT RANGE:	Not available
ODOUR:	Characteristic	FLAMMABILITY LIMITS:	Not available
COLOUR:	Translucent yellow to brown	AUTO-IGNITION TEMPERATURE:	Not available
TASTE:	Not determined	VAPOUR PRESSURE:	No data available
REFRACTIVE INDEX @20°C:	1.370 – 1.550	DENSITY:	Not available
SPECIFIC GRAVITY @20°C:	1.130 – 1.280	VISCOSITY, KINEMATIC:	No data available
WATER SOLUBILITY:	Soluble	OXIDISING PROPERTIES:	Not oxidising
FLASH POINT:	160°C	EXPLOSIVE PROPERTIES:	Not explosive
EVAPORATION RATE:	Non-volatile	BULK DENSITY:	Not applicable
PH:	3.00 – 5.00	RELATIVE VAPOUR DENSITY:	No data available
MELTING/FREEZING POINT:	Not available		

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SECTION 10: STABILITY AND REACTIVITY

REACTIVITY:	See Section 7
CHEMICAL STABILITY:	This product is chemically stable
POSSIBILITY OF HAZARDOUS REACTIONS:	See Section 7
CONDITIONS TO AVOID:	See Section 7
INCOMPATIBLE MATERIALS:	See Section 7
HAZARDOUS DECOMPOSITION PROUCTS:	See Section 5

SECTION 11: TOXICOLOGICAL INFORMATION

INFORMATION ON TOXICOLOGICAL EFFECTION

INHALED:

- The material can cause respiratory irritation in some persons. The body's response to such irritation can cause further lung damage;
- Not normally a hazard due to non-volatile nature of product.

INGESTION:

- Although ingestion is not thought to produce harmful effects (as classified under EC Directives), the material may still be damaging to the health of the individual, following ingestion, especially where pre-existing organ (e.g. liver, kidney (damage is evident;
- Ingestion of large quantities may cause nausea, diarrhoea and vomiting.

SKIN CONTACT:

- The material may accentuate any pre-existing dermatitis condition;
- Skin contact is not thought to have harmful health effects (as classified under EC Directives); the material may still produce health damage following entry though wounds, lesions or abrasions;
- Open cuts abraded, or irritated skin should not be exposed to this material;
- Entry into the blood stream, though, for example, cuts abrasions or lesions, following direct contact or after a delay of some time. Repeated exposure can cause contact dermatitis, which is characterised by redness, swelling and blistering.

EYE:

- Evidence exits, or practical experience predicts, that the material may cause eye irritation in a substantial number of individuals; Prolonged eye contact may cause inflammation characterised by a temporary redness of the conjunctiva (similar to windburn).

CHRONIC:

- Long term exposure to respiratory irritants may result in disease of the airways involving difficult breathing and related systemic problems;
- Substance accumulation, in the human body, may occur and may cause some concern following or long-term occupational exposure.

SCCNFP ALLERGENS ANNEX III – COSMETIC DIRECTIVE 2003/15/EC

7th Amendment Detection Limit 0.001%

CONSTITUENT	IFRA	EFFA	CAS	EC	RANGE
Amyl Cinnamal:	Yes	No	122-40-7	204-541-5	Not detected
Amyl Cinnamyl Alcohol:	Yes	No	101-85-9	202-982-8	Not detected
Anise Alcohol:	No	Yes	105-13-5	203-273-6	Not detected
Benzyl Alcohol:	No	Yes	100-51-6	202-859-9	Not detected
Benzyl Benzoate:	No	Yes	120-51-4	204-402-9	Not detected
Benzyl Cinnamate:	No	Yes	103-41-3	203-109-3	Not detected
Benzyl Salicylate:	No	Yes	118-58-1	204-262-9	Not detected
Cinnamal:	Yes	Yes	104-55-2	203-213-9	Not detected
Cinnamyl Alcohol:	Yes	Yes	104-54-1	203-212-3	Not detected
Citral:	Yes	Yes	5392-40-5	226-394-6	Not detected
Citronellol:	No	Yes	5392-40-5	203-375-0	Not detected
Coumarin:	No	Yes	91-64-5	202-086-7	Not detected
Eugenol:	Yes	Yes	97-53-0	202-589-1	Not detected
Farnesol:	Yes	Yes	4602-84-0	225-004-1	Not detected
Geraniol:	No	Yes	106-24-1	203-377-1	Not detected
Hexyl Cinnamal:	Yes	No	101-86-0	202-983-3	Not detected
Hydroxycitronellal:	Yes	No	107-75-5	203-518-7	Not detected
Isoeugenol:	Yes	Yes	97-54-1	202-590-7	Not detected
Butylphenyl Methylpropional:	Yes	No	80-54-6	201-289-8	Not detected
d-Limonene:	Yes	Yes	5989-27-5	227-813-5	Not detected
Linalool:	Yes	Yes	78-70-6	201-134-4	Not detected
Hydroxyisohexyl 3-Cyclohexene Carboxaldehyde:	No	No	31906-04-4	250-863-4	Not detected
Methyl 2-Octynoate:	Yes	No	111-12-6	203-836-6	Not detected
Alpha-Isomethyl Ionone:	Yes	No	127-51-5/ 90028-68-5	204-846-3/ 289-861-3	Not detected
Evernia Prunastri Extract [Oakmoss]:	Yes	No	9000-50-4/ 6817-10-2		Not detected
			90028-67-4/	289-860-8	
Evernia Furfuracea Extract[Treemoss]:	Yes	No	68648-41-9		Not detected

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ADDITIONAL EFFA LISTED SENSITISERS & IFRA NOTIFIABLE SUBSTANCES

Detection Limit 0.001%

CONSTITUENT	IFRA	EFFA	CAS	EC	RANGE
No Additional Sensitisers:	No	No	Not allocated	Not allocated	Not detected
No Additional Notifiable Substances:	No	No	Not allocated	Not allocated	Not detected

SECTION 12: ECOLOGICAL INFORMATION

TOXICITY:

INGREDIENT	ENDPOINT	TEST DURATION [hr]	SPECIES	VALUE	SOURCE
Glycerin	LC50	96	Fish	>11mg/L	2
Glycerin	EC50	96	Algae or other aquatic plants	77712.039mg/L	3
Glycerin	ECO	24	Crustacea	>500mg/L	1

Legend: *Extracted from 1. IUCLID Toxicity Data 2. Europe ECHA Registered Substance – Eco toxicological Information – Aquatic Toxicity 3. EPIWIN Suite V3.12 – Aquatic Toxicity Data (Estimated) 4. US EPA, Ecotox database – Aquatic Toxicity Data 5. ECETOC Aquatic Hazard Assessment Data 6. NITE (Japan) – Bio concentration Data 7. METI (Japan) – Bio concentration Data 8. Vendor Data*

For Glycerin: Low Kow: -2.66 to -2.47, Atmospheric Fate: Glycerol is broken down in the air by hydroxyl radicals the half-life for this process is 6.8 hours. However, only a negligible amount of the substance will move to the atmospheric compartment. Terrestrial Fate: Only a negligible amount of Glycerin will move into the soil compartment, if released into the environment. Aquatic Fate: Glycerol is considered to be readily biodegradable in the aquatic environment. DO NOT discharge into sewer or waterways.

PERSISTENCE AND DEGRADABILITY:

- LOW persistence level Water/Soil/Air;
- Use according to good working practice; pollution to soil, rivers and the ocean.

BIO-ACCUMULATIVE POTENTIAL:

- Glycerin: LOW (LogKOW = 1.76).

MOBILITY IN SOIL:

- Glycerin: HIGH (KOC = 1).

SECTION 13: DISPOSAL CONSIDERATIONS

WASTE TREATMENT METHODS

PRODUCT/PACKAGING DISPOSAL:

- Legislation addressing waste disposal requirements may differ by country, state and/or territory. Each user must refer to laws operating in their area. In some areas, certain wastes must be tracked;
- A Hierarchy of Control seems to be common – the user should investigate:
  - Reduction;
  - Reuse;
  - Recycle;
  - Disposal [if all else fails].
- DO NOT allow wash water from cleaning or process equipment to enter drains.
- It may be necessary to collect all wash water for treatment before disposal;
- In all cases disposal to sewer may be subject to local laws and regulations and these should be considered first;
- Where in doubt contact the responsible authority;
- Recycle wherever possible or consult manufacturer for recycling options;
- Consult State Land Waste Authority for disposal;
- Bury or incinerate residue at an approved site;
- Recycle containers if possible or dispose of in an authorised landfill.

SECTION 14: TRANSPORT INFORMATION

LABELS REQUIRED	
MARINE POLLUTANT:	No
HAZCHEM:	Not applicable
LAND TRANSPORT [AGD]:	Not regulated for transport of Dangerous Goods
AIR TRANSPORT [ICAO-IATA/DGR];	Not regulated for transport of Dangerous Goods
SEA TRANSPORT [IMDG-Code/GGVSee]:	Not regulated for transport of Dangerous Goods
UN NUMBER:	Not required
PROPER SHIPPING NAME:	Not required
TECHNICAL SHIPPING NAME:	Not applicable
DG CLASS/SUBSIDARY RISK:	Not applicable
PACKAGING GROUP:	Not allocated
SPECIAL PRECAUTIONS:	Not established
HAZCHEM CODE:	Not allocated

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SECTION 15: REGULATORY INFORMATION

SAFETY, HEALTH AND ENVIRONMENTAL REGULATIONS/LEGISLATION SPECIFIC FOR THE SUBSTANCE OR MIXTURE

The substance is not listed as a hazardous chemical under the following international agreements:

- Montreal Protocol on Substances that Deplete the Ozone Layer;
- Stockholm Convention on Persistent Organic Pollutants;
- Rotterdam Convention on the Prior Informed Consent Procedure for Certain Hazardous Chemicals and Pesticides in International Trade;
- Basel convention on the Control of Trans boundary Movements of Hazardous Wastes and their Disposal;
- International Convention for the Prevention of Pollution from Ships (MARPOL);
- Standard for the Uniform Scheduling of Medicines and Poisons (SUSMP);
- Agriculture and Veterinary Chemicals Code Act 1994;
- Australian Inventory of chemical Substances (AICS).

SUBSTANCE CHEMICAL NAME

Melaleuca alternifolia Leaf Extract

NATIONAL INVENTORY	COUNTRY	STATUS ✓✖
Australian Inventory of Chemical Substances (AICS):	AUSTRALIA	✓
Domestic Substances List (DSL):	CANADA	✖
Non-Domestic Substances List (NDSL):	CANADA	✖
Inventory of Existing Chemical Substances Produced for Imported to China (IECSC):	CHINA	✓
European Chemicals Agency (ECHA-EINECS-ELINCS-NLP-COSING):	EUROPE	✓
Japanese Existing and New Chemical Substances Inventory (ENCS):	JAPAN	✖
Korea Existing Chemicals Inventory (KECI):	SOUTH KOREA	✖
New Zealand Inventory (NZIoC):	NEW ZEALAND	✓
Philippines Inventory of Chemicals and chemical Substances (PICCS):	THE PHILLIPPINES	✓
Toxic Substances Control Act (TSCA):	USA	✖
Taiwan Chemical Substance Inventory (TCSI):	TAIWAN	✖
Vietnam National Chemical Database System	VIETNAM	✖

SECTION 16: ADDITIONAL INFORMATION

QUALITY STATEMENT

NATIVE EXTRACTS Pty Ltd specialises in the manufacture and supply of the highest quality, pure, naturally derived phyto-active compounds in hydrophilic extracts, seed oils and pure natural powders; for use in the Cosmetic, Pharmaceutical and Nutraceutical industries globally. Our company's objective is to manufacture and supply the highest quality and purity of natural ingredients across multiple delivery formats that meet the application/formulation objectives and specifications of our customers. Our commitment to quality extends beyond our products and applies to our blends, services, workplace, environmental practices and partnership and relationships engaged with commercial growers and Indigenous communities.
Any quality problems arising will be identified and solved with speed, technical efficiency and economy, stakeholder engagement – focusing our human and technical resources internally and externally to the prevention of quality deficiencies to meet our company goal of "right first time, every time".
The successful operation of our QMS relies on the cooperation, participation and engagement of our personnel across all areas of the company. Our commitment to quality underpins our continued success, the satisfaction of customers and staff, our pursuit to achieve new scientific discoveries and new benchmarks in performance ingredients. We are committed to improving our performance in every aspect of our business.
NATIVE EXTRACTS will to provide high and consistent quality in Botanical extracts and naturally derived phyto-active ingredients, evolving the botanical extract from inferior processes and synthetic standardisation to the delivery of stable, active True to Nature phyto-activity, influencing new innovation in natural product development, new advances in consumer experiences, influencing the emergence of new primary industry partnerships, and participating in socially and environmentally responsible practices. Our commitment is to safety and accurate work to ensure our ingredients conform to various regulatory bodies locally and internationally and are safe to our customers, their clients and the environment. All work is done in conformance to NATIVE EXTRACTS' QMS, the applicable technical and administrative operating policies and procedures of NATIVE EXTRACTS, legal and regulatory requirements, and specific customer requirements.
Through front-line input and management leadership, we will continue to improve our people and processes to anticipate, meet, and exceed the needs of our customers. We support the continually improving quality of our customer's maintenance and other technical operations through the services we provide.

ANIMAL TESTING

NATIVE EXTRACTS Pty Ltd does not test raw materials on animals, neither initially nor as a routine test. The product suppliers for NATIVE EXTRACTS Pty Ltd do not test their products on animals, neither initially nor as a routine test. None of NATIVE EXTRACTS Pty Ltd finished extracts are tested on animals, either initially or as a routine test.

MANUFACTURING PRODUCTS INGREDIENTS DISCLAIMER

As the availability of ingredients and raw materials is not always certain whether due to changes in nature or otherwise, NATIVE EXTRACTS Pty Ltd reserves the right to substitute alternate ingredients/raw materials in the manufacture of its products in order to maintain supply to its customers. Customers should always refer to the ingredients label as affixed to each product or to specification sheets, which are current at all time of supply of the product.

LABELLING DISCLAIMER

NATIVE EXTRACTS Pty Ltd is a manufacturer of extracts. If you intend to re-label our products under your own name/brand for the purpose of on selling or retailing, we thoroughly recommend that you keep up to date with constant changing labelling laws. Please visit www.acco.gov.au or www.nicnas.gov.au. NATIVE EXTRACTS Pty Ltd cannot be held responsible for consequential loss/product recall due to incorrect labelling.

DISCLAIMER

This Safety Data Sheet was prepared according to: Safe Work Australia's Code of Practice for the Preparation of Safety Data Sheets for Hazardous Chemicals, [Publication date: 23/12/2011] and Globally Harmonized System of Classification and Labelling of Chemicals (GHS) [NOHSC:1008(2004)].
The information contained in this Safety Data Sheet is obtained from current and reliable sources. NATIVE EXTRACTS Pty Ltd provides the information contained herein in good faith but makes no representation as to its comprehensiveness or accuracy. This Safety Data Sheet summaries our best current knowledge of the health and safety hazard information of the product but does not claim to be all-inclusive. This document is thus, intended only as a guide to the appropriate precautionary handling of the material by properly trained personnel using this product.
Individuals receiving this information must exercise their independent judgment in determining its appropriateness for a particular purpose. As the ordinary or otherwise use(s) of this product is outside the control of NATIVE EXTRACTS Pty Ltd, no representation or warranty, expressed or implied, is made as to the effect(s) of such use(s), (including damage or injury), or the results obtained. NATIVE EXTRACTS Pty Ltd expressly disclaims responsibility as to the ordinary or otherwise use(s). Furthermore, nothing contained herein should be considered as a recommendation by NATIVE EXTRACTS Pty Ltd as to the fitness for any use. The liability of NATIVE EXTRACTS Pty Ltd is limited to the value of the goods and does not include any consequential loss. NATIVE EXTRACTS Pty Ltd shall not be liable for any errors or delays in the content, or for any actions taken in reliance thereon.
NATIVE EXTRACTS Pty Ltd shall not be responsible for any damage resulting from use of or reliance upon this information. The user of the product is solely responsible for compliance with all laws and regulations applying to the use of the products, including intellectual property rights of third parties.

ACRONYMS

<	Less than	LDLo	LDLo stands for Lethal Dose Low, the minimum amount of a material which tests have shown will be lethal to a specified type of animal. This is normally quoted in mg.kg body weight.
>	Greater than	Lt	Litre
°C	Degrees Celsius	Max.	Maximum
ACCC	Australian Competition and Consumer Commission	Mg	Milligram
ADG	Australian Dangerous Goods	Min.	Minimum
AICS	Australian Inventory of Chemical Substances	ml	Millilitre
AICS	Australian Inventory of Chemical Substances	M^3	Cubic metre
ACGIH	American Conference of Government Industrial Hygienists	mm	Millimetre
AS	Australian Standards	mm Hg	Millimetre of Mercury
BOD	Biochemical Oxygen Demand	N/A NA	Not Applicable
CAS	Chemical Abstracts Service (Registry Number)	NICNAS	The National Industry Chemicals Notification and Assessment Scheme (AUSTRALIA)
Cm^3	Cubic centimetres	NIOSH	The National Institute for Occupational Safety and Health (USA)
COD	Chemical Oxygen Demand	NOHSC	National occupational Health and Safety Commission (AUSTRALIA)
CosIng	The European Commission database with information on Cosmetic Ingredients and Substances	n.o.s.	Not otherwise specified
DG	Dangerous Goods	NZS	New Zealand Standards
EC	European Commission	NZIoC	New Zealand Inventory of Chemicals
EC50	EC stands for the effective concentration. EC50 refers to the concentration of a toxicant, which includes a response halfway between the baseline and maximum after a specified exposure time	OECD	Organisation for Economic Co-operation and Development (Test Method number)
EINECS	European Inventory of Existing Commercial Chemical Substances (Identifying Number)	OSHA	The Occupational Safety and Health Administration (USA)
EFFA	European Flavour Association	PEL	Permissible Exposure Limit
EU	Europe/European Union	Ppb	Parts per billion
g	grams	Ppm	Parts per million
GHS	The Globally Harmonised System of Classification and Labelling of Chemicals	RTECS	The Registry of Toxic Effects of Chemical Substances
GMO	Genetically modified organism	SCCNFP	Scientific Committee on Cosmetic Products and non-Food Products (EUROPE)
Hazchem Code	Emergency action code of numbers and letters that provide information to emergency services especially fire fighters	SDS	Safety Data Sheet
hr	Hour	STEL	Short Term Exposure Limit
HSIS	The Safe Work Australia Hazardous Substances Information System	Subsp.	Subspecies
HSNO	Hazardous Substances Approval Code	Subspecies	Standard for the Uniform Scheduling of Medicine and Poisons (AUSTRALIA)
IATA	The International Air Transport Association	TD	TD stands for Toxic Dose. TD is the amount given all at once, which causes the untoward symptoms in the majority of persons, or in the majority of a group of test animals. This is normally quoted in mg/kg body weight.
ICAO	The International Civil Aviation Organisation	TGA	Therapeutic Goods Administration (AUSTRALIA)
IFRA	The International Fragrance Association	TLV	Threshold Limit Value
IMDG	International Maritime Dangerous Goods	TWA	Time Weighted Average
INCI	The International Nomenclature of Cosmetic Ingredients	UK	United Kingdom
ISO	International Organisation for Standardisation	USA	The United States of America
Kg	Kilograms	µg	Microgram
LC50	LC stands for lethal concentration. LC50 is the concentration of a material in air which causes the death of 50% (one half) of a group of test animals. The material is inhaled over a set period of time, usually 1 or 4 hours. This is normally quoted in mg/kg body weight.	µl	Micro litre
LD50	LD50 stands for Lethal Dose. This is the amount of a material, given all at once, which causes the death of 50% (one half) of a group of test animals. This is normally quoted in mg/kg body weight.		

DATA SOURCE

AICS; Australian Code for the Transport of Dangerous Goods by Rail and Road; Globally Harmonized System of Classification and Labelling of Chemicals (GHS) [NOHSC:1008(2004)]; Work Safe Australia WHS Regulations; CosIng; Supplier Documentation; EFFA; HSIS; IATA Dangerous Goods Regulations; IFRA; IMDG Code; The International Cosmetic Ingredients Dictionary and Handbook; NICNAS; SUSMP; NZIoC; NOHSC Australia.

DOCUMENT PREPARED BY

Vanessa Minnikin, Quality Assurance. Email: vminnikin@nativeextracts.com

Southern Cross University

Southern Cross Plant Science
ANALYTICAL RESEARCH LABORATORY

t: +61 2 6622 3211 • w: scu.edu.au/arl • T Block Level 3, Miltary Road, Lismore NSW 2480 Australia • **ABN:** 41995 651 524 • **TGA:** MI-01122004-LI-000264-1

CERTIFICATE OF ANALYSIS

Melaleuca Alternifolia (Tea Tree) Leaf Extract

SAMPLE NAME	NE Native Snowflower Extract Concentrate SB P3		
FORM	Liquid		
CUSTOMER NAME	Native Extracts Pty Ltd		
CERTIFICATION DATE	02 September 2020		
CUSTOMER REFERENCE	040820-01		
ARL JOB #	A202225	**LAB REF. #**	ARL2005494
ANALYSIS	Cosmetic Allergens	**METHOD**	ARL-TM284-1*

	TEST	SPECIFICATION %w/w		RESULTS %w/w
1	d-limonene **	≥ 0.01 rinse off	≥ 0.001 leave on	nd
2	benzyl alcohol **			nd
3	linalool **			nd
4	methyl heptine carbonate **			nd
5	citronellol **			nd
6a	citral-A neral **			nd
7	geraniol **			nd
6b	citral-B geranial **			nd
8	cinnamic aldehyde **			nd
9	anisyl alcohol **			nd
10	hydroxy-citronellal **			nd
11	cinnamic alcohol **			nd
12	eugenol **			nd
13	coumarin **			nd
14	trans iso-eugenol **			nd
15	γ-methyl ionone **			nd
16	oak moss **			nd
17	tree moss **			nd
18	lilial **			nd
19	cis amyl cinnamaldehyde **			nd
20	lyral **			nd
21	amyl cinnamic alcohol **			nd
22a	cis cis farnesol **			nd
22b	trans trans farnesol **			nd
23	trans hexyl cinnamaldehyde **			nd
24	benzyl benzoate **			nd
25	benzyl salicylate **			nd
26	benzyl cinnamate **			nd

*** Assay by GC (MS detection –area percent report)**

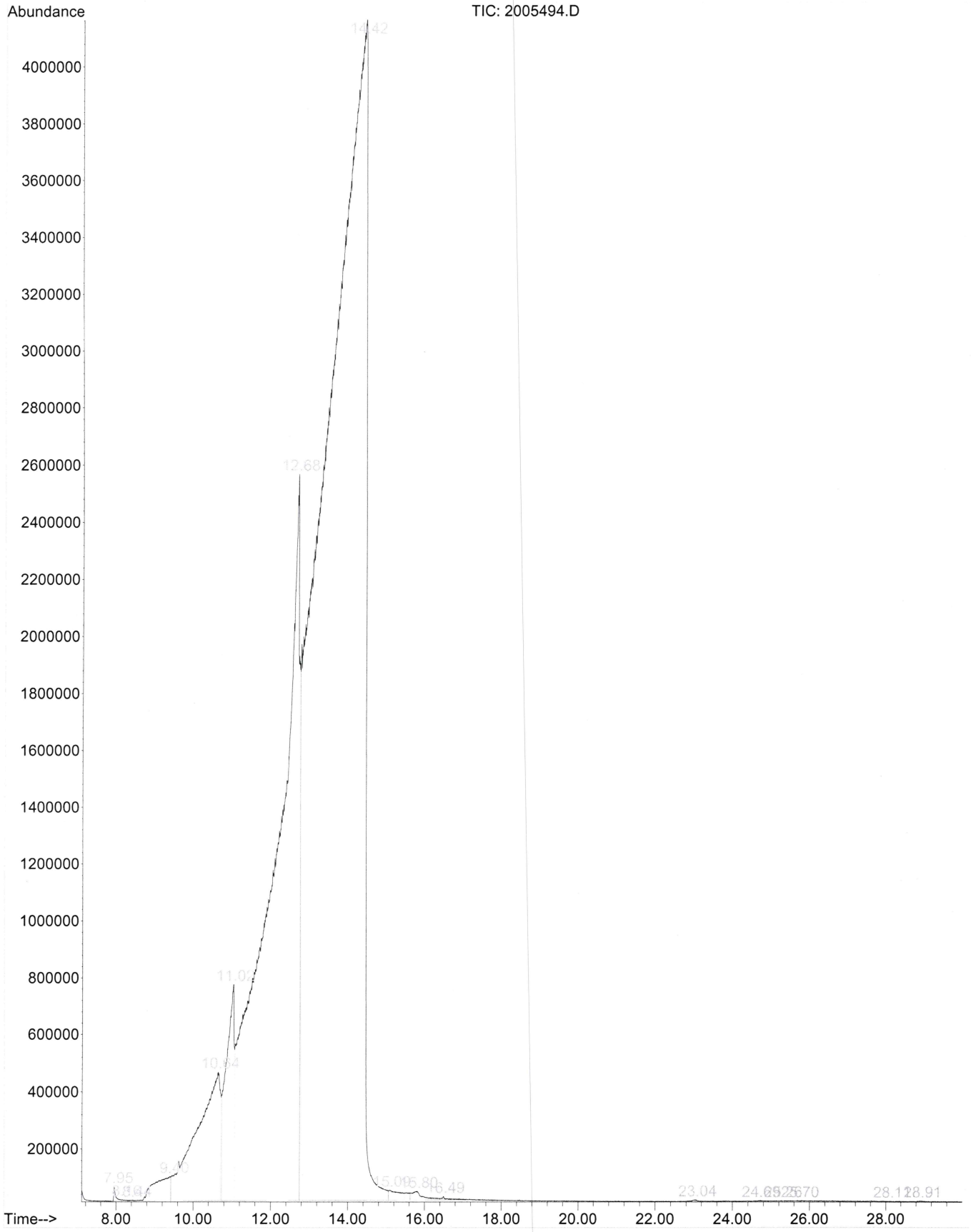
**** The European Cosmetics Directive regarding the potential fragrance allergens requires indicating the presence of 26 fragrance ingredients in finished cosmetic products.**

nd - denotes not detected at 0.001%

MR BENDRIK BAUMEISTER
ANALYTICAL OFFICER

MR ASHLEY DOWELL
MANAGER - ARL

```
File       :Y:\Data\200827\2005494.D
Operator   : BB
Acquired   : 27 Aug 2020  13:40     using AcqMethod 2019 COSMETIC ALLERGENS SCAN.M
Instrument :   Instrument #1
Sample Name: NE Snowflower
Misc Info  :
Vial Number: 3
```



02/09/20

Library Search Report

Data Path : Y:\Data\200827\
Data File : 2005494.D
Acq On : 27 Aug 2020 13:40
Operator : BB
Sample : NE Snowflower
Misc :
ALS Vial : 3 Sample Multiplier: 1

Search Libraries: C:\Database\CO...IC ALLERGENS .L Minimum Quality: 80

Unknown Spectrum: Apex
Integration Events: Chemstation Integrator - EVENTS.E

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	7.95	0.04	C:\Database\COSMETIC ALLERGENS .L No matches found			
2	8.16	0.01	C:\Database\COSMETIC ALLERGENS .L No matches found			
3	8.44	0.01	C:\Database\COSMETIC ALLERGENS .L No matches found			
4	9.40	0.56	C:\Database\COSMETIC ALLERGENS .L No matches found			
5	10.64	4.23	C:\Database\COSMETIC ALLERGENS .L No matches found			
6	11.02	2.44	C:\Database\COSMETIC ALLERGENS .L No matches found			
7	12.68	25.23	C:\Database\COSMETIC ALLERGENS .L No matches found			
8	14.42	67.14	C:\Database\COSMETIC ALLERGENS .L No matches found			
9	15.09	0.15	C:\Database\COSMETIC ALLERGENS .L No matches found			
10	15.80	0.14	C:\Database\COSMETIC ALLERGENS .L No matches found			
11	16.49	0.03	C:\Database\COSMETIC ALLERGENS .L No matches found			
12	23.04	0.01	C:\Database\COSMETIC ALLERGENS .L No matches found			
13	24.69	0.00	C:\Database\COSMETIC ALLERGENS .L No matches found			
14	25.26	0.00	C:\Database\COSMETIC ALLERGENS .L No matches found			
15	25.70	0.00	C:\Database\COSMETIC ALLERGENS .L No matches found			
16	28.12	0.00	C:\Database\COSMETIC ALLERGENS .L No matches found			
17	28.92	0.00	C:\Database\COSMETIC ALLERGENS .L No matches found			

2019 COSMET...RGENS SCAN.M Wed Sep 02 07:35:16 2020

02/09/20

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CERTIFICATE OF ANALYSIS

Melaleuca Alternifolia (Tea Tree) Leaf Extract

SAMPLE NAME	NE Snowflower Extract Concentrate		
FORM	Liquid		
CUSTOMER NAME	Native Extracts Pty Ltd		
CERTIFICATION DATE	22 November 2018		
CUSTOMER REFERENCE	030918-01		
ARL JOB #	A181882	**LAB REF. #**	ARL185724
ANALYSIS	LCMS Compositional analysis	**METHOD**	ARL-TM125
TEST PROFILE (below)	NE Snowflower Extract Concentrate 030918-01		

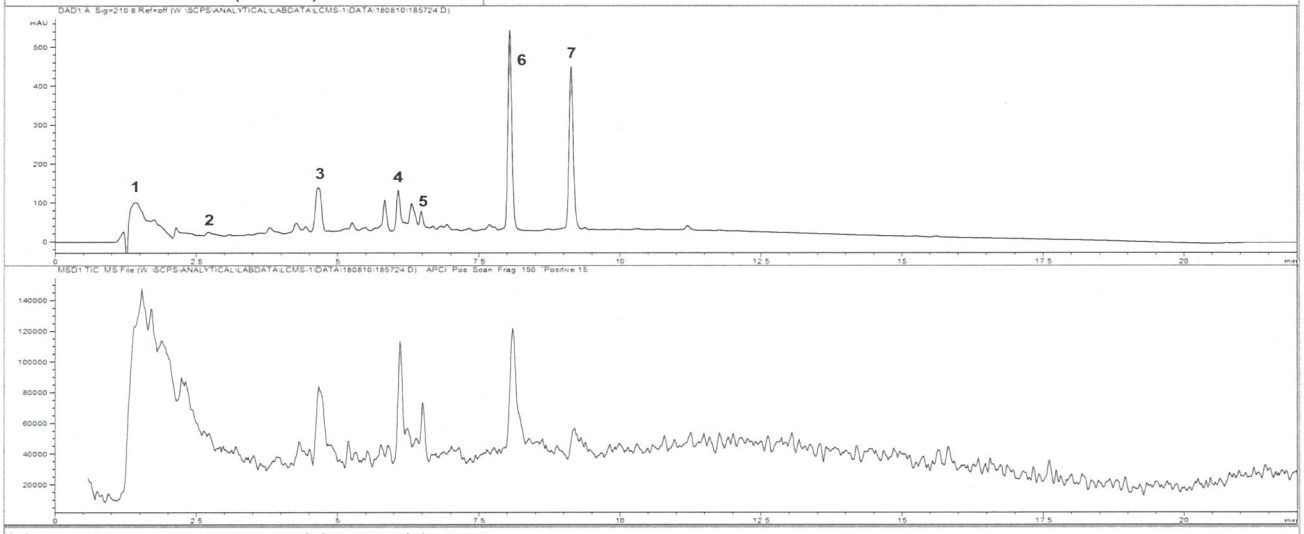


TABLE 1. PEAK IDENTIFICATION

Peak #	RT (min)	Fragment ions [M+H]	Tentative ID (MW)
1	1.5	116, 132, 146, 150	mixed peak - amine, amino acids
2	2.7	-	phenolic (protocatechuic acid)
3	4.7	181, 211	phenolic
4	5.8, 6.1	303, 479	flavonoid glycoside (quercetin glucunuride)
5	6.3, 6.5	287, 463	flavonoid glycoside (kaempferol gluconuride)
6	8.1	167, 182	phenolic
7	9.2	167, 182	phenolic

COMMENTS

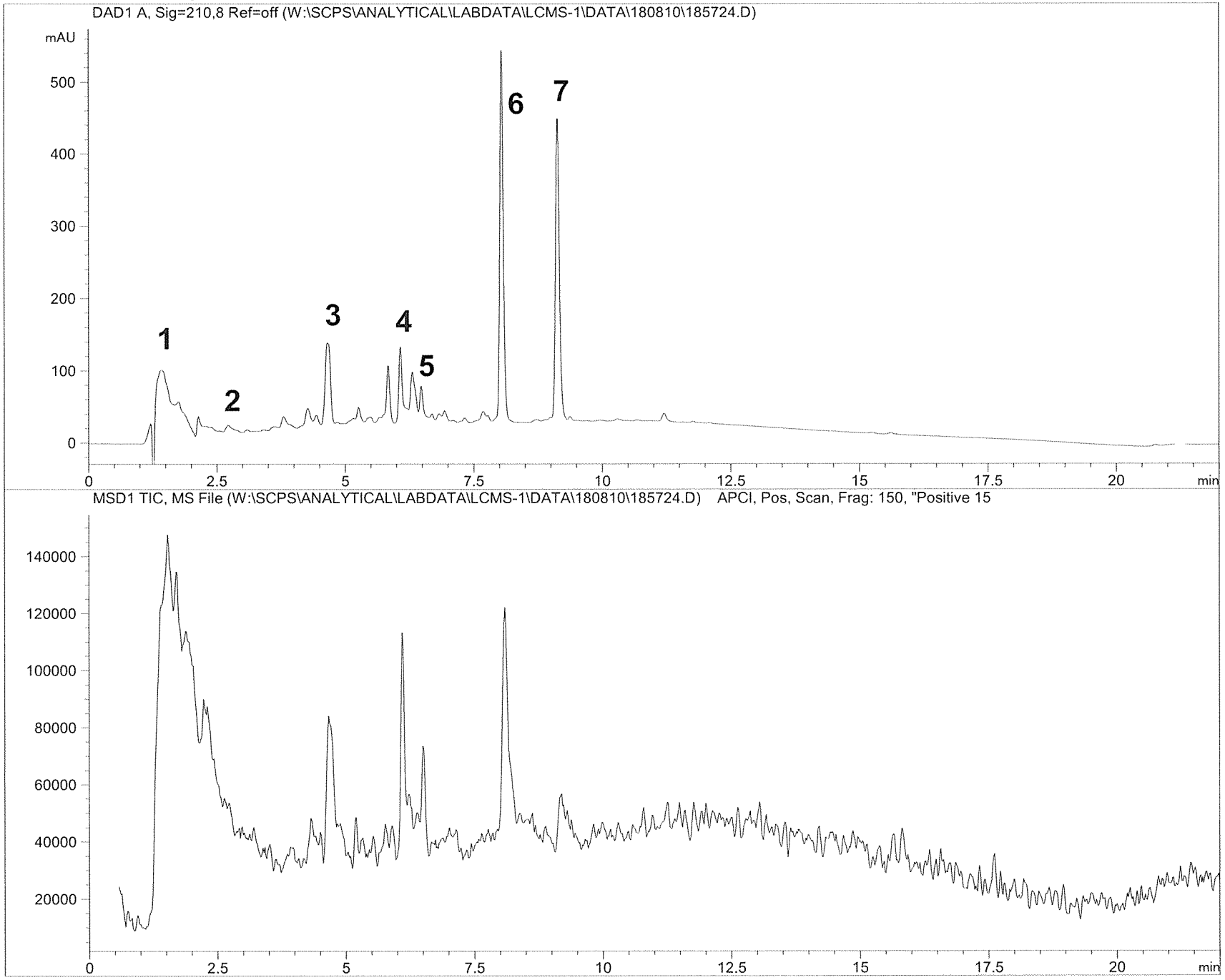
The HPLC-MS profile of the test sample is given above with some major components from the plant extracts indicated. The peaks identified are a range of phenolic and flavonoid derivatives, based on UV-Vis and MS spectra available. Spectral data in support of peak identification is attached.

Peter Mouatt
SENIOR ANALYTICAL OFFICER

P.P Ashley Dowell
MANAGER - ARL

Reference: Dictionary of Natural Products, CRC Press, 2018

Current Chromatogram(s)



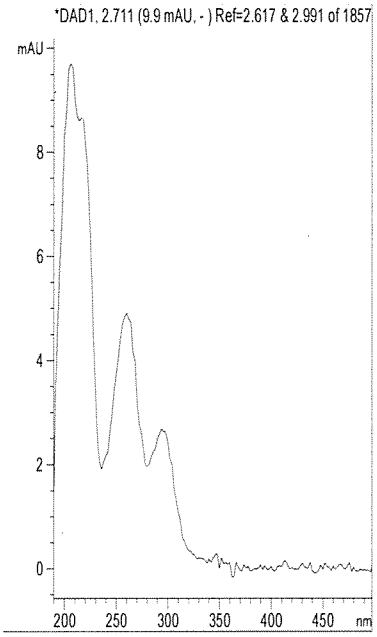


Figure 1. UV-Vis spectra of peak #2, identified as protocatechuic acid based on characteristic UV-Vis spectra

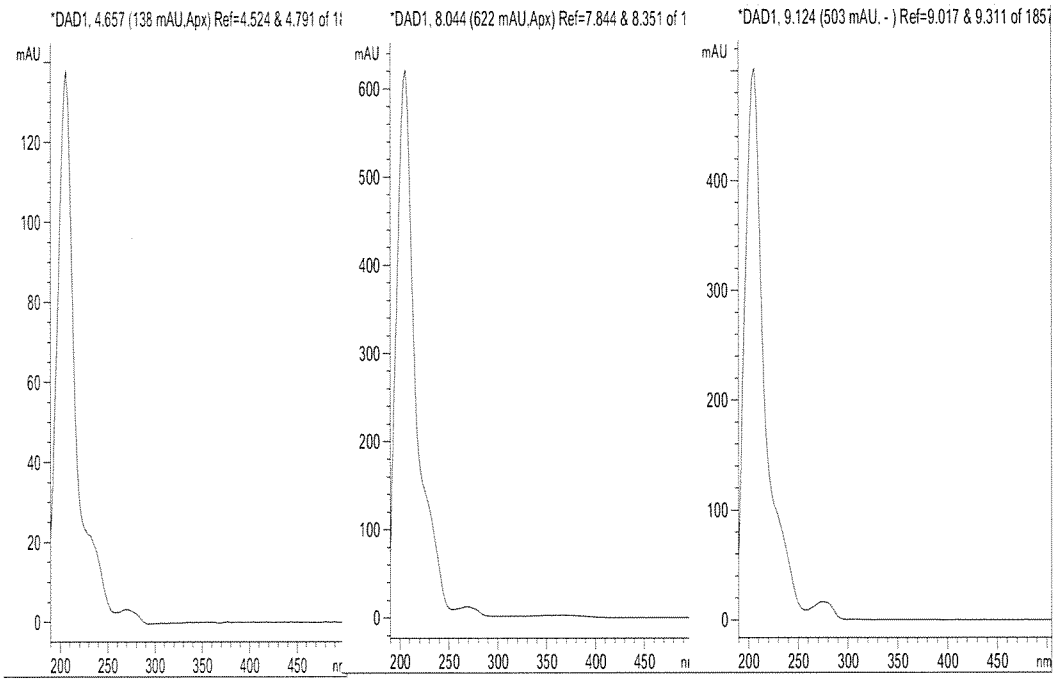


Figure 2. UV-Vis spectra of peaks #3, #6 and #7, identified as phenolics based on absorption maxima ~280nm

*DAD1, 6.071 (108 mAU, -) Ref=5.977 & 6.231 of 185 *DAD1, 6.484 (45.9 mAU,Apx) Ref=6.417 & 6.591 of 18

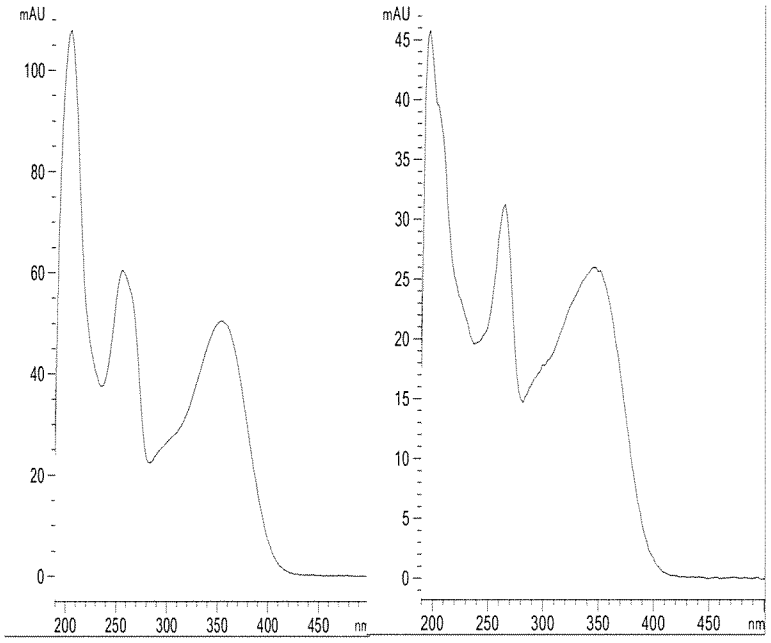
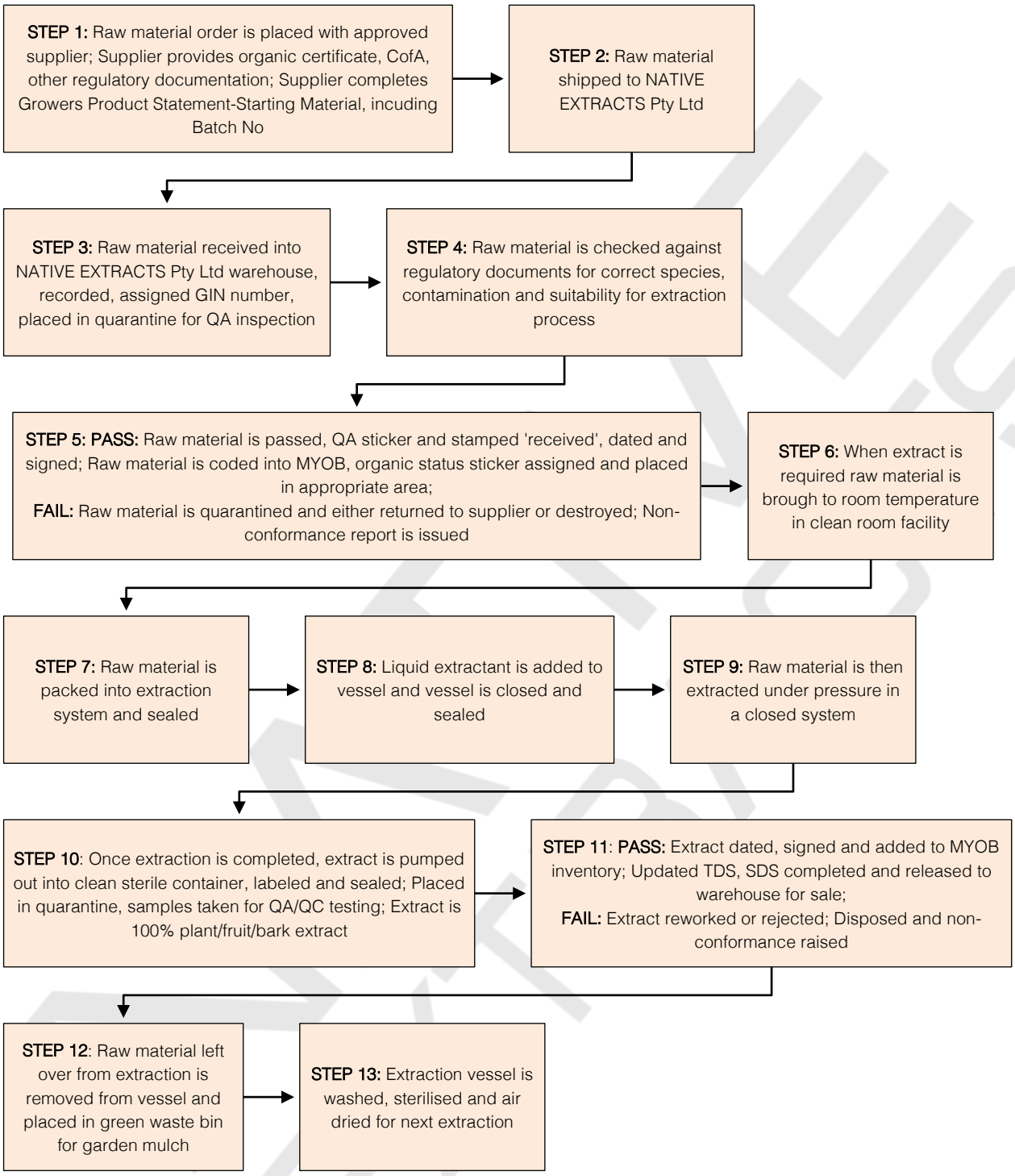


Figure 3. UV-Vis spectra of peaks #4 and #5, identified as flavone glycosides, queretin gluconuride and kaempferol gluconuride based on characteristic Uv-Vis and MS spectra,

MANUFACTURING CONCENTRATE FLOWCHART



A range of commercially cultivated, organic and wild harvested sources grown without the use of pesticides and following environmental practice to meet eco-sustainable and or organic guidelines.

Individual Datasheets, Specifications [TDS], CofA's and Safety Data Sheets [SDS] are available on request.

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NE CONCENTRATE MANUFACTURING FLOWCHART

REVISION No	DATE	NATURE OF CHANGE	APPROVED BY

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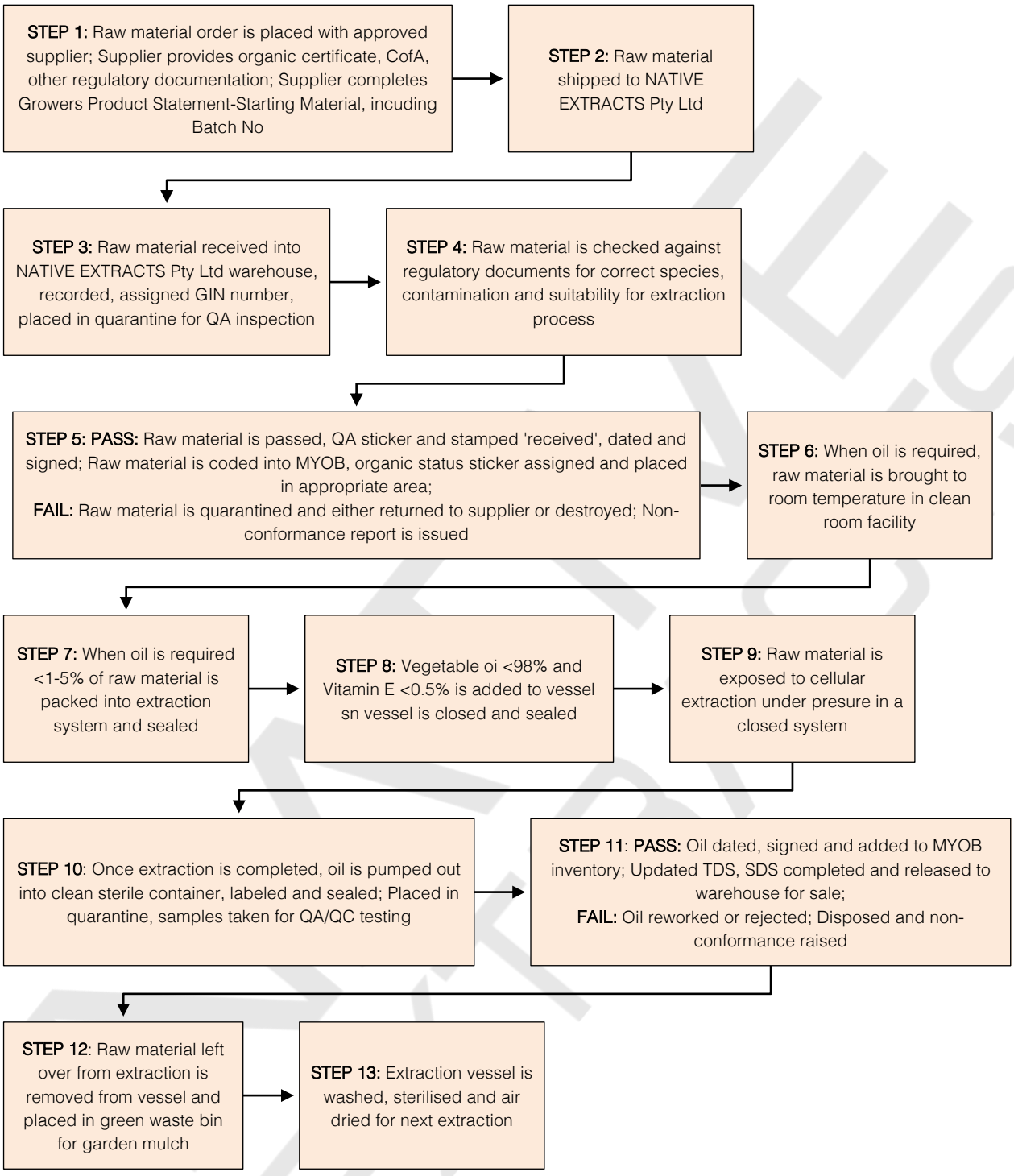
MANUFACTURING OIL FLOWCHART



A range of commercially cultivated, organic and wild harvested sources grown without the use of pesticides and following environmental practice to meet eco-sustainable and or organic guidelines.

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REVISION No	DATE	NATURE OF CHANGE	APPROVED BY

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Southern Cross University

Southern Cross Plant Science
ANALYTICAL RESEARCH LABORATORY

t: +61 2 6622 3211 • w: scu.edu.au/arl • T Block Level 3, Miltary Road, Lismore NSW 2480 Australia • ABN: 41995 651 524 • TGA: MI-01122004-LI-000264-1

CERTIFICATE OF ANALYSIS

Vitis Vinefera (Grape) Seed Oil and Melaleuca Alternifolia (Tea Tree) Leaf Extract

SAMPLE NAME	NSO Native Snowflower Oil		
FORM	Oil		
CUSTOMER NAME	Native Extracts Pty Ltd		
CERTIFICATION DATE	02 September 2020		
CUSTOMER REFERENCE	010619-01		
ARL JOB #	A202225	**LAB REF. #**	ARL2005495
ANALYSIS	Cosmetic Allergens	**METHOD**	ARL-TM284-1*

	TEST	SPECIFICATION %w/w		RESULTS %w/w
1	d-limonene **	≥ 0.01 rinse off	≥ 0.001 leave on	nd
2	benzyl alcohol **			nd
3	linalool **			nd
4	methyl heptine carbonate **			nd
5	citronellol **			nd
6a	citral-A neral **			nd
7	geraniol **			nd
6b	citral-B geranial **			nd
8	cinnamic aldehyde **			nd
9	anisyl alcohol **			nd
10	hydroxy-citronellal **			nd
11	cinnamic alcohol **			nd
12	eugenol **			nd
13	coumarin **			nd
14	trans iso-eugenol **			nd
15	γ-methyl ionone **			nd
16	oak moss **			nd
17	tree moss **			nd
18	lilial **			nd
19	cis amyl cinnamaldehyde **			nd
20	lyral **			nd
21	amyl cinnamic alcohol **			nd
22a	cis cis farnesol **			nd
22b	trans trans farnesol **			nd
23	trans hexyl cinnamaldehyde **			nd
24	benzyl benzoate **			nd
25	benzyl salicylate **			nd
26	benzyl cinnamate **			nd

*** Assay by GC (MS detection –area percent report)**

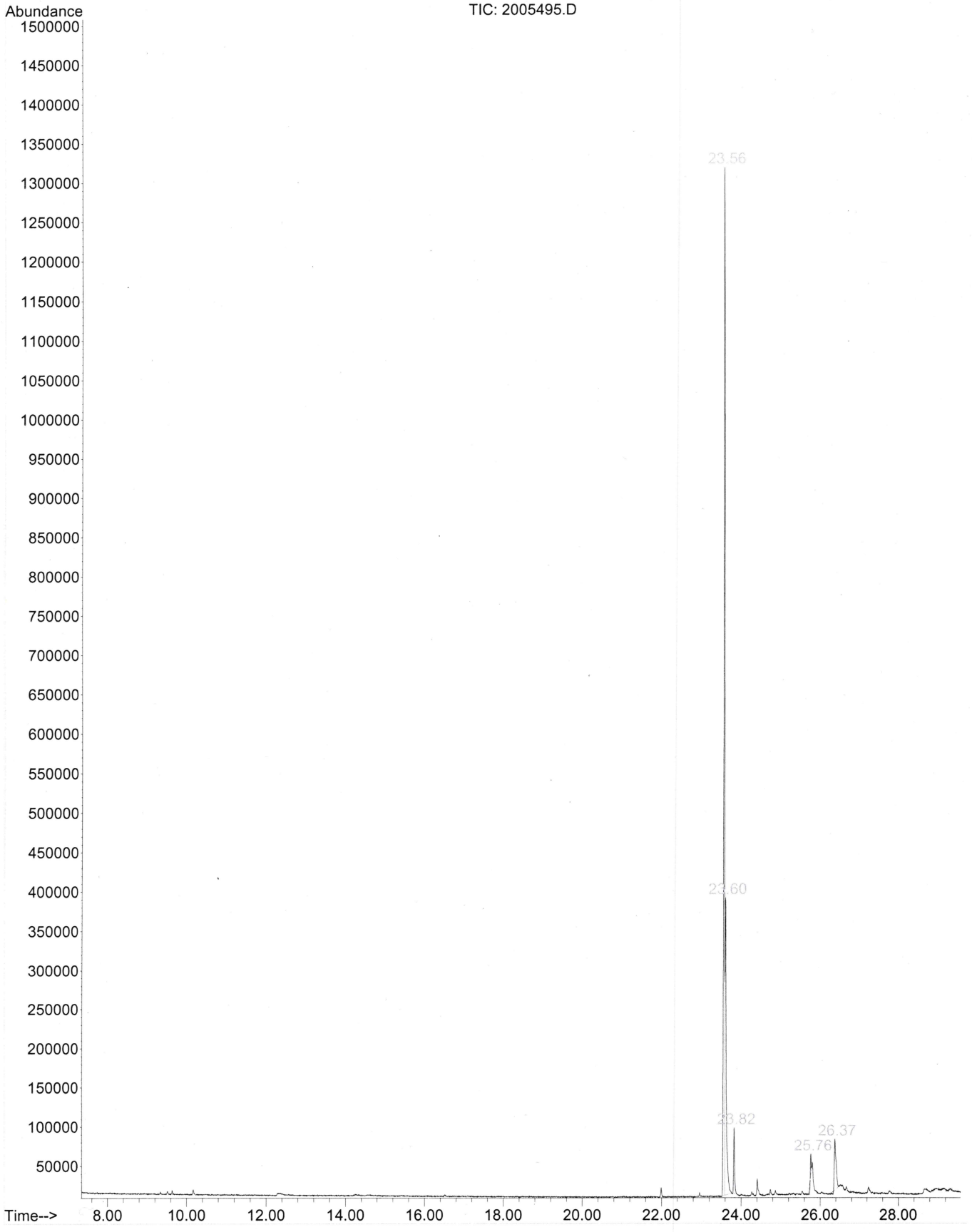
**** The European Cosmetics Directive regarding the potential fragrance allergens requires indicating the presence of 26 fragrance ingredients in finished cosmetic products.**

nd - denotes not detected at 0.001%

MR BENDRIK BAUMEISTER
ANALYTICAL OFFICER

MR ASHLEY DOWELL
MANAGER - ARL

File :Y:\Data\200827\2005495.D
Operator : BB
Acquired : 27 Aug 2020 14:58 using AcqMethod 2019 COSMETIC ALLERGENS SCAN.M
Instrument : Instrument #1
Sample Name: NSO Snowflower
Misc Info :
Vial Number: 4



02/09/20

Library Search Report

Data Path : Y:\Data\200827\
Data File : 2005495.D
Acq On : 27 Aug 2020 14:58
Operator : BB
Sample : NSO Snowflower
Misc :
ALS Vial : 4 Sample Multiplier: 1

Search Libraries: C:\Database\CO...IC ALLERGENS .L Minimum Quality: 80

Unknown Spectrum: Apex
Integration Events: Chemstation Integrator - autoint1.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	23.56	62.16	C:\Database\COSMETIC ALLERGENS .L No matches found			
2	23.60	27.25	C:\Database\COSMETIC ALLERGENS .L No matches found			
3	23.82	4.41	C:\Database\COSMETIC ALLERGENS .L No matches found			
4	25.76	0.22	C:\Database\COSMETIC ALLERGENS .L No matches found			
5	26.37	5.97	C:\Database\COSMETIC ALLERGENS .L No matches found			

2019 COSMET...LERGEN SIM.M Wed Sep 02 07:36:51 2020

02/09/20

SECTION 1. IDENTIFICATION OF THE SUBSTANCE AND SUPPLIER

PRODUCT IDENTIFER	
Product Name:	**NSO Snowflower Oil**
Botanical Name:	*Melaleuca alternifolia (and) Vitis vinifera*
Product Code:	**ANE0513**
INCI Name:	Vitis vinifera (Grape) Seed Oil (and) Melaleuca alternifolia (Tea Tree) Leaf Extract
CAS:	Not allocated
EC:	Not allocated
REACH Restriction:	Exempt from registration ex Annex V
UN Number:	Not required
HS Code:	1515.90.94
RECOMMENDED USE OF THE CHEMICAL AND RESTRICTIONS OF USE	
Relevant identified uses:	Cosmetic ingredient; Topical application; Not to be ingested
Usage:	2.0 - 5.0%
SUPPLIER DETAILS	
Name:	NATIVE EXTRACTS Pty Ltd
Address:	24 Kays Lane ALSTONVILLE NSW 2477 AUSTRALIA
Telephone:	+61 2 6686 5725
Email:	enquiries@nativeextracts.com
Website:	www.nativeextracts.com
EMERGENCY TELEPHONE NUMBERS [24/H/24H] – INTERNATIONAL CENTRES WITHIN YOUR COUNTRY	
AUSTRALIA:	Poisons Information Centre 13 11 26
USA:	Poison Control Centre 1-800-222-1222
GERMANY	Federal Institute for Risk Assessment
ITALY:	National Institute of Health
UNITED KINGDOM:	National Poison Information Services
OTHER COUNTRIES:	Please contact relevant government services

SECTION 2. HAZARDS IDENTIFIED

CLASSIFICATION OF THE SUBSTANCE OR MIXTURE	
POISONS SCHEDULE:	Unscheduled

NON-HAZARDOUS CHEMICAL – NON-DANGEROUS GOODS:
According to the WHS Regulations and the ADG Code; Globally Harmonized System of Classification and Labelling of Chemicals [GHS]; Regulation (EC) No 1223/2009 of the European Parliament and the Council of 30 November 2009 on cosmetic products (the "Cosmetics Regulation" or the "Regulation"), Governing the composition, labelling and packaging of finished cosmetic products.

CLASSIFICATION:	Not applicable
LABEL ELEMENTS	
GHS LABEL ELEMENTS:	Not applicable
SIGNAL WORD:	Not applicable
HAZARD STATEMENT[S]	
	Not applicable
PRECAUTIONARY STATEMENT[S]	
PREVENTION:	
	Not applicable
RESPONSE:	
	Not applicable
STORAGE	
	Not applicable
DISPOSAL:	
	Not applicable

SECTION 3: COMPOSTIONAL INFORMATION ON INGREDIENTS

SUBSTANCE CHEMICAL NAME	CAS No	EC	[%w/w]
Vitis vinifera (Grape) Seed Oil	8024-22-4 / 84929-27-1	284-511-6 / -	<98%
Melaleuca alternifolia Leaf	85085-48-9	285-377-1	<1.0 – 5.0%
Tocopherols [Mixed, low α-type]	1406-66-2	Not allocated	<0.5%

Cellular Extraction of manufactured in Australia

SECTION 4: FIRST AID MEASURES

DESCRIPTION OF FIRST AID MEASURES

EYE CONTACT: If this product comes into contact with the eye:
- Wash out immediately with fresh running water;
- Ensure complete irrigation of the eye by keeping eyelids apart and away from eye and moving the eyelids by occasionally lifting the upper and lower lids;
- Seek medical attention without delay; if pain persists or recurs seek medical attention;
- Removal of contact lenses after an eye injury should only be undertaken by skilled personnel.

SKIN CONTACT:
- First aid is not generally required; If in doubt, contact a Poisons Information Centre or doctor.

INHALATION:
- If fumes or combustion products are inhaled remove from contaminated area;
- Lay patient down. Keep warm and rested;
- Prostheses such as false teeth, which may block airway, should be removed, where possible, prior to initiating first aid procedures;
- Apply artificial respiration if not breathing, preferably with a demand value resuscitator, bag-valve mask device, or pocket mask as trained. Perform CPR if necessary;
- First aid is not generally .

SWALLOWED:
- Immediately give a glass of water;
- First aid is not generally required; If in doubt, contact a Poisons Information Centre or doctor.

INDICATION OF ANY IMMEDIATE MEDICAL ATTENTION AND SPECIAL TREATMENT NEEDED: Treat symptomatically.

SECTION 5: FIRE FIGHTING MEASURES

EXTINGUISHING MEDIA

Water spray or fog; Foam

SPECIAL HAZARDS ARISING FROM THE SUBSTANCE

FIRE INCOMPATIBILITY: Not applicable.

ADVICE FOR FIRE FIGHTERS

FIRE FIGHTING: Use water delivered as a fine spray to control fore and cool adjacent area.

FIRE/EXPLOSION HAZARD: Not applicable.

HAZCHEM: Not applicable.

SECTION 6: ACCIDENTIAL RELEASE MEASURES

PERSONAL PRECAUTIONS, PROTECTIVE EQUIPMENT AND EMERGENCY PROCEDURES

See Section 8.

ENVIRONMENTAL PRECAUTIONS

See Section 12.

METHODS OF MATERIAL FOR CONTAMINATION AND CLEAN UP

MINOR SPILLS:
- Remove all ignition sources;
- Clean up all spills immediately;
- Avoid breathing vapours and contact with skin and eyes;
- Control personal contact with the substance, by using protective equipment.

MAJOR SPILLS:
- MODERATE HAZARD: Clear area of personnel and move upwind;
- Alert Fire Brigade and tell them location and nature of hazard;
- Wear breathing apparatus plus protective gloves.

SECTION 7: HANDLING AND STORAGE

PRECAUTIONS FOR SAFE HANDLING

SAFE HANDLING:
- Avoid all personal contact, including inhalation;
- Wear protective clothing when risk of exposure occurs;
- Prevent concentration in hollows and sumps;
- DO NOT allow clothing wet with substance to stay in contact with the skin.

OTHER INFORMATION:
- Store in original containers;
- Keep containers securely sealed;
- No smoking, naked lights or ignition sources;
- Store in a cool, dry, well-ventilated area.

CONDITIONS FOR SAFE STORAGE, INCLUDING AND INCOMPATIBILITES

SUITABLE CONTAINERS:
Packaging as recommended by manufacturer;
Check all containers are clearly labelled and free from leaks.

STORAGE INCOMPATIBILITY: Avoid reaction with oxidising agents

X: Must not be stored together; **O:** May be stored together with specific preventions; **+:** May be stored together

Flammable	Explosive	Poison	Oxidising	Respiratory	Warning	Corrosive
+	X	O	O	+	+	+

SECTION 8: EXPOSURE CONTROLS/PERSONAL PROTECTION

CONTROL PARAMETERS

The product is not classified. No control parameters are to be mentioned.

EXPOSURE CONTROLS

APPROPRIATE ENGINEERING CONTROLS:

- Engineering controls are used to remove a hazard or place a barrier between the worker and the hazard. Well-designed engineering controls can be highly effective in protecting workers and will typically be independent of worker interactions to provide third high level of protection;
- The basic types of engineering controls are; Process controls which involve changing the way a job activity or process is done to reduce the risk;
- Enclosure and/or isolation of emission source which keeps a selected hazard 'physically' away from the worker and ventilation that strategically 'adds' and removes' air in the work environment.

PERSONAL PORTECTION:

EYE AND FACE PROTECTION:

- Safety glasses with side shield;
- Chemical goggles;
- Contact lenses may pose a special hazard; soft contact lenses may absorb and concentrate irritants. A written policy document, describing the wearing of lenses or restrictions on use, should be created for each workplace or task.

SKIN PROTECTION: See Hand Protection below.

HAND/FEET PROTECTION:

- Wear chemical protective gloves, e.g. PVC;
- Wear safety footwear or safety gumboots, e.g. Rubber;
- The selection of suitable gloves does not only depend on the material, but also on further marks of quality, which vary from manufacturer to manufacturer;
- Where the chemical is a preparation of several substances, the resistance of the glove material cannot be calculated in advance and has therefore to be checked prior to the application;
- The exact break through time for substances has to be obtained from the manufacturer of the protective gloves and has to be observed when making a final choice;
- Personal hygiene is a key element of effective hand care.

BODY PROTECTION: See Other Protection below.

OTHER: Overalls; PVC Apron; Barrier Cream.

STANDARDS: The following Australian Standards will provide general advice regarding safety clothing and equipment:

AS/NZS 1715:	Respiratory Equipment
AS 1161:	Protective Gloves
AS2919:	Industrial Clothing
AS1336/AS/NZS 1337:	Industrial Eye Protection
AS/NZS2210:	Occupational Protective Footwear

THERMAL HAZARDS: Not available

SECTION 9: PHYSICAL AND CHEMICAL PROPERTIES

PHYSICAL/CHEMICAL PROPERTIES	RESULT	PHYSICAL/CHEMICAL PROPERTIES	RESULT
APPEARANCE:	Viscous liquid	**WATER SOLUBILITY:**	Insoluble
ODOUR:	Characteristic	**FLASH POINT:**	<100°C [Closed cup]
COLOUR:	Yellow to green	**MELTING/FREEZING POINT:**	Not available
TASTE:	Not determined	**BOILING POINT RANGE:**	Not available
REFRACTIVE INDEX @20°C:	1.450 – 1.490	**VAPOUR PRESSURE:**	No data available
SPECIFIC GRAVITY @20°C:	1.900 – 0.940	**VAPOUR DENSITY:**	Not available
PEROXIDE VALUE:	2.87 mEq/Kg	**VISCOSITY, KINEMATIC:**	No data available
SOUABILITY:	Soluble in vegetable oils		

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SECTION 10: STABILITY AND REACTIVITY

REACTIVITY:	Oxidation with atmospheric oxygen; Formation of free fatty acids.
CHEMICAL STABILITY:	Stable under recommended handling and storage conditions; this material presents no significant reactivity hazard.
POSSIBILITY OF HAZARDOUS REACTIONS:	Reacts with oxidants.
CONDITIONS TO AVOID:	Avoid heat, flames, sunlight and other sources of ignition.
MATERIALS TO AVOID:	Strong oxidising agents.
INCOMPATIBLE MATERIALS:	See Section 7
HAZARDOUS DECOMPOSITION PROUCTS:	Product does not decompose with proper handling.

SECTION 11: TOXICOLOGICAL INFORMATION

INFORMATION ON TOXICOLOGICAL EFFECTION

INHALED: Not expected to be an irritant.

INGESTION: Not expected to be an irritant.

SKIN CONTACT: Not expected to be an irritant.

EYE: Not expected to be an irritant

CHRONIC: Not expected to be an irritant.

SCCNFP ALLERGENS ANNEX III – COSMETIC DIRECTIVE 2003/15/EC

7th Amendment Detection Limit 0.001%

CONSTITUENT	IFRA	EFFA	CAS	EC	RANGE
Amyl Cinnamal:	Yes	No	122-40-7	204-541-5	Not detected
Amyl Cinnamyl Alcohol:	Yes	No	101-85-9	202-982-8	Not detected
Anise Alcohol:	No	Yes	105-13-5	203-273-6	Not detected
Benzyl Alcohol:	No	Yes	100-51-6	202-859-9	Not detected
Benzyl Benzoate:	No	Yes	120-51-4	204-402-9	Not detected
Benzyl Cinnamate:	No	Yes	103-41-3	203-109-3	Not detected
Benzyl Salicylate:	No	Yes	118-58-1	204-262-9	Not detected
Cinnamal:	Yes	Yes	104-55-2	203-213-9	Not detected
Cinnamyl Alcohol:	Yes	Yes	104-54-1	203-212-3	Not detected
Citral:	Yes	Yes	5392-40-5	226-394-6	Not detected
Citronellol:	No	Yes	5392-40-5	203-375-0	Not detected
Coumarin:	No	Yes	91-64-5	202-086-7	Not detected
Eugenol:	Yes	Yes	97-53-0	202-589-1	Not detected
Farnesol:	Yes	Yes	4602-84-0	225-004-1	Not detected
Geraniol:	No	Yes	106-24-1	203-377-1	Not detected
Hexyl Cinnamal:	Yes	No	101-86-0	202-983-3	Not detected
Hydroxycitronellal:	Yes	No	107-75-5	203-518-7	Not detected
Isoeugenol:	Yes	Yes	97-54-1	202-590-7	Not detected
Butylphenyl Methylpropional:	Yes	No	80-54-6	201-289-8	Not detected
d-Limonene:	Yes	Yes	5989-27-5	227-813-5	Not detected
Linalool:	Yes	Yes	78-70-6	201-134-4	Not detected
Hydroxyisohexyl 3-Cyclohexene Carboxaldehyde:	No	No	31906-04-4	250-863-4	Not detected
Methyl 2-Octynoate:	Yes	No	111-12-6	203-836-6	Not detected
Alpha-Isomethyl Ionone:	Yes	No	127-51-5/ 90028-68-5	204-846-3/ 289-861-3	Not detected
Evernia Prunastri Extract [Oakmoss]:	Yes	No	9000-50-4/ 6817-10-2		Not detected
			90028-67-4/	289-860-8	
Evernia Furfuracea Extract[Treemoss]:	Yes	No	68648-41-9		Not detected

ADDITIONAL EFFA LISTED SENSITISERS & IFRA NOTIFIABLE SUBSTANCES

Detection Limit 0.001%

CONSTITUENT	IFRA	EFFA	CAS	EC	RANGE
No Additional Sensitisers:	No	No	Not allocated	Not allocated	Not detected
No Additional Notifiable Substances:	No	No	Not allocated	Not allocated	Not detected

SECTION 12: ECOLOGICAL INFORMATION

ECO-TOXICITY: None established; Use according to good working practices; Avoid pollution to soil, rivers and the ocean.

PERSISTENCE AND DEGRADABILITY:

- LOW persistence level and readily biodegradable; During natural decomposition;
- No dangerous products are developed; Use according to good working practice; pollution to soil, rivers and the ocean.

BIO-ACCUMULATIVE POTENTIAL: None established.

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MOBILITY IN SOIL: None established.

SECTION 13: DISPOSAL CONSIDERATIONS

WASTE TREATMENT METHODS

PRODUCT/PACKAGING DISPOSAL:

- Legislation addressing waste disposal requirements may differ by country, state and/or territory. Each user must refer to laws operating in their area. In some areas, certain wastes must be tracked;
- A Hierarchy of Control seems to be common – the user should investigate:
  - Reduction;
  - Reuse;
  - Recycle;
  - Disposal (if all else fails).
- It may be necessary to collect all wash water for treatment before disposal;
- In all cases disposal to sewer may be subject to local laws and regulations and these should be considered first;
- Where in doubt contact the responsible authority;
- Recycle wherever possible or consult manufacturer for recycling options;
- Consult State Land Waste Authority for disposal;
- Bury or incinerate residue at an approved site;
- Recycle containers if possible, or dispose of in an authorised landfill.

SECTION 14: TRANSPORT INFORMATION

LABELS REQUIRED

MARINE POLLUTANT:	No
HAZCHEM:	Not applicable
LAND TRANSPORT (AGD):	Not regulated for transport of Dangerous Goods
AIR TRANSPORT (ICAO-IATA/DGR);	Not regulated for transport of Dangerous Goods
SEA TRANSPORT (IMDG-Code/GGVSee):	Not regulated for transport of Dangerous Goods
UN NUMBER:	Not required
PROPER SHIPPING NAME:	Not required
TECHNICAL SHIPPING NAME:	Not applicable
DG CLASS/SUBSIDARY RISK:	Not applicable
PACKAGING GROUP:	Not allocated
SPECIAL PRECAUTIONS:	Not established
HAZCHEM CODE:	Not allocated

SECTION 15: REGULATORY INFORMATION

SAFETY, HEALTH AND ENVIRONMENTAL REGULATIONS/LEGISLATION SPECIFIC FOR THE SUBSTANCE OR MIXTURE

The substance is not listed as a hazardous chemical under the following international agreements:

- Montreal Protocol on Substances that Deplete the Ozone Layer;
- Stockholm Convention on Persistent Organic Pollutants;
- Rotterdam Convention on the Prior Informed Consent Procedure for Certain Hazardous Chemicals and Pesticides in International Trade;
- Basel convention on the Control of Trans boundary Movements of Hazardous Wastes and their Disposal;
- International Convention for the Prevention of Pollution from Ships (MARPOL);
- Standard for the Uniform Scheduling of Medicines and Poisons (SUSMP);
- Agriculture and Veterinary Chemicals Code Act 1994;
- Australian Inventory of chemical Substances (AICS).

SUBSTANCE CHEMICAL NAME

Vitis vinifera (Grape) Seed Oil (and) Melaleuca alternifolia (Tea Tree) Leaf Extract

NATIONAL INVENTORY	COUNTRY	STATUS ✓×
Australian Inventory of Chemical Substances (AICS):	AUSTRALIA	×
Domestic Substances List (DSL):	CANADA	×
Non-Domestic Substances List (NDSL):	CANADA	×
Inventory of Existing Chemical Substances Produced for Imported to China (IECSC):	CHINA	×
European Chemicals Agency (ECHA-EINECS-ELINCS-NLP-COSING):	EUROPE	×
Japanese Existing and New Chemical Substances Inventory (ENCS):	JAPAN	×
Korea Existing Chemicals Inventory (KECI):	SOUTH KOREA	×
New Zealand Inventory (NZIoC):	NEW ZEALAND	×
Philippines Inventory of Chemicals and chemical Substances (PICCS):	THE PHILLIPPINES	×
Toxic Substances Control Act (TSCA):	USA	×
Taiwan Chemical Substance Inventory (TCSI):	TAIWAN	×
Vietnam National Chemical Database System	VIETNAM	×

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SECTION 16: ADDITIONAL INFORMATION

QUALITY STATEMENT

NATIVE EXTRACTS Pty Ltd specialises in the manufacture and supply of the highest quality, pure, naturally derived phyto-active compounds in hydrophilic extracts, seed oils and pure natural powders; for use in the Cosmetic, Pharmaceutical and Nutraceutical industries globally. Our company's objective is to manufacture and supply the highest quality and purity of natural ingredients across multiple delivery formats that meet the application/formulation objectives and specifications of our customers. Our commitment to quality extends beyond our products and applies to our blends, services, workplace, environmental practices and partnership and relationships engaged with commercial growers and Indigenous communities.
Any quality problems arising will be identified and solved with speed, technical efficiency and economy, stakeholder engagement – focusing our human and technical resources internally and externally to the prevention of quality deficiencies to meet our company goal of "right first time, every time".
The successful operation of our QMS relies on the cooperation, participation and engagement of our personnel across all areas of the company. Our commitment to quality underpins our continued success, the satisfaction of customers and staff, our pursuit to achieve new scientific discoveries and new benchmarks in performance ingredients. We are committed to improving our performance in every aspect of our business.
NATIVE EXTRACTS will to provide high and consistent quality in Botanical extracts and naturally derived phyto-active ingredients, evolving the botanical extract from inferior processes and synthetic standardisation to the delivery of stable, active True to Nature phyto-activity, influencing new innovation in natural product development, new advances in consumer experiences, influencing the emergence of new primary industry partnerships, and participating in socially and environmentally responsible practices.
Our commitment is to safety and accurate work to ensure our ingredients conform to various regulatory bodies locally and internationally and are safe to our customers, their clients and the environment. All work is done in conformance to NATIVE EXTRACTS' QMS, the applicable technical and administrative operating policies and procedures of NATIVE EXTRACTS, legal and regulatory requirements, and specific customer requirements.
Through front-line input and management leadership, we will continue to improve our people and processes to anticipate, meet, and exceed the needs of our customers. We support the continually improving quality of our customer's maintenance and other technical operations through the services we provide.

ANIMAL TESTING

NATIVE EXTRACTS Pty Ltd does not test raw materials on animals, neither initially nor as a routine test. The product suppliers for NATIVE EXTRACTS Pty Ltd do not test their products on animals, neither initially nor as a routine test. None of NATIVE EXTRACTS Pty Ltd finished extracts are tested on animals, either initially or as a routine test.

MANUFACTURING PRODUCTS INGREDIENTS DISCLAIMER

As the availability of ingredients and raw materials is not always certain whether due to changes in nature or otherwise, NATIVE EXTRACTS Pty Ltd reserves the right to substitute alternate ingredients/raw materials in the manufacture of its products in order to maintain supply to its customers. Customers should always refer to the ingredients label as affixed to each product or to specification sheets, which are current at all time of supply of the product.

LABELLING DISCLAIMER

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ACRONYMS

<	Less than	LDLo	LDLo stands for Lethal Dose Low, the minimum amount of a material which tests have shown will be lethal to a specified type of animal. This is normally quoted in mg.kg body weight.
>	Greater than	Lt	Litre
°C	Degrees Celsius	Max.	Maximum
ACCC	Australian Competition and Consumer Commission	Mg	Milligram
ADG	Australian Dangerous Goods	Min.	Minimum
AICS	Australian Inventory of Chemical Substances	ml	Millilitre
AICS	Australian Inventory of Chemical Substances	M^3	Cubic metre
ACGIH	American Conference of Government Industrial Hygienists	mm	Millimetre
AS	Australian Standards	mm Hg	Millimetre of Mercury
BOD	Biochemical Oxygen Demand	N/A NA	Not Applicable
CAS	Chemical Abstracts Service (Registry Number)	NICNAS	The National Industry Chemicals Notification ants Assessment Scheme (AUSTRALIA)
Cm^3	Cubic centimetres	NIOSH	The National Institute for Occupational Safety and Health (USA)
COD	Chemical Oxygen Demand	NOHSC	National occupational Health and Safety Commission (AUSTRALIA)
CosIng	The European Commission database with information on Cosmetic Ingredients and Substances	n.o.s.	Not otherwise specified
DG	Dangerous Goods	NZS	New Zealand Standards
EC	European Commission	NZIoC	New Zealand Inventory of Chemicals
EC50	EC stands for the effective concentration. EC50 refers to the concentration of a toxicant, which includes a response halfway between the baseline and maximum after a specified exposure time	OECD	Organisation for Economic Co-operation and Development (Test Method number)
EINECS	European Inventory of Existing Commercial Chemical Substances (Identifying Number)	OSHA	The Occupational Safety and Health Administration (USA)
EFFA	European Flavour Association	PEL	Permissible Exposure Limit
EU	Europe/European Union	Ppb	Parts per billion
g	grams	Ppm	Parts per million
GHS	The Globally Harmonised System of Classification and Labelling of Chemicals	RTECS	The Registry of Toxic Effects of Chemical Substances

24 Kays Lane ALSTONVILLE NSW 2477 AUSTRALIA **P +61 2 6686 5725**

GMO	Genetically modified organism	SCCNFP	Scientific Committee on Cosmetic Products and non-Food Products (EUROPE)
Hazchem Code	Emergency action code of numbers and letters that provide information to emergency services especially fire fighters	SDS	Safety Data Sheet
hr	Hour	STEL	Short Term Exposure Limit
HSIS	The Safe Work Australia Hazardous Substances Information System	Subsp.	Subspecies
HSNO	Hazardous Substances Approval Code	Subspecies	Standard for the Uniform Scheduling of Medicine and Poisons (AUSTRALIA)
IATA	The International Air Transport Association	TD	TD stands for Toxic Dose. TD is the amount given all at once, which causes the untoward symptoms in the majority of persons, or in the majority of a group of test animals. This is normally quoted in mg/kg body weight.
ICAO	The International Civil Aviation Organisation	TGA	Therapeutic Goods Administration (AUSTRALIA)
IFRA	The International Fragrance Association	TLV	Threshold Limit Value
IMDG	International Maritime Dangerous Goods	TWA	Time Weighted Average
INCI	The International Nomenclature of Cosmetic Ingredients	UK	United Kingdom
ISO	International Organisation for Standardisation	USA	The United States of America
Kg	Kilograms	µg	Microgram
LC50	LC stands for lethal concentration. LC50 is the concentration of a material in air which causes the death of 50% (one half) of a group of test animals. The material is inhaled over a set period of time, usually 1 or 4 hours. This is normally quoted in mg/kg body weight.	µl	Micro litre
LD50	LD50 stands for Lethal Dose. This is the amount of a material, given all at once, which causes the death of 50% (one half) of a group of test animals. This is normally quoted in mg/kg body weight.		

DATA SOURCE

AICS; Australian Code for the Transport of Dangerous Goods by Rail and Road; Globally Harmonized System of Classification and Labelling of Chemicals (GHS) [NOHSC:1008(2004)]; Work Safe Australia WHS Regulations; Coslng; Supplier Documentation; EFFA; HSIS; IATA Dangerous Goods Regulations; IFRA; IMDG Code; The International Cosmetic Ingredients Dictionary and Handbook; NICNAS; SUSMP; NZIoC; NOHSC Australia.

DOCUMENT PREPARED BY

Vanessa Minnikin, Quality Assurance. Email: vminnikin@nativeextracts.com

Southern Cross University

Southern Cross Plant Science
ANALYTICAL RESEARCH LABORATORY

t: +61 6622 3211 • w: scu.edu.au/scps • T Block Level 3, Miltary Road, Lismore NSW 2480 Australia • ABN: 41995 651 524 • TGA: MI-01122004-LI-000264-1

CERTIFICATE OF ANALYSIS

Vitis Vinifera (Grape) Seed Oil and Melaleuca Alternifolia (Tea Tree) Leaf Extract

SAMPLE NAME	NSO Snowflower Oil		
FORM	Oil		
CUSTOMER NAME	Native Extracts Pty Ltd		
CERTIFICATION DATE	17 September 2018		
CUSTOMER REFERENCE	040918-01		
ARL JOB #	A182122	**LAB REF. #**	ARL186664
ANALYSIS	FAMES	**METHOD**	ARL-TM149

TEST	SPECIFICATION	RESULTS
	Area %	
Myristic acid	Not Specified	0.05
Palmitic acid		6.02
Palmitoleic acid		0.06
Magaric		0.03
Stearic acid		3.53
Oleic acid		14.84
cis-vaccenic acid		0.78
Linoleic acid		68.11
a-linolenic acid		0.35
arachidic acid		0.19
11-ecosenoic acid		0.19
8,11,14 Eicosatrienoic		0.05
Behenic acid		0.09

*** Assay by GC (FID detection –Area percent report)**

Bijayalakshmi . N.

DR BIJAYALAKSHMI NONGMAITHEM
ANALYTICAL OFFICER

MR ASHLEY DOWELL
MANAGER - ARL

Data File D:\DATA\180912A\2018-09-12 16-54-28\A186664.D
Sample Name: NSO Snowflower oil

```
==========================================================================
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Acq. Instrument  : GC-1                             Location : Vial 6
Injection Date   : 9/13/2018 1:22:45 AM                  Inj :   1
                                                  Inj Volume : 1 µl
Acq. Method      : D:\DATA\180912A\2018-09-12 16-54-28\FAMES35L.M
Last changed     : 7/31/2018 1:18:22 PM by Bijaya
Analysis Method  : D:\METHODS\QA METHODS\SNOWFLOWER.M
Last changed     : 9/17/2018 1:44:13 PM by Bijaya
Method Info      : FAMES BPX70
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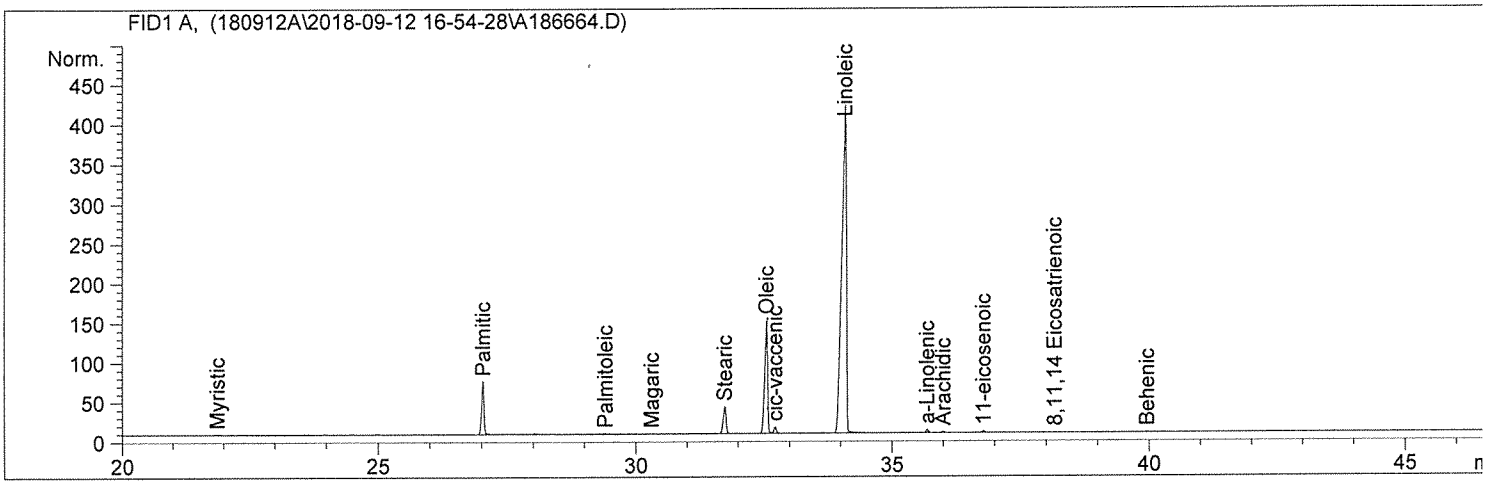



```
==========================================================================
                          Area Percent Report
==========================================================================

Sorted By             :      Signal
Calib. Data Modified  :      9/17/2018 1:39:21 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: FID1 A,

Peak RetTime  Type    Width     Area      Area     Name
  #   [min]           [min]    [pA*s]       %
----|-------|------|-------|----------|--------|--------------------
   1  21.875 VV      0.0665    1.87823  0.04903 Myristic
   2  27.026 VV      0.0545  230.72142  6.02322 Palmitic
   3  29.404 VB      0.0558    2.28595  0.05968 Palmitoleic
   4  30.308 VV      0.0566    1.14824  0.02998 Magaric
   5  31.737 VV      0.0629  135.08960  3.52665 Stearic
   6  32.561 BV      0.0584  568.59802 14.84383 Oleic
   7  32.721 VV      0.0557   30.03362  0.78406 cic-vaccenic
   8  34.107 VV      0.0817 2608.92334 68.10861 Linoleic
   9  35.682 VV      0.0515   13.49355  0.35226 a-Linolenic
  10  35.999 VV      0.0587    7.34527  0.19176 Arachidic
  11  36.779 VV      0.0545    7.34650  0.19179 11-eicosenoic
  12  38.166 VV      0.0622    2.04365  0.05335 8,11,14 Eicosatrienoic
  13  39.956 VV      0.0825    3.56269  0.09301 Behenic

Totals :                    3612.47008  94.3072
```

Literature review on tea tree oil

Toxicity profiles for tea tree oil, constituents of tea tree oil and known oxidation products

By
Jesper Bo Nielsen, PhD

November 2005

Contents

1 Preface

1.1 Background

In 2003, Jesper Nielsen PhD provided RIRDC with a review of the data gaps that existed to demonstrate the toxicology and safety of tea tree oil. This review of data was measured against criteria to be satisfied in the following European regulatory arenas: Cosmetics, Pharmaceuticals, and biocides. The review clearly highlighted relevant data gaps and outlined weaknesses in existing data. Since that report in 2003, the SCCP has issued an opinion on Tea Tree Oil which has concluded that the committee has insufficient data to make an assessment on its safety. As a result of the previous literature review and the publication of the SCCP opinion, the Australian tea tree industry has together with the Australian government (through RIRDC) commissioned a literature search on the toxicity of individual tea tree oil components including potential products formed due to oxidation of the oil. The present report has therefore to be seen as a supplement to the initial report with focus on the toxicity profiles of the individual components and potential oxidation products of tea tree oil. The following Terms of Reference were developed.

1.2 Terms of Reference

Using the data provided by RIRDC the review should critically evaluate the available literature and cover the following terms:

1) Acute toxicity
2) Skin and eye irritation
3) Skin and respiratory sensitization
    a. Skin sensitization/allergenicity
        i. Animal data
        ii. Human data
        iii. Rate of allergic reactions
        iv. Existence of subgroups with increased susceptibility
        v. Identification of causative agent/s
4) Dermal/percutaneous absorption
5) Repeat dose toxicity
6) Mutagenicity/genotoxicity
7) Carcinogenicity
8) Reproductive toxicity
9) Toxicokinetics
10) Phototoxicity

Further, the review should try to identify an appropriate No Observed Adverse Effect Level (NOAEL) for tea tree oil, which could be used in the calculation of a Margin of Safety for tea tree oil.

The level of detail used in the review should be sufficient to allow the SCCP evaluator to independently verify the conclusions. Studies not covered in the SCCP opinion should be highlighted to assist the SCCP.

2 Executive summary

The present review is based on the publicly available literature and summarizes the toxicity profiles of the 14 individual constituents of TTO with an expected concentration in newly refined TTO above 0.5 % and five known oxidative degradation products from TTO.

The purpose has been to supply SCCP and other regulatory agencies with an updated review of the relevant literature on the human toxicity of TTO and TTO constituents including suggestions for "No observed adverse effect levels" (NOAELs) for specified targets as well as an overall NOAEL for TTO. The review will also identify potential problems related to the use of TTO products and provide possible approached to be considered by the industry.

- TTO products and formulations have generally, except for the neat products, been reported to be without significant risk for acute human toxicity. Oral exposure to neat TTO does, however, have a clear potential for servere human toxicity.

- The known toxicokinetics indicate transport to the liver, hepatic biotransformation followed by renal elimination. The relatively short elimination half-lives expected on the basis of the presently known information on TTO constituents does not indicate significant accumulation over time of either parent compound or metabolites.

- The NOAEL for irritative effects of TTO is expeted to be equal to or above 25% based on human studies and considering the experimental studies probably below 50%.

- The allergic potential of freshly produced *Melaleuca alternifolia* oil is presumed to be low on healthy skin, whereas photoaged *Melaleuca alternifolia* oil must be considered to be a stronger sensitizer due to formation of oxidative degradation products.

- The prevalence of positive findings following exposure of pre-sensitized dermatological patients in the clinical studies is generally around 0.4-0.6%. Thus TTO has probably a weak sensitizing potential among pre-sensitised people, though the present known number may be an overestimate due to problems with aged testing material and selection bias in some clinical studies.

- Oxidative degradation products from TTO appear to possess a clear sensitizing potency.

- The formation of oxidation products in TTO and TTO products need to be controlled. Whether this apparently technical problem can be dealt with during production, through addition of anti-oxidants, or through documented shelf-lives for the products is an issue that needs appropriate consideration.

- Several constituents of TTO do not cause toxicity themselves, but enhance the percutaneous penetration of other substances.

- The relative occurrences of individual constituents of TTO differ between what is applied on the skin and what is absorbed. The penetration rates for those TTO constituents eventually penetrating the skin, i.e. terpinen-4-ol and α-terpineol (the least lipophilic) are relatively low.

- Based on the available information on the repeat dose toxicity, the renal effects would have the lowest estimated NOAEL. Present data suggest a NOAEL of 510 mg/kg with a worst case scenario estimate of 117 mg/kg b.w.

- Two TTO constituents (1,8-cineole and phellandrene) may act as weak promoters. There is no strong evidence that any of the TTO constituents are mutagenic. The carcinogenic mechanism explaining the gender and species specific renal tumors induced by limonene in male F344 rats is not seen in humans. Based on the available information, neither TTO nor its constituents are expected to pose any carcinogenic risk to humans.

- Among constituents of TTO for which evidence of potential foetotoxicity is available, α-terpinene has the lowest estimate of a NOAEL (30 mg/kg bw) and the highest relative occurrence (9% on average) in TTO. Based on reproductive toxicity, a NOAEL for TTO can tentatively be set at 330 mg TTO/kg bw following oral exposure.

- An overall NOAEL for TTO based on the presently available scientific information is based on the potential foetotoxicity of a TTO constituent and is estimated at 330 mg/kg b.w. A margin of safety estimate for dermal use of TTO products based on this value would need to incorporate the fraction of an applied dose absorbed and the actual concentration of TTO in the product besides an estimate of the amount of TTO applied on the skin.

3 Literature search strategy

A search was made for each of the relevant components/products in scientific literature databases and on the internet. These search results were then combined with various keywords to limit the results to information relating to the toxicity of the components.

3.1 Data sources

The following literature databases were searched in August 2005:

- Medline (via UWA library)
- Biological Abstracts (via UWA library)
- Agricola (via UWA library)
- Scopus (http://www.scopus.com/scopus/search/form.url)
- Web of knowledge (including Current Contents) (via UWA library)
- PubMed (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi)
- Ingenta (http://www.ingentaconnect.com/)

Documents and data were also sourced from the internet. In particular, the following websites were searched;

- National Toxicology Program (http://ntp-server.niehs.nih.gov/)
- Toxnet (http://toxnet.nlm.nih.gov/)
- IPCS Intox databank (http://www.intox.org/databank/index.htm)

Data was requested from the Research Institute for Fragrance Materials (RIFM). Reports were generated for many of the components. These were reviewed and any additional references added to the Endnote database. RIFM also has unpublished reports on the dermal irritation and sensitisation capacity of several components of tea tree oil (eg. α-pinene). These reports have not been available for this review.

Bibra Information Systems Ltd. has published toxicity profiles for the following compounds; Eucalyptol (1991), Terpinolene (1993), Linalool (1995), α- and γ-terpinene (1992), α-terpineol (2001), α-pinene (2002) and α-phellandrene (1993). These profiles are generally short summaries of published literature at the time of publication. As most reports are of older data, we have refrained from including them in our review.

3.2 Search terms

The present review is based on the publicly available literature and summarizes the toxicity profiles of the 14 individual constituents of TTO with an expected concentration in newly refined TTO above 0.5 % given by the official ISO-norm for TTO (Table 1). Besides these 14 constituents, the review includes toxicity profiles on five known oxidative degradation products from TTO. For a thorough review of the toxicological profile of TTO, the reader is referred to a review on TTO toxicity for RIRDC (Nielsen 2003) or a recently published review article (Hammer et al. 2005).

Reading and understanding toxicological profiles requires appreciation of the difference between hazard and risk. Thus, exposure to a hazardous chemical may occur without any significant health risk, given that the exposure/dose is

sufficiently low. During the discussion of potential risks associated with exposure to the individual constituents of TTO, the concentrations of these constituents in the oil and in products will be considered.

Table 1. Main constituents of TTO with expected range and average percentages for premium grade TTO. The main constituents of TTO are terpenes (C_{10}); sesquiterpenes (C_{15}) constitutes only a small fraction.

Constituent	Min-Max (%)	Average (%)
Terpinen-4-ol	37 – 45	41.0
γ-terpinene	10 – 28	19.0
α-terpinene	5.0 – 13	9.0
p-cymene	0.5 – 12	6.0
1,8-cineole	3.0 – 7.0	5.0
α-terpineol	1.5 -8.0	4.8
δ-cardinene	Traces – 8.0	4.0
Aromadendrene	Traces – 7.0	3.5
α-pinene	1.0 – 6.0	3.5
Terpinolene	1.5 – 5.0	3.3
Limonene	0.5 – 4.0	2.3
Sabinene	Traces – 3.5	1.7
Globulol	Traces – 3.0	1.5
Viridiflorol	Traces – 1.5	0.7

Data on components was searched for using the following component names: terpinen-4-ol, terpinene, 1,8-cineole, eucalyptol, terpinolene, cymene, pinene, terpineol, aromadendrene, cadinene, limonene, sabinene, globulol, and viridiflorol. Searches were also conducted for the autoxidation products ascaridol(e), isoascaridol(e) and 1,2,4-trihydroxymenthane.

Alternate names and synonyms such as terpinenol, carvomenthenol, and eucalyptol were also used.

Search results for each component were combined with search results for each of the terms listed in Table 2. Additional search terms such as NOAEL and Draize were also used. Terms were truncated so that permutations of each search term would be identified.

Table 2. Terms used to search for data relating to the toxicity of Tea tree oil components (asterisk indicates a wildcard)

Allerg*	Hepato*	Poison*
Carcino*	Irrit*	Rat
Chronic	Metaboli*	Sedat*
Embryo*	Mutagen*	Sensiti*
Foeto/Feto*	Nephro*	Teratogen*
Genotox*	Neuro*	Toxic*

4 Summary of toxicity profiles for TTO constituents

4.1 Acute toxicity

Human evidence based on casuistic reports clearly demonstrates that TTO may cause severe acute toxicity following oral exposure to neat TTO. Temporary depression of the central nervous system has been reported in children drinking no more than a few teaspoons of 100% TTO. Based on the published cases, intoxicated children have not experienced prolonged and severe sequela. However, more serious and potentially fatal effects following unintentional oral intake of neat TTO can not be excluded, and such risks should be minimized. There are no reports available on human intoxications due to oral intake or dermal use of diluted/formulated TTO products.

In experimental animals, the oral as well as dermal LD_{50} values are generally in the range of 1000-5000 mg/kg b.w. for TTO constituents, which is in accordance with the LD_{50} value above 5000 mg/kg b.w. reported for TTO.

However, in experimental studies intraperitoneal administration of high doses (100 or 200 mg/kg b.w.) of myrcene or limonene to mice caused sedative as well as motor relaxant effects (Gurgel do Vale, Couto Furtado et al. 2002). Further, TTO administered orally at doses greater than 1500 mg/kg b.w. to female rats appeared to induce persistent neurotoxic lesions in pathways controlling limb movements (Kim, Cerven et al. 2002). The implication of this observation is limited due to the high dose needed. Should new studies demonstrate this kind of neurotoxicity at significantly lower doses, this may have implications for the safety assessment of TTO.

A human LD_{50} value between 500 and 5000 mg/kg b.w. has been suggested for limonene (Gosselin, Hodge et al. 1976). As limonene only constitutes approximately 2.5% of TTO, this value does not change the overall conclusion regarding the acute toxicity of TTO products/formulations. Thus, TTO products and formulations have generally, except for the neat products, been reported to be without significant risk for acute human toxicity.

4.2 Skin and eye irritation

4.2.1 In vitro and animal data

ρ-Cymene and γ-terpinene were not irritating when assessed in vitro using the HET-CAM assay (Demirci, Paper et al. 2004).

Irritation was not evident when 1,8-cineole (Opdyke 1975) and terpinolene (Opdyke 1976) were applied to intact or abraded rabbit skin for 24 h with occlusion. α-Pinene applied neat to the skin of mice and swine was not irritating (Urbach and Forbes cited in (Opdyke 1978)).

Moderate irritation was seen when terpinen-4-ol (100%) (Opdyke 1982), ρ-cymene (100%) (Opdyke 1974), γ-terpinene (100%) (Opdyke 1976), terpineol (Opdyke 1974), *d*-limonene (Opdyke 1975), α-phellandrene (100%) (Opdyke 1978), α-pinene (Opdyke 1978) and myrcene (Opdyke 1976) were applied to intact or abraded rabbit skin for 24 h with occlusion.

Evaluation of skin damage and cytotoxicity of a range of terpenes on rat abdominal skin showed no irritation for 1,8-cineole and α-terpineol, whereas significant histopathological changes and cytotoxicity against human keratinocytes were evident for terpinolene, α-terpinene and limonene at very low concentrations (Kitahara, Ishiguro et al. 1993). The irritancy of α-terpinene, terpinolene and limonene to rabbits was further evaluated by the Draize test, and terpinolene was more irritating than limonene, which was in turn more irritating than α-terpinene (Okabe, Obata et al. 1990). The interpretation of the in vitro observations in relation to irritation of human skin is complicated, as evidence from the studies in rabbits and clinical studies in human do not appear to demonstrate the same degree of toxicity to the skin.

Investigation of the irritant capacity of several terpenes by transepidermal water loss (TEWL) and histological observations suggested that α-terpineol is potentially irritating (Fang, Hung et al. 2003).

Based on the information that no eye irritation in rabbits was observed at 1% sabinene (Yao and Chiou 1993) and that sabinene constitutes below 2% of TTO, it can be anticipated that an irritant response due to sabinene in a TTO product is unlikely.

In a report on acute dermal irritation in the rabbit of TTO, the skin irritation index was determined by the Draize method using NZ White rabbits exposed to undiluted TTO (batch 88/375). The Draize irritation index for undiluted TTO was found to be 5.0, indicating a severe irritant (Bolt 1989). This result has been observed in several studies with neat TTO. In a study in rabbits from 1996 (Pharmatox) following OECD guideline 404, TTO was applied for 4 hours with a semi-occlusive patch application followed by a 14 days observation period. The study demonstrated that: TTO (75%) was found to be a mild to moderate irritant, TTO (50%) was found to be a minimal irritant, TTO (25%) was found to be a non-irritant, and TTO (12.5%) was found to be a non irritant. Thus, a clear and expected dose relationship between concentration of TTO and irritancy was observed.

Primary eye irritation of TTO was studied in the rabbit (female, Japanese White) under GLP conditions (Oyama 2000). Two groups of three rabbits were given a single ocular dose (0.1 mL) of TTO (1% or 5% in liquid paraffin). After instillation of the test substance, no abnormal signs in the clinical conditions were observed among the rabbits. Ocular responses using Draize's criteria demonstrated a conjunctival discharge lasting for up to six hours following instillation of 1% TTO and conjunctival redness and discharge for up to 24 hours following instillation of 5% TTO. In both groups, the maximal response was observed after one hour. Based on these observations, the author concludes, that both TTO solutions can be classified as "minimally irritating" (Oyama 2000).

4.2.2 Human data

When Patch testing human volunteers, the following TTO constituents were non-irritating: terpinen-4-ol (5-10%) (Opdyke 1982; Knight and Hausen 1994), γ-

terpinene (5%) (Opdyke 1976; Southwell, Freeman et al. 1997), α-terpinene (5%) (Opdyke 1976; Knight and Hausen 1994), 1,8-cineole (4-28%) (Opdyke 1975; Knight and Hausen 1994; Southwell, Freeman et al. 1997), ρ-cymene (4%) (Opdyke 1974; Knight and Hausen 1994), terpinolene (20%) (Opdyke 1976; Knight and Hausen 1994), terpineol (12%) (Opdyke 1974; Knight and Hausen 1994), α-pinene (10%) (Opdyke 1978; Knight and Hausen 1994), cadinene (10%) (Opdyke 1973), myrcene (4%) (Opdyke 1976; Knight and Hausen 1994), α-phellandrene (4-8%) (Opdyke 1978; Knight and Hausen 1994) aromadendrene (1%) (Knight and Hausen 1994) and limonene (1%) (Knight and Hausen 1994).

In a larger multicenter study, a set of 5 to 10 fragrances at 2 concentrations was patch tested. Besides scores for allergic response, the researchers indicated the frequency of doubtful or irritant reaction that was not seen as allergy. A total of 1323 patients were patch tested in 11 centres and none of them demonstrated irritancy to α-terpineol (Frosch, Pilz et al. 1995). A later study by six of the same dermatological departments demonstrated that among 18 fragrances tested in 1606 consecutive patients, the lowest reactivity was observed with α-terpineol, yelding only 1 positive (<0.1%) allergic respons and 11 (0.7 %) doubtful/irritant reactions in a patch test with 5% α-terpineol (Frosch, Johansen et al. 2002).

Eye irritation thresholds between 100ppm and 1000 ppm for ρ-cymene, 1,8-cineole, α- and γ- terpinene, α-pinene, limonene have also been determined (Cometto-Muñiz, Cain et al. 1998; Cometto-Muñiz, Cain et al. 1998).

Limonene was not a respiratory irritant when tested in humans at concentrations of 10, 225, and 450 mg/m^3. At the highest exposure level a temporary decrease in lung capacity was observed (Falk-Filipsson, Lof et al. 1993).

Using a protocol based on the original Draize method, the potential of six TTO products to induce skin irritancy and/or allergenicity in humans was tested (Skin&CancerFoundationAustralia 1997). A total of 311 persons were included in the study and exposed to 100% TTO, 25% TTO in cream, 25% TTO in ointment, 25% TTO in gel, 5% TTO in cream and 5% TTO + 5% synergist in cream. No information as to the synergist was given. The test substances were applied to the skin a minimum of seven times during a three-week induction period. These observations indicate that products with concentrations of TTO below or equal to 25% are not causing irritancy to the participants. Likewise, undiluted TTO is not an irritant for the vast majority of the participants, but a small fraction of the population (in this study 5.5%) seems to be more susceptible to TTO and demonstrates positive skin reactions towards undiluted TTO. The small fraction of participants with an increased susceptibility to TTO was not further characterised regarding previous incidensies of skin irritation.

All data indicate that the irritative effects of TTO and TTO constituents depend on the dose. A range of individual TTO constituents as well as TTO has been demonstrated to be irritants when applied undiluted. Experimental studies in rabbits demonstrate that 75-100% TTO is a strong irritant, 50% TTO a mild irritant, and that 25% TTO and lower concentrations are non-irritative. Studies in humans are limited to studies on neat TTO (or constituents) and concentrations from 25% and below. The human data support the experimental data in so far as the neat oil is a significant irritant, whereas irritative effects are not observed when the concentration of TTO is below 25%.

Thus, the no-observed-effect-level for irritative effects of TTO is expeted to be at least 25% based on human studies and considering the experimental studies

probably below 50%. Moreover, the assumption that cineole should be a main culprit is not supported by the published evidence of irritative effects following exposure to TTO.

4.3 Skin sensitization

A skin sensitizer is an agent that is able to cause an allergic response in susceptible individuals. The consequence of this is that following subsequent exposure via the skin, the characteristic adverse health effects of allergic contact dermatitis may be provoked. As yet, there is not a validated in vitro test method accepted for skin sensitisation. Two validated in vivo laboratory animal tests to evaluate the potential of a substance to cause skin sensitisation exist: The local lymph node assay (LLNA, OECD 429) and the guinea pig maximisation test (GPMT, OECD 406).

4.3.1 In vitro and animal data

α-Pinene (10%) was sensitising using an open epicutaneous test in guinea pigs whereas l-carvone (1%), ρ-cymene (4%), d-limonene (8%), terpinene-4-ol (5%), 1,8-cineole (16%) and 1-terpineol (12%) were not (Klecak 1985). Likewise, no sensitizing capacity of 1,8-cineole was observed in guinea pigs (Hausen, Reichling et al. 1999). The amount of α-pinene in TTO is 3.5%, and a 25% TTO product will therefore have around 0.9% α-pinene.

d-Limonene did not produce sensitisation reactions when applied to guinea pigs whereas oxidised d-limonene did (Karlberg, Boman et al. 1991). A more recent study supporting the initial observation demonstrated that only the oxidation products of *d*-limonene, (R)-(-)-carvone, (+)-limonene oxide, along with air oxidized *d*-limonene, were potent sensitizers in the Freund complete adjuvant test and in the guinea pig maximization test (Haneke 2002). Limonene at 25 and 50% did not produce a response in the local lymph node assay, but 100% did (Warbrick, Dearman et al. 2001). This was regarded as being a weak response. The concentration of limonene in TTO is 2-3%, and the amount of limonene in a 25% TTO product would be reduced to 0.6%.

In a report on skin sensitisation in the guinea pig following exposure to TTO (Bolt 1989), groups of 20 albino guinea pigs (HA strain) were tested according to the Magnusson & Kligman method. The induction procedure consisted of two intradermal injections (5% TTO in paraffin or 5% TTO with Freund´s complete adjuvant) or an epidermal induction application (undiluted TTO). The dose used for challenge was 30% TTO. The erythemal reactions were measured 24 hours after removal of the patch of the challenge test. There were no responses in either group. The experimental methodology stated in the report appears to follow OECD 406 guidelines.

These in vitro and animal data do not suggest that any of the TTO constituents tested (except for oxidation products) are sensitizers.

4.3.2 Human data

Using a maximisation test and 25 human volunteers, the following components did not produce sensitisation reactions; terpinen-4-ol (5%) (Opdyke 1982; Klecak 1985), γ-terpinene (5%) (Opdyke 1976), α-terpinene (5%) (Opdyke 1976), 1,8-cineole (16%) (Opdyke 1975; Klecak 1985), terpinolene (20%) (Opdyke 1976; Klecak 1985), ρ-cymene (4%) (Opdyke 1974; Klecak 1985), *d*-limonene (8%) (Opdyke 1975; Klecak 1985), cadinene (10%) (Opdyke 1973), l-carvone (1%)

(Klecak 1985) and myrcene (4%) (Opdyke 1976), whereas α-pinene (10%) did (Klecak 1985). An interesting observation in relation to the pinenes was that β-pinene did not cause sensitisation reactions (Klecak 1985).

A maximization test on 25 volunteers with α-phellandrene at a concentration of 4% in petrolatum produced one sensitization reaction (Opdyke 1978). In view of the autoxidation problems, it was decided that the maximization procedure should be repeated on α-phellandrene using a freshly distilled sample processed under a blanket of nitrogen and containing an antioxidant. The same maximization test was carried out on another 25 volunteers using 8% in petrolatum of this freshly processed sample, and no sensitization reactions were observed (Opdyke 1978). However, phellandrene was identified as a sensitizer in another study on the sensitizing potential of some essential oils and their constituents (Woeber and Krombach 1969), and α-phellandrene induced a positive patch test in four of the eleven patients included in a study on patients from a dermatological department (Hausen, Reichling et al. 1999). To what extent the positive findings in the two latter studies are caused by oxidative degradation products of phellandrene is not clear.

4.3.2.1 Contact dermatitis

The results of patch testing of TTO-sensitised individuals with TTO components in three large studies (Knight and Hausen 1994; Southwell, Freeman et al. 1997; Hausen, Reichling et al. 1999) are summarised below (Table 3). A high fraction of TTO-sensitised patients demonstrated positive patch tests against 5% ascaridol (9 out of 11), 5% α-Terpinene (15 out of 21), and terpinolene when tested with 10% oil in ethanol (17 out of 18) (Knight and Hausen 1994; Hausen, Reichling et al. 1999). Positive patch test results were also recorded for aromadendrene (5 out of 18), limonene (6 out of 18), α-Phellandrene (5 out of 18), and 1,2,4-Trihydroxymenthane (4 out of 11) (Knight and Hausen 1994; Hausen, Reichling et al. 1999). It is, however, noteworthy that clear differences occur between skin-reactions recorded in different studies. Thus, α-Terpinene tested at comparable concentrations cause significantly different results in the studies from the group around Hausen (Knight and Hausen 1994; Hausen, Reichling et al. 1999) and Southwells study published in 1997 (Southwell, Freeman et al. 1997). Likewise, limonene and aromadendrene caused skin reactions in five or six out of seven participants in the Knight and Hausen study from 1994 when applied in 1% as compared to zero or one in eleven subjects exposed to 5% aromadendrene or limonene in the study from 1999 (Hausen, Reichling et al. 1999). Differences do occur between dermal reactions recorded in different studies with limited number of participants. However, these differences are often equally well explained by presense of impurities or oxidative product in test oils. The present data from these studies do not allow a closer evaluation on the potential presence of oxidation products.

Table 3. Number of presensitized dermatological patients reacting to TTO components (% of component tested)

Component	Hausen *et al*., 1999 n = 11	Southwell *et al*., 1997 n = 3	Knight & Hausen, 1994 n = 7
Aromadendrene	0 (5)		5 (1)
Ascaridol	9 (5)		
d-Carvone	0 (5)		0 (5)
l-Carvone	0 (5)		
1,8-Cineole	0 (5)	0 (1.4)	0 (5)
ρ-Cymene	0 (5)	0 (1.5)	1 (1)
Limonene	1 (5)	0 (0.7)	6 (1)
Myrcene	2 (5)		0 (1,5)
α-Phellandrene	4 (5)		1 (1)
α-Pinene	0 (10)	0 (0.7)	
β-Pinene		0 (0.9)	
Sesquiterpene hydrocarbons		3 (1.5)	
α-Terpinene	7 (5)	1 (5.9)	7 (5)
γ-Terpinene		0 (5.2)	
Terpinen-4-ol	0 (10)	0 (9.5)	2 (10)
α-Terpineol		0 (1.3)	0 (1,10)
Terpinolene	11 (10)	0 (1.1)	0 (1)
			6 (10)
1,2,4-Trihydroxymenthane	4 (5)		
Viridiflorene	1 (5)		

Limonene cause skin reactions in six of seven participants in the Knight and Hausen study from 1994 when applied in 1% as compared to only one in eleven subjects exposed to 5% limonene (Knight and Hausen 1994; Hausen, Reichling et al. 1999). Other studies have, however, not supported the high fraction of positive reactons recorded in the study from 1994 (Knight and Hausen 1994). Thus, patch testing with limonene (1%) produced 1 irritant or doubtful positive reaction in 192 participants, whereas 0.1% limonene produced no reactions (Frosch, Pilz et al. 1995). Further, patch testing with 3% limonene produced only 7 positive in 1606 dermatology patients (Frosch, Johansen et al. 2002). Whether the positive reactions observed in the 1994 study on limonene were caused by impurities or oxidative products is not to say, but positive patch test reactions to oxidised limonene are common amongst dermatology patients (Karlberg, Dooms-Goossens et al. 1997; Matura, Goossens et al. 2002; Matura, Karlberg et al. 2003).

In contrast to the study in guinea pigs, α-pinene produced no dermal sensitization when tested at concentration of 10% and 12% in petroleum, respectively in a dermal human sensitization study (EPA 2005). In experiments with oil of turpentine and α-pinene, it was shown that only the autoxidation products of oil of turpentine and not the terpenes themselves were eczematogenic. Autoxidation of a-pinene in the presence of air and light was sufficient to produce the eczematogenic agent, but its formation could be prevented by addition of inhibitors such as hydroquinone and pyrogallol (Opdyke 1978).

Patch testing of 100 dermatological patients with 1% and 5% terpineol produced no irritant reactions (Frosch, Pilz et al. 1995). Consecutive testing of 1606 patients attending the patch test clinic of 6 European departments of dermatology demonstrated that the standard fragrance mix produced the highest reactivity in all centres (mean 11.4%; range 9.3–17.9%), whereas caryophyllene caused positive reactions in 0.6% and α-terpineol in less than 0.1% of the patients (Frosch, Johansen et al. 2002). In a more recent study, 1511 consecutive dermatitis patients in 6 European dermatology centres were patch tested with oxidized fragrance terpenes and some oxidation fractions and compounds. About 0.5% of the patients reacted to oxidized caryophyllene (Matura, Sköld et al. 2005).

There have been a number of human contact dermatitis cases due to topical application of TTO with well over a dozen published cases within the last ten years (Apted 1991; De Groot and Weyland 1992; Selvaag, Eriksen et al. 1994; Van Der Valk, De Groot et al. 1994; De Groot 1996; Bhushan and Beck 1997). The applications included 100% TTO as well as lower concentrations of TTO in different formulated products.

In an older study on occupational skin disorders, terpinolene was found not to be a sensitizer for human skin (Woeber and Krombach 1969) and a high fraction of TTO-sensitised patients with existing skin disease demonstrated positive patch tests against terpinolene when tested with 10% oil in ethanol (17 out of 18), whereas patch testing with terpinolene (1%) did not show any positive respons (Knight and Hausen 1994; Hausen, Reichling et al. 1999).

Using a protocol based on the original Draize method, the potential of six TTO products to induce skin irritancy and/or allergenicity in humans was tested (Skin&CancerFoundationAustralia 1997). A total of 311 persons were included in the study and exposed to 100% TTO, 25% TTO in cream, 25% TTO in ointment, 25% TTO in gel, 5% TTO in cream and 5% TTO + 5% synergist in cream. The report concludes that TTO is a mild allergen as only 1% of the participants (3/308) were sensitised, ie. made allergic, to TTO by means of the Draize test (Skin&CancerFoundationAustralia 1997).

Based on an Italian study in 725 persons patch tested according to GIRDCA guidelines, the authors conclude that the sensitization potential of Melaleuca oil is poor, and that the response in patch tests appears to be dose dependent, and primarily observed after exposure to undiluted TTO. Positive responses to patch tests were more frequent in subjects with existing allergic contact dermatitis or atopic dermatitis (Lisi, Meligeni et al. 2000).

The prevalence of hypersensitivity to a number of allergens was tested in a group of 219 volunteers (Greig, Carson et al. 2000). The findings were slightly higher than in other studies. The prevalence for hypersensitivity to TTO was found to be 2.3%. The authors argue that the prevalence found might be too high due to selection bias as the population studied were self-selected (Greig, Carson et al. 2000).

In 1997, 1216 patients were patch tested at a dermatologic clinic (Fritz, Burg et al. 2001). Products containing *Melaleuca alternifolia* oils were tested concentrated or diluted. Seven patients with an allergic contact dermatitis due to TTO were identified. Two of them also exhibited delayed type IV hypersensitivity towards fragrance-mix or colophony suggesting the possibility of cross reaction or an allergic group reaction. The allergic potential of low concentrations of freshly produced *Melaleuca alternifolia* oil is presumed to be low on healthy skin, whereas photoaged *Melaleuca alternifolia* oil must be considered to be a stronger sensitizer due to formation of oxidative degradation products (Fritz, Burg et al. 2001).

By 2003 close to 7000 patients at German dermatological clinics had been tested epicutaneously with a 5% dilution of oxidised TTO containing the original constituents as well as oxidation products (Hausen 2004). Seventy patients (1%) had a positive reaction to TTO (Hausen 2004). The most important allergens of TTO appears to be terpinolene, ascaridol, α-terpinene, and 1,2,4-trihydroxy menthane for which the prevalence of allergic respons among patients visiting dermatological clinics vary between 0.4% and 0.6% (Hausen 2004). Ascaridol and 1,2,4-trihydroxy menthane have repeatedly been found as oxidation products in aged TTO products.

The more recent appreciation of the potential presence of oxidative degradation products in TTO and TTO formulations is important as most of the earlier studies do not describe the age or storage condition for the TTO, TTO products or individual constituents applied. Thus earlier studies may have been conducted with potentially partly oxidised oils, which may explain some of the apparently contradicting results obtained between studies and the observation that a low concentration may induce a positive response in one study, whereas a repetition with a higher dose does not. This is the case with α-pinene, α-terpinene and terpinolene. Further, the test concentrations applied are considerable higher than what would be expected from the use of TTO products. To what extent aging of test formulation has been a problem in the larger clinical studies is equally uncertain, but several studies clearly demonstrate that replacement of old test samples with fresh TTO reduces the occurrence of positive findings. The prevalence of positive findings following exposure of pre-sensitized dermatological patients in the clinical studies is generally around 0.4-0.6%. Thus TTO has probably a weak sensitizing potential, though the present known numbers may be an overestimate due to problems with aged test material. Further surveillance of skin sensitization due to exposure to TTO should therefore be encouraged with due focus on the test material used.

On the other hand, oxidative degradation products from TTO appear to possess a clear sensitizing potency. The formation of oxidation products in TTO and TTO products need therefore to be controlled. Whether these degradation products are formed during distillation, product formulation or during storage at retailers or consumers is not clear. Whether this apparently technical problem can be dealt with during production, through addition of anti-oxidants, or through documented shelf-lives for the products is an issue that needs consideration.

4.4 Dermal/percutaneous absorption

Several terpenes (thymol, menthone and 1,8-cineole) do not cause toxicity themself, but enhance the percutaneous penetration of other substances (e.g. propranolol, piroxicam, zidovudine, insulin, haloperidol) (Doliwa, Santoyo et al. 2001; Vaddi, Ho et al. 2002; Pillai and Panchagnula 2003; Narishetty, Panchagnula et al. 2004; Amnuaikit, Ikeuchi et al. 2005).The degree of enhancement depends on the lipophilicity of the terpene as well as the lipophilicity of the drug in question (El-Kattan, Asbill et al. 2001). The levels of terpenes absorbed or deposited in the skin are seldom reported.

The effect of three cyclic terpenes (carveol, terpinene-4-ol, α-terpineole) on the transdermal penetration of water was studied in vitro. The maximum increase in permeability coefficients of carveol, terpinen-4-ol and α-terpineol was 10.6, 8.7 and 10.9, respectively (Magnusson, Runn et al. 1997), thus demonstrating clear effects on skin integrity. Likewise, treatment of human epidermis with terpene penetration enhancers has been shown to increase electrical conductivity. The increase in ion transport suggests that terpenes open new polar pathways across the stratum corneum. A correlation between increases in ion transport and previously reported increases in 5-fluorouracil penetration suggests that terpene enhancers may create micro-pores in the intercellular lipids through which both ions and polar drugs may pass (Cornwell and Barry 1993).

A quantitative study in mice and rabbits demonstrated that p-cymene is well absorbed through the skin (Wepierre 1963; Wepierre 1963) . Following absorption,

the ring substituent is oxidized to yield aromatic alcohol and carboxylic acid metabolites that are excreted free or conjugated in the urine.

The presently available data on penetration through human skin demonstrates that in experimental studies on dermal penetration of different ingredients of TTO, the first component to penetrate the skin and reach the subcutaneous fat layer (within 1 hour) was terpinen-4-ol. After two hours exposure α-terpineol was also found in the subcutaneous fat layer (Hayes, Leach et al. 1997). As exposure time was increased, more ingredients were detected (1,8-cineole, α-terpinene, p-cymene, α-terpinolene), but all in considerably lower amounts (Hayes, Leach et al. 1997).

A more recent study revealed that among seven major constituents of TTO (terpinene-4-ol, 1,8-cineole, p-cymene, terpinolene, α-terpineol, α-terpinene, γ-terpinene) present on the upper side of the skin, only three (terpinen-4-ol, *α*-terpineol, eucalyptol) could positively be identified as being absorbed through the skin (Nielsen and Nielsen 2006). γ–Terpinene which was found to appear in higher amounts than *α*-terpineol and 1,8-cineole in the TTO applied to the skin was not detected as absorbed. The three constituents absorbed were those compounds among the seven constituents with the lowest log P_{ow} values – the least lipophilic (Nielsen and Nielsen 2006). Thus, the relative occurrences of individual constituents of TTO differ between what is applied to the skin and what eventually get absorbed (Nielsen and Nielsen 2006). The penetration rates for the TTO constituents eventually penetrating the skin were low, and a the penetration coefficient (Kp) around 20μm/h for terpinene-4-ol was reported as was a lag-time from 4-6 hours for terpinene-4-ol (Nielsen and Nielsen 2006).

The penetration of TTO through human epidermal membranes was also evaluated experimentally by use of Franz cells (static diffusion cells) (Edwards-Jones, Buck et al. 2004). TTO was applied topically as the pure oil and as a 20 % formulation in ethanol. Following the 24 hr experimental period, terpinen-4-ol, α-terpineol, and 1,8-cineole were detected in the receptor phase. None of the other TTO constituents could be detected in the receptor phase, but a fraction of sesqui-terpinene compounds together with terpinen-4-ol and α-terpineol was seen in epidermis (Cross and Roberts 2006).

These recent observations are in agreement with studies using a matrix-type transdermal system describing the levels of terpenes and their effects on the stratum corneum after dermal application (Cal, Janicki et al. 2001). In this study, dermis did not present a barrier for penetration of terpenes. For all terpenes the penetration was, however, slower in the presence of epidermis, and large amounts of terpenes were found in epidermis indicating that affinity of these compounds to the stratum corneum is very high (Cal, Janicki et al. 2001).

When the difference in thickness of epidermis and dermis is taken into consideration, the higher affinity of terpenes to epidermis than dermis can be demonstrated. The dry mass of epidermis is approximately 2–3 mg/cm2. Thus, the amounts of terpenes found in epidermis, most probably to stratum corneum, correspond to over 50% of the total mass (Cal, Janicki et al. 2001).

Penetration of limonene, terpinolene, and cineole had lag-times close to two hours and an absorption through the matrix-type barrier between 8% and 13% of the applied amount (Cal, Janicki et al. 2001)

4.5 Repeat dose toxicity

Based on the toxicokinetic evidence, accumulation of TTO, its constituents, or metabolites is not expected. Metabolism occurs primarily in the liver followed by renal excretion. Relevant target organs for non-genotoxic effects following repeated and intended use of TTO products is therefore the liver and the kidneys. After acute high dose exposure, effects on the gastrointestinal tract (intestinal atony) and the central nervous system have been observed (see section on acute toxicity). The mutagenic/carcinogenic potential of TTO and constituents is discussed in section 4.6.

Terpinen-4-ol did not induce changes in the morphology or function of the kidneys of male Sprague-Dawley rats following 28 days of repeated oral exposure to 400 mg/kg b.w., and was considered to be non-toxic (Schilcher and Leuschner 1997). The available literature on systemic effects of terpinen-4-ol is very limited. Based on the 28-days study on kidney toxicity in rats, the NOAEL after oral exposure may be estimated to be 400 mg/kg. As terpinen-4-ol on average constitutes 40% of TTO, this NOAEL for terpinen-4-ol corresponds to an oral NOAEL for TTO (based on renal toxicity of terpinen-4-ol) of 1000 mg/kg.

Cineole given to B6C3F1 mice by gavage for 28 days at doses up to 1200 mg/kg/day did not result in any changes. When given encapsulated at doses corresponding to 600 – 5607 mg/kg/day, some hypertrophy of hepatocytes was seen, but was not considered significant (National Toxicology Program, cited in (De Vincenzi, Silano et al. 2002)). Cineole (8 or 32 mg/kg/body weight) was given by gavage to male SPF CFLP mice 6 days per week for 80 weeks. No changes were evident in mice given cineole when compared to control mice (Roe, Palmer et al. 1979). Based on the studies on hepatic and renal toxicity evaluated by BIBRA, a NOAEL might be estimated as 300 mg/kg body weight, which is in agreement with the evaluation from the Norwegian Food Control Authorities in 1999. As 1,8-cineole on average constitutes 5% of TTO, this NOAEL for 1,8-cineole corresponds to an oral NOAEL for TTO (based on liver and kidney toxicity of 1,8-cineole) of 6000 mg/kg.

Exposure to α-terpinene (125 or 250 mg/kg b.w.) for nine consecutive days caused decreased body weight gain in pregnant Wistar rats (Araujo, Souza et al. 1996). No maternal toxicity was observed at 60 mg/kg b.w., and a NOAEL of 60 mg/kg b.w. for systemic effects following repeated exposure to α-terpinene is suggested. Based on the amount of α-terpinene present in TTO, this corresponds to a NOAEL of 660 mg/kg b.w. for TTO.

The effects of ρ-cymene on the brain chemistry of rats was studied by exposing male Long-Evans rats to 0, 50 or 250 ppm p-cymene by inhalation (Lam, Ladefoged et al. 1996). Rats were exposed for 6 hours per day, 5 days per week for four weeks and then had an 8 week wash-out period. No obvious toxicity was seen during the exposure period and body weights did not differ after the 12 week trial period. Levels of synaptosomal protein were significantly reduced in treated rats, whereas relative amounts of noradrenaline and dopamine were increased.

A limited number of relevant repeat-dose studies are available and the inhalation route is often used for cumene. A NOAEL of 488 ppm based on inhalation might be suggested as might also a LOAEL of 769 mg based on the only study with oral exposure. Based on the oral study and using an uncertainty factor of 10, a NOAEL for cumene/p-cymene of 75 mg/kg body weight is suggested. As p-cymene on average constitutes 6% of TTO, this NOAEL for p-cymene corresponds to an oral

NOAEL for TTO (based on possible renal effects of p-cymene) of 1200 mg/kg body weight.

In a 3-month oral toxicity study, rats were fed an alpha-pinene resin or pinene polymer made predominantly from alpha-pinene. (The ratio of alpha-and beta-pinene was 10:1.) The dose levels were 0, 1, 3 or 5% in the diet. Effects seen at 5% (3967 mg/kg/day) included an increase in relative liver weight in both sexes, and absolute liver weight in females only. Increased relative thyroid weights in males were noted at the 3 and 5% dose levels. In the absence of histopathological alterations, these changes were not considered treatment related. No effects were noted at 1%, which corresponds to roughly 800 mg/kg/day (EPA 2005). Based on the amount of pinene present in TTO, this corresponds to a NOAEL for TTO above 5000 mg/kg b.w.

Based on the study using dietary exposure of rats to concentrations of α-terpineol corresponding to 500 mg/kg b.w. (Hagan, Hansen et al. 1967), a NOAEL for α-terpineol of 500 mg/kg bw can be suggested as the study did not demonstrate any toxicity. As α-terpineol on average constitutes 5% of TTO, this NOAEL for α-terpineol corresponds to an oral NOAEL for TTO (based on the only available study on systemic toxicity for α-terpineol) of 10.000 mg/kg body weight might be suggested.

Adult beagle dogs were gavaged twice daily for 6 months with 100 or 1000 mg d-limonene/kg body weight per day. Limonene ingestion did not affect feed consumption or body weight. Increased kidney weight was seen but no histopathological kidney changes were seen. No nephropathy was evident (Webb, Kanerva et al. 1990).

An activated immune response from alveolar macrophages has been observed in rats following oral exposure to limonene at doses from and above 250 mg /kg b.w. It is unclear how these observations would add to a potential risk following dermal exposure to a TTO product containing around 2.5% limonene. However, if the data was used to estimate a NOAEL for TTO, this NOAEL would probably be above 2000 mg/kg b.w.

Methyleugenol administered by gavage at a maximum dosage of 1000 mg/kg body weight to F344/N rats and B6C3F1 mice for 14 weeks resulted in erythrocyte microcytosis and thrombocytosis in rats (Abdo, Cunningham et al. 2001). Other results were suggestive of impaired liver function and protein digestion. Adverse effects seen in the salivary glands, adrenal glands, testis and uterus were considered to be secondary to the liver and stomach effects. The no-observed-effect level (NOEL) was estimated to be 10 mg/kg for both species (Abdo, Cunningham et al. 2001). Methyl eugenol is present in trace amounts in TTO (below 0.1%) and the estimated NOAEL for TTO based on the repeated dose toxicity of this minor constituent (methyl eugenol) would exceed 1000 mg/kg b.w.

A range of toxic effects have been reported after repeated exposure to TTO or TTO constituents and used to estimate NOAEL values. For α-terpinene the estimated NOAEL is based on weight loss in pregnant rats, and given the presence of 9% α-terpinene in TTO, this would equal a NOAEL for TTO of 660 mg/kg. The direct extrapolation from a NOAEL for a constituent to a NOAEL for TTO is only acceptable when no other constituent is reported to affect the same target. In case of TTO, three constituents have been reported to affect the kidneys.

TTO constituent	Target toxicity	Estimated NOAEL	Conc. in TTO
Terpinen-4-ol	renal	400 mg/kg	40%
Cineole	renal	300 mg/kg	4.5%
Cumene	renal	75 mg/kg	6%

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

(40%/400mg/kg + 4.5%/300mg/kg + 6%/75mg/kg) x NOAEL = 100%

This formula gives an estimated NOAEL for TTO of 510 mg/kg

Lack of data on possible renal effects of the remaining constituents may decrease the NOAEL further. A worst case scenario would be that the remaining 49.5% of TTO had a constituent-specific NOAEL equal to cumene. Incorporating this estimate in the calculation of a NOAEL for TTO gives an adjusted formula:

(40%/400mg/kg + 4.5%/300mg/kg + 6%/75mg/kg + 49.5%/75mg/kg) x NOAEL = 100%

The worst case scenario estimate for a NOAEL for TTO would be 117 mg/kg

Based on the available information on the repeat dose toxicity, the renal effects would have the lowest estimated NOAEL of 117 mg/kg b.w.. A margin of safety estimate for dermal use of TTO products based on this value would need to incorporate the fraction of an applied dose absorbed and the actual concentration of TTO in the product besides an estimate of the amount of TTO applied on the skin.

4.6 Mutagenicity/carcinogenicity

4.6.1 Bacterial assays

The mutagenic potential of tea tree oil (Melaleuca alternifolia) was examined using the Ames Test. One of the major components, the monoterpenoid terpinen-4-ol, was also examined to determine if it demonstrated any mutagenic potential. Salmonella typhimurium (TA102, TA100 and TA98) was utilised in the Ames test. Commercially available tea tree oils were tested. No mutagenic effect was determined in any of the brands of tea tree oil on any of the strains of Salmonella examined with or without metabolic activation (Fletcher, Cassella et al. 2005). The same negative results were obtained for the terpinen-4-ol component examined. There was a clear evidence of toxicity of tea tree oil on all Salmonella strains and also by terpinen-4-ol at higher dose levels. It is suggested that terpinen-4-ol may contribute significantly to the widely reported antibacterial activity of tea tree oil (Fletcher, Cassella et al. 2005).

Further, the following TTO constituents were found to be non-mutagenic using bacterial assays such as the Ames test: α-terpinene (Gomes-Carneiro, Viana et al. 2005), 1,8-cineole (Yoo 1985; Gomes-Carneiro, Felzenszwalb et al. 1998), α-terpineol (Florin, Rutberg et al. 1980), limonene (Florin, Rutberg et al. 1980; Watabe, Hiratsuka et al. 1981; Connor, Theiss et al. 1985), α-pinene (Rockwell and Raw 1979; Florin, Rutberg et al. 1980; Connor, Theiss et al. 1985; Gomes-Carneiro, Viana et al. 2005), cymene (Rockwell and Raw 1979), and β-myrcene (Gomes-Carneiro, Viana et al. 2005).

Though no mutagenicity was observed when tested directly, weak mutagenic activity toward TA100, but not TA98, was observed in an older study with ether extracts of urine from rats fed β-terpineol (Rockwell and Raw 1979). Repetition of this finding has not been published and it is difficult to evaluate the implications of this observation given that the effect is observed in β-terpineol and it is the α-form that occurs in TTO. Terpineol was negative using the *Bacillus subtilis* rec- assay (Oda, Hamano et al. 1978), but caused a slight increase in the number of revertants for one of four test strains (Gomes-Carneiro, Felzenszwalb et al. 1998).

α-Terpineol was negative in 5 out of six salmonella strains. However, the result from the last strain (TA102) can not be ignored as a false positive finding because of dose-related toxicity. However, in support of a lack of genotoxic potential, α-terpineol did not induce lung tumors in mice following repeated intraperitoneal administrations.

4.6.2 Tests with mammalian cells

γ-Terpinene increased DNA strand breakage in human lymphocytes at high doses (0.2 mM) when tested in the Comet assay, but significantly reduced chemically-induced DNA damage at lower doses (Aydin, Basaran et al. 2005).

Cineole, *d*-(+)-limonene, *l*-phellandrene and β-pinene at concentrations ranging from 10 – 1000 μM did not increase the frequency of spontaneous sister-chromatid exchanges in Chinese hamster ovary cells (Sasaki, Imanishi et al. 1989).

β-Myrcene is non-mutagenic in mammalian cells (Kauderer, Zamith et al. 1991) and is not genotoxic in bone marrow cells of rats administered β-myrcene orally (Zamith, Vidal et al. 1993).

Limonene produced renal tumors in male F344 rats (Turner, Tinwell et al. 2001; Sekihashi, Yamamoto et al. 2002). No tumors are found in female F344 rats, other rats or mice. It is a non-genotoxic carcinogen in male F344 rats, but is considered to be non-mutagenic and of no cancer risk to humans (Flamm and Lehman-McKeeman 1991; Whysner and Williams 1996; Rivedal, Mikalsen et al. 2000).

Cineole, d/l-carvone, d-limonene, terpineol, and thymol did not induce primary lung tumors in male or female A/He mice following 24 ip injections during an eight week period with 24 weeks follow-up. The doses used were either MTD (maximal tolerated dose) or 20% of MTD (Stoner, Shimkin et al. 1973).

In conclusion, two of the TTO constituents (1,8-cineole and phellandrene) may act as weak promoters. There is no strong evidence that any of the TTO constituents are mutagenic. The carcinogenic mechanism explaining the gender and species specific renal tumors induced by limonene in male F344 rats is not seen in humans. Based on the available information, neither TTO nor its constituents are expected to pose any carcinogenic risk to humans.

4.7 Reproductive toxicity

The available literature on reproductive toxicity of TTO and constituents is limited. Therefore, results from studies on myrcene, linalool, and cumene which are terpenes/terpenoids with some structural and chemical resemblancies with the major components of TTO, are included.

α-Terpinene was given to female Wistar rats at 30, 60, 125 and 250 mg/kg body weight on days six to 15 of pregnancy. The two highest doses were maternally toxic, and the highest dose also caused a reduction in the proportion of pregnant females. Foetuses from rats given 250 mg/kg had reduced body weights and increased kidney weights. Abnormal ossification of bones and minor skeletal abnormalities were evident in foetuses from females given 60 mg/kg or more. Thus the NOAEL for embryofoetotoxicity was set at 30 mg/kg body weight (oral route) (Araujo, Souza et al. 1996).

β-Myrcene was given to female Wistar rats at 250, 500, 1000 and 1500 mg/kg by gavage from day 15 of pregnancy until postnatal day 21. Offspring from rats given 250 mg/kg did not show adverse effects but those given 500 mg/kg or more showed decreased birth weight, increased perinatal mortality and delayed postnatal development. Fertility of female offspring of rats given 1000 or 1500 mg/kg was impaired. The data suggest a NOAEL for peri- and postnatal developmental of 250 mg β-myrcene/kg body weight (Delgado, De Almeida Nogueira et al. 1993).

In a similar study, β-Myrcene (0, 100, 300 and 500 mg/kg) was given by gavage to male and female Wistar rats prior to mating, during mating and pregnancy, and up to postnatal day 21. Male and female rats showed increased liver and kidney weights but no other signs of toxicity. β-Myrcene did not affect the proportion of females impregnated nor the pregnancy index. There was no evidence of maternal toxicity or external malformations at any dose. At 500 mg/kg there was an increased resorption rate and more skeletal abnormalities in fetuses. Myrcene did not affect postnatal weight gain but developmental milestones were slightly delayed. These data suggested a NOAEL for toxic effects on fertility and general reproductive performance of 300 mg β-myrcene/kg body weight (Paumgartten, De-Carvalho et al. 1998).

Studies with cumene (which is closely related to ρ-cymene) have indicated a low potential for reproductive toxicity (EPA, cited in (The Flavour and Fragrance High Production Volume Consortia - the Terpene Consortium 2002)). The effects of cumene vapour on development in CD rats and New Zealand white rabbits has been examined (Darmer Jr, Neeper-Bradley et al. 1997).

The effects of coriander oil (72.9% linalool, 22.3% other terpenoids, balance unknown) on reproduction and development has been studied in rats (United National Environmental Program 2002). The NOAEL for linalool based on foetotoxicity is suggested at a level of 365 mg linalool/kg bw (United National Environmental Program 2002).

Among constituents of TTO for which evidence of potential foetotoxicity is available, α-terpinene has the lowest estimate of a NOAEL (30 mg/kg bw) and the highest relative occurrence (9% on average) in TTO. Based on reproductive toxicity, a NOAEL for TTO can tentatively be set at 330 mg TTO/kg bw following oral exposure.

4.8 Toxicokinetics

A discussion of the toxicokinetics of TTO is essentially meaningless since TTO is a mixture of some 14+ individual substances. However, data do exist on some of the constituents and parallels exist between different constituents belonging to the same chemical groups. Thus, the structural formulas of TTO constituents illustrated in chapter 7 demonstrate the striking structural resemblances among the main constituents of TTO.

Most dat on the toxicokinetics of TTO is based on oral exposures. Chemicals absorbed via the gastrointestinal tract may undergo metabolism in the liver before reaching the general circulation, whereas chemicals absorbed through the skin avoid this first pass metabolism. However, in relation to elimination kinetics, biotransformation, and organ deposition, data from oral exposures are also relevant to the dermal exposure situation.

Major biotransformation is expected to take place in the liver and to a lesser extent in other organs by the cytochrome P-450 dependent monooxigenases. These phase I reactions play a key role in converting more lipophilic terpenes into more hydrophilic compounds, which may then conjugate with glucoronic acid (or other phase II reactions) to generate even more hydrophilic metabolites that eventually are excreted. Excretion is expected to be dominated by renal elimination, but bile excretion followed by faecal elimination will also occur. Conjugates will be expected to be excreted within 2-3 days post exposure.

An important notion is that intestinally absorbed chemicals will be transported directly to the liver before entering the systemic circulation. In this way the body has an evolutionary developed defence against oral exposure to toxicants. Following dermal absorption, however, this first pass metabolism is circumvented with the consequence that absorbed chemicals may enter critical organs for toxicity before having passed the liver and thus before being metabolised.

A simple toxicokinetic model for metabolic pathways for the terpenes 1,8-cineole, p-cymene and terpinen-4-ol (major terpenes found in melaleuca oil) has been described by Villar et al. (Villar, Knight et al. 1994) In this model, less than 10% of the absorbed oil is expected to be eliminated through the faeces, and 60-80% of an oral dose is expected to be eliminated through the urine within 48-72 hours (Villar, Knight et al. 1994).

A number of terpenes (α-pinene, d-limonene, α-terpinene, β-myrcene, terpineol, and 1,8-cineole) have been demonstrated in vitro (liver microsomes prepared from phenobarbital-treated rats) to dose-dependently inhibit the liver enzyme CYP2B1 (De-Oliveira, Ribeiro-Pinto et al. 1997; De-Oliveira, Fidalgo-Neto et al. 1999). An inhibited isoenzyme will affect the metabolism of those chemicals.that depend on CYP2B1, though IC_{50}-values between 0.1µM and 15µM in microsomal preparations will be expected to require a substantial in vivo dose to reach significant target organ concentrations.

Based on observations from in vivo intoxications in man and animals, the expected target organs for toxicity during the acute phase will be the gastrointestinal tract and the central nervous system. Effects on these targets will be expected to be clearly dose dependent with existence of non-toxic exposure levels.

At lower doses no acute systemic toxicity is expected. The known toxicokinetics indicate transport to the liver, hepatic biotransformation followed by renal elimination. The relatively short elimination half-lives expected on the basis of the presently known information on TTO constituents does not indicate significant accumulation of either parent compound or metabolites. Therefore, toxicity following low-dose repeated exposure has focussed on targets like hepatic toxicity, renal toxicity, mutagenicity of parent constituent as well as metabolites, and foetotoxicity.

4.9 Degradation/oxidation products

Like most natural oils several constituents of TTO may undergo oxidative degradation during storage as well as metabolism after being absorbed. Photooxidation may also be an issue for some chemicals. A few descriptions of degradation of TTO with time are described in the literature. Section 6.15 includes a discussion of the most prominent degradation/oxidation products. In section 6.15 the known toxic effects of these compounds have been included along with the parent compounds and stratified according to toxicity target.

4.10 Phototoxicity

At a concentration of 100%, TTO did not produce phototoxic effects when applied to the skin of hairless mice, but some irritation was noted (Forbes and Davies 1982). The potential phototoxic effects of TTO and its constituents are expected to be covered by the inclusion of degradative and oxidative products in the previous sections. All methods have their strengths and weaknesses and this study from 1982 is not outstanding. But if 100 % TTO does not produce phototoxic reactions, those products that may be used on the skin and exposed to sun light, which may contain 5-10 % TTO can generally be regarded as safe. However, as with several other questions on toxicity of TTO, the question that needs focus is the degradation and oxidation products formed in aged TTO products.

5 Toxicity profile for TTO

Tea tree oil (TTO) is a mixture of many individual constituents. From a clinical point of view, toxicity testing of that specific mixture of constituents that makes TTO is the most relevant, but for scientific and preventive purposes the toxicity profiles of the individual constituents are equally important. Thus, knowledge on individual profiles will allow the focus to be directed against the constituents that are most problematic (lowest margin of safety) and allow a discussion how these constituents may be eliminated, reduced, or controlled during processing and storage of TTO products. The present chapter will focus on the literature that has tested TTO either as neat oil, as mixtures with different carriers or as sales products, whereas chapter 6 will focus on the individual constituents and their known degradation/oxidation products.

Acute toxicity

Oral exposure

TTO can be toxic if ingested, as evidenced by experimental studies in rats and from cases of human poisoning. The oral LD_{50} for TTO in a rat model is 1.9 – 2.6 ml/kg (Russell 1999). Rats dosed with 1500 mg TTO/kg body weight appeared lethargic and ataxic and showed depressed activity levels 72 h post dosing (Kim, Cerven et al. 2002). By day 4, however, all but one animal given this dose had regained all locomotor functions. Although values determined in animal models are not necessarily directly related to human toxicity, the experimental data do indicate that TTO dose-dependently is orally toxic.

Published cases of oral poisoning in humans tend to be more dramatic in children because of their low body weight compared to an adult. One such case report involved a 23-month-old child who drank less than 10 ml of 100% pure TTO (Jacobs and Hornfeldt 1994). After a nap of approximately 30 minutes, he was unsteady on his feet and appeared as if ‘drunk’. The child was taken to a hospital and treated with activated charcoal and sorbitol via a naso-gastric tube, and approximately 5 h later he appeared to be asymptomatic. All other signs (such as respiratory rate, oxygen saturation, pupil reactivity, electrolytes and blood glucose) were normal throughout (Jacobs and Hornfeldt 1994). The authors attributed the clinical symptoms to a central nervous system depression caused by the ingested TTO. Similar symptoms were reported in a 17-month-old boy beginning 10 minutes after the ingestion of an unknown but less than 10ml volume of 100% pure TTO (Del Beccaro 1995). Under observation in hospital, complete resolution of symptoms occurred after approximately 5 h. In a third case, the ingestion of 2 teaspoons of 100% pure TTO by a 4-year-old boy led to symptoms of ataxia within 30 minutes followed by unconsciousness and unresponsiveness requiring intubation (Morris, Donoghue et al. 2003). The boy’s neurologic status improved gradually over 10 h and he was discharged from hospital 24 h after admission without respiratory or neurologic sequelae.

A case of poisoning in an adult occurred when a patient drank approximately half a tea cup of TTO corresponding to a dose of approximately 0.5-1.0 ml/kg body weight (Seawright 1993). The patient was comatose for 12 h, and semi-conscious and hallucinatory for the following 36 h. Symptoms of abdominal pain and diarrhoea continued for approximately 6 weeks after this. In another incident, a 60-year-old man who swallowed one and a half teaspoonfuls of TTO as a preventative for a cold presented with a red rash which covered his feet, knees, upper body and

arms including his palms and elbows (Elliott 1993). His hands, feet and face were also swollen. The rash and other symptoms gradually disappeared and approximately one week later he had more or less recovered.

Dermal exposure
Toxicity following dermal application of inappropriately high doses of melaleuca oil to cats or dogs treated for fleas has been described. Animals had typical signs of depression, weakness, uncoordination and muscle tremors. However, the treatment of clinical signs has been sufficient to achieve recovery without sequelae within 2-3 days (Villar, Knight et al. 1994). For the same reason (fleas) three cats each had 120 ml of 100 % pure TTO applied to their shaved but intact skin (Bischoff and Guale 1998). All three cats experienced severe symptoms (hypothermia, uncoordination, dehydration, trembling), and one died after three days. The other two cats recovered within 24 and 48 h, respectively. The authors noted that the cat that died had elevated blood urea and persistent dehydration, which suggests that the animal may have had pre-existing renal damage unrelated to the TTO poisoning (Bischoff and Guale 1998).

The toxicity observed in cats and dogs are parallel to the effects observed in orally intoxicated humans (i.e. effects on the central nervous system). Dose comparisons are not possible between the dermal exposure of cats or dogs and the oral human exposure given that the skin area with dermal exposure and the absorption of TTO are not known. However, a dose of 120 mL on a cat appear to be a dose resembling the estimated dose from the study by Seawright from 1993.

In a study following OECD guideline 402 – limit test for acute dermal toxicity a group of 5 male and 5 female rabbits (NZ whites) were treated with TTO dermally (2000 mg/kg bw, undiluted sample, batch 88/375, skin area appr. 175 cm^2, 24 hours exposure) (Bolt 1989). Slight diarrhoea was observed on day three in one animal. No weight loss or other signs of toxicity was recorded during the two weeks observation period. The author concludes that the test sample is essentially non-toxic at a dose level of 2000 mg/kg bw.

In another study, the acute dermal LD_{50} in rabbits was recorded as in excess of 5000 mg/kg bw since this dose caused 2/10 deaths in rabbits (FragranceRawMaterialsMonograph 1988).

Dermal penetration
Experimental studies on dermal penetration of different ingredients of TTO demonstrated that the first component to penetrate the skin and reach the subcutaneous fat layer (within 1 hour) was terpinen-4-ol. After two hours exposure α-terpineol was also found in the subcutaneous fat layer (Hayes, Leach et al. 1997). As exposure time was increased, more ingredients were detected (1,8-cineole, α-terpinene, p-cymene, α-terpinolene), but all in considerably lower amounts (Hayes, Leach et al. 1997).

The penetration of TTO through human epidermal membranes was also evaluated experimentally by use of Franz cells (static diffusion cells) (Edwards-Jones, Buck et al. 2004). TTO was applied topically as the pure oil and as a 20 % formulation in ethanol. Following the 24 hr experimental period, terpinen-4-ol, α-terpineol, and 1,8-cineole were detected in the receptor phase. None of the other TTO constituents could be detected in the receptor phase, but a fraction of sesqui-terpinene compounds together with terpinen-4-ol and α-terpineol was seen in epidermis (Cross and Roberts 2006). Close to 15 % of the applied amounts of terpinen-4-ol and α-terpineol were recovered in the receptor phase, but over all less than 3% of the applied TTO penetrated the skin within the 24 hr experimental

period (Cross and Roberts 2006). There was a general experimental problem relating to recovery in these studies, which the authors explain by evaporation of volatile constituents during the experimental period. The results on fractional dermal penetration of the TTO are, however, in good agreement with earlier studies described below (Hayes, Leach et al. 1997).

Another experimental study using Franz cells evaluated the influence of topical application of TTO on the penetration of benzoic acid and methiocarb through human skin and identified the same three TTO constituents (terpinen-4-ol, α-terpineol, 1,8-cineole) as the only TTO constituents to quantifiable penetrate the skin (Nielsen and Nielsen 2006). Further, this study demonstrated that TTO significantly decreased the penetration rate as well as the total amount of benzoic acid and the pesticide methiocarb penetrating the skin (Nielsen and Nielsen 2006).

An important observation from these studies is that apparently only the least lipophilic constituents of TTO penetrate the human skin (Nielsen and Nielsen 2006). Thus, despite a low overall dermal penetration of TTO, the constituents that do penetrate the skin, penetrate in higher amounts. The low (lack of) penetration of the more lipophilic constituents of TTO may also have implications for the risk assessment related dermal exposure to these constituents.

An experimental study on human skin discs with 12 hours exposure to 200 µL (180 mg) TTO demonstrated a skin penetration of 4 mg TTO. This number was used to estimate risk in a worst case scenario based on topical application of 10 mL (9 g) neat TTO, which would correspond to a skin penetration of 200 mg TTO. This would equate some 2.8 mg/kg bw for an adult (70 kg) and 20 mg/kg for a child (10 kg). When this calculation is repeated on a 4% TTO product, assuming identical dermal penetration and exposure to 10 mL, an expected exposure would be 0.11 mg/kg for an adult and 0.8 mg/kg for a child. Histopathological assessment of skin discs exposed to 4% TTO indicated no major cellular damage apart from a few sporadic vacuolated cells (Hayes, Leach et al. 1997).

Skin irritation

Human data

In an assessment of the skin sensitivity and irritant potential of TTO, twenty-eight volunteers received applications of 1, 2.5, 5 or 10% TTO in sorbolene cream in a double blind placebo controlled pattern in occlusive patch testing for 21 days (5 days a week for three weeks) (Altman 1991). Irritancy was rated on a scale from 0 (no irritation) to 4 (erythema with oedema and blistering). Four persons exhibited slight irritation on one or two days out of 15 observations (concentrations of TTO used in four persons experiencing one day with slight irritation were 1% TTO, 2.5% TTO, 5% TTO, 5% TTO, respectively). One person reported slight irritation on 11 out of 15 days using the 10% formulation. No volunteers treated with placebo (sorbolene) reported any skin irritation.

Using a protocol based on the original Draize method, the potential of six TTO products to induce skin irritancy and/or allergenicity in humans was tested (Skin&CancerFoundationAustralia 1997). A total of 311 persons were included in the study and exposed to 100% TTO, 25% TTO in cream, 25% TTO in ointment, 25% TTO in gel, 5% TTO in cream and 5% TTO + 5% synergist in cream. No information as to the synergist was given. The test substances were applied to the skin a minimum of seven times during a three-week induction period. Skin reactions were scored on a scale ranging from no reaction (grade 0), erythema (grade 1), erythema and oedema (grade 2), vesiculation (grade 3) to bulla formation (grade 4). Irritancy was only observed after exposure to the undiluted TTO. The report argues that based on this study and the use of average values for irritancy,

TTO should be considered to be a low-irritant substance (Skin&CancerFoundationAustralia 1997). However, use of average values in inhomogeneous populations may not be correct. Thus, more than one third (118/306) of the participants had a positive reading for TTO on one of nine days in contrast to below 1% following exposure to the other products tested and in controls. Further, 17 persons (5.5%) had a stronger reaction than erythema on at least one day during the induction phase. Based on these observations, the conclusion might look different: The products with concentrations of TTO below or equal to 25% are not causing irritancy to the participants. Likewise, undiluted TTO is not an irritant for the vast majority of the participants, but a small fraction of the population (in this study 5.5%) seems to be more susceptible to TTO and demonstrates positive skin reactions towards undiluted TTO.

Unfortunately, the report does not give information as to the distribution of skin reactions between observation days for the individuals. This information could have been valuable for a discussion of the lengths and severity of the adverse reactions in the susceptible individuals given undiluted TTO.

Experimental data
The effect of TTO on wound healing was evaluated in a study on rabbits with or without surgically produced wounds. The rate of wound closure was not affected by exposure to undiluted TTO during a seven days observation period (Bolt 1989).

In a report on acute dermal irritation after exposure to TTO, the skin irritation index was determined by the Draize method using NZ White rabbits exposed to undiluted TTO (batch 88/375). The Draize irritation index for undiluted TTO was found to be 5.0, indicating a severe irritant (Bolt 1989). To what extent this finding is affected by occlusion causing an overestimate of the Draize index is not clear.

In a report on dermal irritation in the rabbit due to TTO exposure, the test sample of TTO was applied to the dorsal skin region of six rabbits (NZ Whites) at a rate of 0.5 mL of initially undiluted TTO, but from day 2 a 25% solution in paraffin oil over an area of 15 cm^2 during 30 days (Bolt 1989). Assessment of irritation was made on days 2, 7, 14, 21 and 30. Terminal skin biopsies were carried out and histological analysis performed. The undiluted TTO caused severe irritation after 24 hours. Hence the concentration was reduced to 25% (It is surprising that the authors initiate the study with undiluted TTO given that they 2 months before in an earlier report conclude that undiluted TTO is a severe irritant). The 25% solution of TTO in paraffin oil was not a visible irritant to the skin, but did cause minor (grade 1+) pathological changes consistent with mild irritation. The changes were seen as consistent with a non-specific dermatitis due to topical application of an irritant preparation. The authors conclude that the observed lesions following exposure to 25% TTO in paraffin were superficial and reversible.

Two studies on acute dermal irritation/corrosion following OECD guidelines have been reported in 1996:

1 Pharmatox. Acute dermal irritation/corrosion of TTO in the rabbit (T1836.A):

Guideline:	OECD 404
Species/strain:	Rabbit / New Zealand White
Group size:	3 female rabbits
Test substance:	Tea tree oil (TTO)
Batch No.:	28220296
Dose:	500 µL undiluted applied on 4x4 cm patches
GLP:	in compliance

TTO (100%) was found to be a mild irritant at 60 minutes post exposure, a severe irritant at 24 and 48 hours, a moderate irritant at 72 hours and a mild irritant 7 and 14 days following a 4 hour semi-occlusive patch application on intact skin. At 21 days the skin had returned to normal.

2. Pharmatox. Acute dermal irritation/corrosion of 75, 50, 25 and 12.5% TTO solutions in the rabbit (T1836.B):

Guideline:	OECD 404
Species/strain:	Rabbit / New Zealand White
Group size:	3 female rabbits
Test substance:	Tea tree oil (TTO)
Batch No.:	28220296
Dose:	500 µL diluted to with peanut oil and applied on 4x4 cm patches
GLP:	in compliance

TTO was applied for 4 hours with a semi-occlusive patch application followed by a 14 days observation period. The study demonstrated that:
TTO (75%) was found to be a mild to moderate irritant,
TTO (50%) was found to be a minimal irritant,
TTO (25%) was found to be a non-irritant,
TTO (12.5%) was found to be a non irritant

Based on the experimental studies with TTO, it is concluded that irritant reactions will not be expected to occur at TTO concentrations below 25 %.

Mucous membrane irritation
The guidelines describe that tests for mucous membrane irritation should follow either OECD 405 or the alternative bovine cornea opacity-permeability test. However, the HET-CAM test (Hen's Egg Test – ChorioAllantoic Membrane) (Gilleron, Coecke et al. 1997) is an alternative method often used in screening studies for finished cosmetic products. It is presently being validated, and may be taken up in the legislation of some EU member states (e.g. France).

Using the HET-CAM test TTO products were screened for eye irritation potential under GLP conditions (Schilcher and Leuschner 1997). TTO powder and TTO ground leaf were both evaluated as non-irritant. Undiluted TTO, water-soluble TTO, 25% TTO with 5% surfactant and 10% TTO with 10% surfactant were all rated as severe irritants, whereas 5% TTO with 8% surfactant was rated as a slight irritant. However, the placebo group (0% TTO and 10% surfactant) was also rated as a severe irritant. As the surfactant by it self caused a high irritation index, the results obtained with diluted TTO cannot be used for evaluating the irritancy of TTO. It is not clear from the report whether the water-soluble TTO was tested as undiluted or as a 10% solution. Further, no information is available as to the composition of the surfactant used.

These data demonstrates the importance of differentiation between testing of TTO and testing of a TTO product. Thus, the irritancy of a TTO-product need not be due to TTO, and the absence of irritancy of TTO does not assure the safety of a TTO-product.

The primary eye irritation of TTO was also studied in the rabbit (female, Japanese White) under GLP conditions (Oyama 2000). Two groups of three rabbits were given a single ocular dose (0.1 mL) of TTO (1% or 5% in liquid paraffin). After instillation of the test substance, no abnormal signs in the clinical conditions were observed among the rabbits. Ocular responses using Draize's criteria demonstrated

a conjunctival discharge lasting for up to six hours following instillation of 1% TTO and conjunctival redness and discharge for up to 24 hours following instillation of 5% TTO. In both groups, the maximal response was observed after one hour. Based on these observations, the author concludes, that both TTO solutions can be classified as “minimally irritating” (Oyama 2000).

Skin sensitisation

Human data

The human data on contact dermatitis was recently reviewed in a PhD-thesis (Hayes, Leach et al. 1997). The thesis states, that there has been an increase in the number of human contact dermatitis cases due to topical application of TTO with well over a dozen published cases within the last ten years (Apted 1991; De Groot and Weyland 1992; Selvaag, Eriksen et al. 1994; Van Der Valk, De Groot et al. 1994; De Groot 1996; Bhushan and Beck 1997). The applications included 100% TTO as well as lower concentrations of TTO in different formulated products.

In 1997, 1216 patients were patch tested at a dermatologic clinic (Fritz, Burg et al. 2001). Fourteen of them used products containing TTO. The patients used creams, hair products and essential oils containing *Melaleuca alternifolia* oil for cosmetic reasons and to treat skin infections. They were patch tested for a standard panel of allergens, topical emulgators, perfumes, plants, topical medications, metal, gloves, topical disinfectants and preservatives, dental products and rubber derivatives. Products containing *Melaleuca alternifolia* were tested concentrated or diluted. Seven patients with an allergic contact dermatitis due to tea tree oil were identified. Two of them also exhibited delayed type IV hypersensitivity towards fragrance-mix or colophony suggesting the possibility of cross reaction or an allergic group reaction caused by contamination of the colophony with the volatile fraction of turpentines. The allergic potential of low concentrations of *Melaleuca alternifolia* oil is presumed to be low on healthy skin. Photoaged *Melaleuca alternifolia* oil must be considered to be a stronger sensitizer (Fritz, Burg et al. 2001).

The prevalence of hypersensitivity to a number of allergens was tested in a group of volunteers (Greig, Carson et al. 2000). The findings were slightly higher than in other studies. The prevalence for hypersensitivity to TTO was found to be 2.3%. The authors argue that the prevalence found might be too high due to selection bias as the population studied were self-selected (Greig, Carson et al. 2000). 219 volunteers took part in the study. Close to 50% of the volunteers demonstrated hypersensitivity to dust mites and rye grass. This is a high number compared to the around 30% fraction of people expected to react to prick tests for dust mites or rye grass. 2.4% - 4.3% demonstrated marked irritancy to 100% TTO, whereas 7.2% - 10.1% demonstrated mild irritancy to 100% TTO (Greig, Carson et al. 2000). No participants demonstrated irritancy of any kind to 10% TTO. The bias could, however, go both ways and the prevalence for hypersensitivity to TTO is close to the study by (Leach 2000).

Leach concluded that TTO must be considered a mild allergen as only 2% of the 150 panellists showed an allergic reaction (Leach 2000).

Based on an Italian study in 725 persons patch tested according to GIRDCA guidelines, the authors conclude that the sensitization potential of Melaleuca oil is poor, and that the response in patch tests appears to be dose dependent, and primarily observed after exposure to undiluted TTO. Positive responses to patch tests were more frequent in subjects with existing allergic contact dermatitis or atopic dermatitis (Lisi, Meligeni et al. 2000).

Using a protocol based on the original Draize method, the potential of six TTO products to induce skin irritancy and/or allergenicity in humans was tested (Skin&CancerFoundationAustralia 1997). A total of 311 persons were included in the study and exposed to 100% TTO, 25% TTO in cream, 25% TTO in ointment, 25% TTO in gel, 5% TTO in cream and 5% TTO + 5% synergist in cream. No information as to the synergist was given. The test substances were applied to the skin a minimum of seven times during a three-week induction period. After a two-week period without skin exposure to TTO, a single 48-hour challenge for each product was applied on a new area of skin. The report concludes that TTO is a mild allergen as only 1% of the participants (3/308) were sensitised, ie. made allergic, to TTO by means of the Draize test (Skin&CancerFoundationAustralia 1997).

Experimental data
In a report on skin sensitisation in the guinea pig of TTO (Bolt 1989), groups of 20 albino guinea pigs (HA strain) were tested according to the Magnusson & Kligman method. The induction procedure consisted of two intradermal injections (5% TTO in paraffin or 5% TTO with Freund´s complete adjuvant) or an epidermal induction application (undiluted TTO). The dose used for challenge was 30% TTO. The erythemal reactions were measured 24 hours after removal of the patch of the challenge test. There were no irritant responses in either group. The experimental methodology stated in the report appears to follow OECD 406 guidelines.

Mutagenicity
Several in vitro genotoxicity tests are available. The SCCNFP for the in vitro base level testing of cosmetic ingredients recommend three assays (adopted by SCCNFP in December 2003):

1. Gene mutation tests
   Bacterial reverse mutation test (Ames test, OECD 471)
2. Test for clastrogenicity
   In vitro mammalian cell chromosome aberration test (OECD 473)
3. Test for aneugenicity and non-disjunction
   In vitro micronucleus test (OECD 474)

The first two tests are usually considered to provide sufficient evidence of mutagenic and/or genotoxic potential. There are, however, situations in which mutagenicity testing beyond the base level (two tests) may be required. Normally, if there is clear structural alert for mutagenicity or when some concern is raised by positive results from in vitro tests, further testing may be justified, e.g. micronucleus test in mammalian cells (OECD 474)

In vitro data
The mutagenicity of complete TTO was evaluated by the Salmonella/microsome assay (TA98, TA100, TA102) with and without metabolic activation (Bolt 1989). The sample of TTO was markedly antibacterial and doses above 50 µg were toxic to *S. Typhimurium*. At doses of 50 µg or less TTO did not demonstrate toxicity against the indicator strains. At these dose levels no reversion-inducing activity towards either of the indicator strains was observed. This study therefore indicates that TTO at doses below 50 µg is not mutagenic in this assay (Bolt 1989). A more recent study gave supporting evidence of the absence of a mutagenic potential of TTO when tested by the bacterial reverse mutation assay on the TA98 and TA100 *Salmonella* strains (Evandri, Battinelli et al. 2005).

A study following OECD guideline 474 and testing the potential of TTO to induce micronucleis in bone marrow demonstrated absence of any chromosomal damage at 48 hours following in vivo oral exposure of mice to TTO at doses ranging from

1000 to 1750 mg/kg (Firefly 2005). At the highest dose (1750 mg/kg), TTO induced toxicity (decreased weight gain) in the mice (Firefly 2005).

6 Toxicity profiles for individual TTO constituents

6.1 Terpinen-4-ol

The acute toxicity of terpinen-4-ol has been studied in mice, rats and rabbits with different administration routes (oral, subcutaneous, intraperitoneal, intramuscular, and dermal) (National Toxicology Program 2005). The dermal LD_{50} value in rabbits was described as above 2500 mg/kg (Opdyke 1982; National Toxicology Program 2005).

Terpinen-4-ol at 400 mg/kg was administered orally to male Sprague-Dawley rats for 28 days to assess nephrotoxicity. Terpinen-4-ol did not induce changes in the morphology or function of the kidneys, and was considered to be non-toxic at this dose level (Schilcher and Leuschner 1997).

The effect of terpinen-4-ol on intestinal relaxation was studied in vitro in rabbit duodenum. The intestinal relaxation induced by terpinen-4-ol is consistent with previous studies undertaken in the guinea-pig and rat ileum. The effect was dose related and was achieved at relatively low concentrations (200 μM). The relaxation of the rabbit duodenum and the decrease in spontaneous mechanical activity induced by terpinen-4-ol were promptly reversed by washing out the compound from the bath, showing functionally that terpinen-4-ol did not cause damage to the tissue contractile apparatus (Nascimento, Leal-Cardoso et al. 2005).

Terpinen-4-ol (2% in gel) significantly enhanced the percutaneous permeation of hydrocortisone formulated in HPMC (hydroxypropylmethyl Cellulose) gel systems (El-Kattan, Asbill et al. 2000).

Moderate irritation was seen when terpinen-4-ol (100%) was applied to intact or abraded rabbit skin for 24 h with occlusion (Opdyke 1982). Using 48 h closed patch tests and human volunteers, terpinen-4-ol (5%) in petrolatum was found to be non-irritating (Opdyke 1982). Patch testing of 10 volunteers with terpinen-4-ol (5-10%) did not show any irritant reactions (Knight and Hausen 1994).

Terpinen-4-ol (5%) was not sensitising using an open epicutaneous test in guinea pigs (Klecak 1985). Using a maximisation test and 25 human volunteers, terpinen-4-ol (5%) did not produce sensitisation reactions (Opdyke 1982). In a patch test of seven dermatological patients with positive patch tests for TTO and seven control persons, one pre-sensitised patient gave a positive reaction to terpinen-4-ol at 1% and an additional patient when the concentration of terpinen-4-ol was increased to 10%. None of the non-sensitized controls gave positive reactions to 10% terpinen-4-ol (Knight and Hausen 1994). Likewise, 10% terpinen-4-ol gave no response when tested in 10 guinea pigs (Knight and Hausen 1994). In a more recent study by the same group, terpinen-4-ol (10%) was applied on 11 dermatological patients, and none of them gave positive reactions to terpinen-4-ol (Hausen, Reichling et al. 1999).

The mutagenic potential of terpinen-4-ol was examined in the Ames test using *Salmonella typhimurium* (TA102, TA100 and TA98). No mutagenic effect was determined for the terpinen-4-ol component of TTO in any of the strains of *Salmonella* examined with or without metabolic activation. There was a clear evidence of toxicity against all *Salmonella* strains by terpinen-4-ol at higher dose levels. It is suggested that terpinen-4-ol may contribute significantly to the widely reported antibacterial activity of tea tree oil (Fletcher, Cassella et al. 2005).

Evaluation:

Irritancy:	neat terpinen-4-ol induce irritancy, but not at 5-10%.
Sensitisation:	possibly a weak sensitiser at 10% in pre-sensitised patients.
Mutagenicity:	not mutagenic.
Systemic toxicity:	The available literature on systemic effects of terpinen-4-ol is limited. Based on the 28-days study on kidney toxicity in rats, the NOAEL after oral exposure may be estimated to be 400 mg/kg. As terpinen-4-ol on average constitutes 40% of TTO, this NOAEL for terpinen-4-ol corresponds to an oral NOAEL for TTO (based on renal toxicity of terpinen-4-ol) of 1000 mg/kg.

6.2 γ-Terpinene

Most of the literature on γ-terpinene is more than 30 years old and several reports were not published in international journals. For regulatory purposes this literature was reviewed by Opdyke in 1976 (Opdyke 1976).

The acute oral LD_{50} in rats was reported as 3.7 g/kg body weight and the dermal LD_{50} in rabbits exceeded 5 g/kg body weight (Opdyke 1976).

Neat γ-terpinene was moderately irritating to intact and abraded rabbit skin when applied for 24 hours under occlusion, whereas 48 hours closed-patch test of human exposed to 5% γ-terpinene in petrolatum produced no irritation (Opdyke 1976). γ-Terpinene did not demonstrate any irritative effects or toxicity in the CAM (Chorioallantoic Membrane) assay (Demirci, Paper et al. 2004). An investigation on skin reactions to γ-terpinene (5% in soft white paraffin) using an occlusive patch test on 25 human subjects for 21 days demonstrated neither irritation nor allegic response (Southwell, Freeman et al. 1997).

A maximization test carried out in 1975 by Kligman et al. on 25 volunteers using 5% γ-terpinene in petrolatum revealed no sensitization reactions. A study published in german including 20 pre-sensitized persons found one positive reaction to γ-terpinene (Opdyke 1976).

γ-terpinene induced DNA damage in human lymphocytes in the comet assay at concentrations from and above 0.2mM (Aydin, Basaran et al. 2005). In contrast, it was found that below DNA damaging concentrations γ-terpinene protected lymphocytes against DNA damage induced by other chemicals (Aydin, Basaran et al. 2005). The interpretation of these data in relation to human risk is difficult as dose-comparisons between these in vitro studies and the human exposure situation with dermal exposure is complicated.

Evaluation:

Irritancy:	neat γ-terpinene was moderately irritating in rabbits, whereas more relevant concentrations of γ-terpinene did not induce irritation in humans.
Sensitisation:	possibly a weak sensitiser in patients pre-sensitised to terpenes. In volunteers without a past history of allergic reactions to cosmetic products, 5% γ-terpinene did not induce any allergic response during 21 days observation
Mutagenicity:	No data from Ames test available. γ-terpinene induce DNA damage at high doses when tested in the Comet assay.
Systemic toxicity:	The available literature on systemic effects of γ-terpinene is not sufficient to reach conclusions on chronic toxicity or estimate a NOAEL. The dermal LD_{50} value above 5 mg/kg indicates low toxicity.

6.3 α-Terpinene

The oral LD_{50} value of α-terpinene was reported to be 1680 mg/kg body weight in rats (Opdyke 1976).

α-terpinene has been demonstrated in vitro (liver microsomes prepared from phenobarbital-treated rats) to dose-dependently inhibit the liver enzyme CYP2B1 (De-Oliveira, Ribeiro-Pinto et al. 1997; De-Oliveira, Fidalgo-Neto et al. 1999). An inhibited isoenzyme will affect the metabolism of those chemicals,.that depend on CYP2B1, though IC_{50}-values between 0.1µM and 15µM in microsomal preparations will be expected to require a substantial in vivo dose to reach significant target organ concentrations.

α-Terpinene (30, 60, 125 and 250 mg/kg body weight) in corn oil was given by gavage to female Wistar rats from day 6 to 15 of pregnancy. Caesarean sections were performed on day 21 of pregnancy. The number of implantation sites, living and dead foetuses, resorptions and corpora lutea was recorded. All foetuses were weighed, examined for externally visible malformations. A reduction in body weight minus uterine weight at term indicated that the two highest oral doses tested (125 and 250 mg α-terpinene/kg body weight) were maternally toxic. Signs of delayed ossification (poorly ossified and not ossified bones as well as irregular spongy bones) and a higher incidence of minor skeletal malformations were observed at doses of 60 mg/kg body weight or more. These findings indicate that the no-observed-adverse-effect level for α-terpinene-induced embryofoetotoxicity can be set at 30 mg/kg body weight by the oral route (Araujo, Souza et al. 1996).

Ten hours following application of 0.1% α-terpinene on rat abdominal skin histopathology demonstrated effects on epidermis (liquefaction, desquamation) as well as dermis/hypodermis (collagen fiber swelling) (Kitahara, Ishiguro et al. 1993). The same authors also demonstrated that α-terpinene at a concentration in cell cultures of 0.1% caused significant toxicity by affecting the cell survival of human keratinocytes (Kitahara, Ishiguro et al. 1993). The irritancy of α-terpinene, terpinolene and limonene to rabbits was evaluated by the Draize test (Okabe, Obata et al. 1990). Terpinolene was more irritating than limonene, which was in turn more irritating than α-terpinene. The interpretation of the in vitro observations in relation to irritation of human skin is complicated, as evidence from the study in rabbits and human evidence does not appear to demonstrate the same degree of toxicity to the skin. Thus, using 48 h closed patch tests and human volunteers α-terpinene (5%) in petrolatum was non-irritating (Opdyke 1976).

Using a maximisation test and 25 human volunteers, α-terpinene (5% in petrolatum) did not produce sensitisation reactions (Opdyke 1976). However, in a more recent study α-terpinene appears to be among the most important allergens of TTO for which the prevalence of allergic response among patients visiting dermatological clinics vary between 0.4% and 0.6% (Hausen 2004).

The mutagenicity of α-terpinene was evaluated by the *Salmonella*/microsome assay (TA100, TA98, TA97a and TA1535 tester strains), without and with addition of an extrinsic metabolic activation system (rat liver S9 fraction induced by Aroclor 1254). Results from the present study indicated that α-terpinene is not mutagenic in the Ames test (Gomes-Carneiro, Viana et al. 2005).

Evaluation:

Irritancy:	based on human evidence, α-terpinene will not be expected to induce irritancy at a concentration of 5%.
Sensitisation:	equivocal evidence. α-terpinene is probably a weak sensitiser in pre-sensitised patients.
Mutagenicity:	not mutagenic.
Systemic toxicity:	There is limited evidence of systemic toxicity following exposure to α-terpinene. However, the study on α-terpinene-induced embryofoetotoxicity suggests a NOAEL for α-terpinene of 30 mg/kg body weight. As α-terpinene on average constitutes 9% of TTO, this NOAEL for α-terpinene corresponds to an oral NOAEL for TTO (based on α-terpinene embryotoxicity) of 330 mg/kg.

6.4 1,8-Cineole – eucalyptol

The acute oral LD_{50} in rats 2480 mg/kg bw (Jenner, Hagan et al. 1964).

The European Commission (European Commission 2002) states that eucalyptol undergoes oxidation in vivo with the formation of hydroxycineole which is excreted as glucuronide. In rats, 2-hydroxycineole, 3-hydroxycineole and 1,8-dihydroxycineol-9-oic acid were identified as main urinary metabolites (Madyastha and Chadha 1986).

Single subcutaneous doses of 250 or 500 mg/kg bw increased the activity of drug-metabolizing enzymes and stimulated bile flow (Jori, Di Salle et al. 1972). Liver microsomal-enzyme activity was greatly enhanced in adult rats treated with eucalyptol both during and after pregnancy and was also increased in the foetal and newborn offspring of such rats. In these offspring, a more marked stimulation of the generally poor drug-metabolizing capacity was demonstrated in connection with the O-demethylation of p-nitroanisole than with the p-hydroxylation of aniline. Suckling rats treated directly with eucalyptol also showed an increase in liver-enzyme activity, but administration of the oil to lactating mothers did not lead to any enzyme induction in the suckling rats. It thus appears that while eucalyptol is able to penetrate the placental barrier and reach a concentration in the foetal blood high enough to stimulate hepatic enzyme activity, it is unable to cross the blood-milk barrier to any effective extent. Its placental mobility is compatible with its high lipid solubility, a property reported to have a direct bearing on placental penetration (Jori and Briatico 1973). In relation to another member of the CYP-family of liver enzymes, 1,8-cineole has been found in vitro (liver microsomes prepared from phenobarbital-treated rats) to dose-dependently inhibit the liver enzyme CYP2B1 (De-Oliveira, Ribeiro-Pinto et al. 1997; De-Oliveira, Fidalgo-Neto et al. 1999). Apparently, 1,8-cineole induces or inhibite different liver enzymes, thus potentially affecting hepatic metabolism.

Cineole in a concentration of 5% enhance the skin permeation of propranolol in polymer films significantly (Amnuaikit, Ikeuchi et al. 2005). However, in another study on the enhancing effect of naturally occurring terpenes, 1,8-cineole was demonstrated to be a poor enhancer of the in vitro percutaneous absorption of diclofenac sodium from carbopol gels containing propylene glycol (Arellano, Santoyo et al. 1996). 1-8-cineole acts to reduce the intensity of lipid based reflections. Decreases in reflection intensities may be linked to a disruption of lipid packing within the bilayers and/or to a disturbance in the stacking of the bilayers (Cornwell, Barry et al. 1996). Ten hours following application of 0.1% cineole on rat abdominal skin histopathology demonstrated no effects on epidermis (liquefaction, desquamation) as well as dermis/hypodermis (collagen fiber swelling) (Kitahara, Ishiguro et al. 1993). The same authors also demonstrated that cineole at a concentration in cell cultures of 0.1% did not cause cell toxicity by affecting the cell survival of human keratinocytes (Kitahara, Ishiguro et al. 1993).

Groups of 6 male and 6 female B6C3F1 mice were fed eucalyptol for 28 days either by stomach tube on 5 days/wk at doses of 150, 300, 600 and 1200 mg/kg bw or in encapsulated form at concentrations of 3750, 7500, 15000 and 30000 mg/kg, equivalent to 600 – 5607 mg/kg bw/day for male and 705-6777 mg/kg bw/day for female mice. The liver weight/body weight ratio in males was increased at all but the lowest dose given in encapsulated form as was the brain weight/body weight ratio in females at the top dose level. Microscopic examination revealed a minimal hypertrophy of centrilobular hepatocytes in animals of both sexes fed the encapsulated compound, especially at the two highest dose levels (Wolff et al, 1987b). A parallel study with groups of 6 male and 6 female Fischer 344 rats exposed the animals to eucalyptol for 28 days either by stomach tube on 5 days/wk

at doses of 150, 300, 600 and 1200 mg/kg bw or in encapsulated form with the diet at concentrations of 3750, 7500, 15000 and 30000 mg/kg, equivalent to 381 – 3342 mg/kg bw/day for the male rats and to 353 – 3516 mg/kg bw/day for the female rats (Wolff et al., 1987a cited in (European Commission 2002)). At dose levels of 600 mg/kg bw and higher, dose-related decrease of body weight gain and absence of a normal degree of hepatic centrilobular cytoplasmic vacuolization was observed in male rats. In addition, other dose-related lesions in the liver, kidneys and parotid salivary glands were found at all dose levels in male rats fed encapsulated eucalyptol (Wolff et al., 1987a cited in (European Commission 2002)).

Groups of 10 male Wistar rats were given 0, 500, or 1000 mg eucalyptol/kg bw/day by gavage for 28 days. Statistically significant decreases in the terminal body weight and increased relative liver and kidney weights were found in both dose groups, whereas the relative brain weight was increased only in the highest dose group. No macroscopical changes were seen. Only brain, liver and kidneys were examined histopathologically, showing no changes in the brain and minor focal infiltration of mononuclear cells in the liver among all groups. In kidneys, a dose-related accumulation of eosinophilic protein droplets containing α2u-globulin in the cytoplasma of proximal tubular epithelial cells was induced (Kristiansen and Madsen 1995).

Eucalyptol was tested as constituent of toothpaste in an oral long-term study with specific pathogen-free CFLP mice. Groups of 52 male mice were given 0, 8 and 32 mg eucalyptol/kg bw/day in 1 ml toothpaste base/kg bw/day by gavage 6 days/week for 80 weeks followed by an observation period between 16 and 24 weeks according to the number of survivors. No treatment-related effects on body weight, food consumption, survival, weight of adrenals, kidneys, liver, lungs or spleen, on the microscopic appearance of brain, lungs, liver and kidneys and on the tumour incidence were observed (Roe, Palmer et al. 1979).

Evaluation of skin damage and cytotoxicity of 1,8-cineole on rat abdominal skin showed no irritation (Kitahara, Ishiguro et al. 1993). Using 48 h closed patch tests and human volunteers, 1,8-cineole (16%) in petrolatum was non-irritating (Opdyke 1975). Patch testing of 7 volunteers with 1,8-cineole did not show any irritant reactions (Knight and Hausen 1994).Skin irritancy following occlusive patch testing for 21 days was not detected in 28 humans exposed to any of eight preparations of pure cineole in concentrations ranging from 3.8% to 28.1% in soft paraffin (Southwell, Freeman et al. 1997).

Among a group of 25 human subjects without prior allergic reactions to cosmetic products none gave a positive response when tested with up to 28.8% 1,8-cineole in a TTO mixture with terpinen-4-ol in the occlusive patch test (daily readings and replacement for 21 days) (Southwell, Freeman et al. 1997). Three participants in the same study initially gave an allergic reaction to TTO, but when retested with new and substantially pure 1,8-cineole (1.4%) no reactions were found. The authors argue that impurities or oxidation products might have influenced their first trial and that 1,8-cineole is not an allergen (Southwell, Freeman et al. 1997). Likewise, in a study in 11 human subjects sensitized to TTO none demonstrated allergic reaction to the 1,8-cineole constituent (5%) nor could its sensitizing capacity be shown in experimentally sensitized guinea pigs exposed to 5% 1,8-cineole (Hausen, Reichling et al. 1999).

1,8-cineole was not mutagenic when evaluated by the Salmonella/microsome assay (TA97a, TA98, TA100 and TA102 tester strains), without and with addition of an extrinsic metabolic activation system (lyophilized rat liver S9 fraction induced by Aroclor 1254) (Gomes-Carneiro, Felzenszwalb et al. 1998). Eucalyptol did not

show mutagenic effects in the following strains of *Salmonella typhimurium* with or without metabolic activation: TA 98, TA 100, TA 1535 and TA 1537 (Haworth, Lawlor et al. 1983). In CHO cells, eucalyptol did not induce chromosome aberrations with or without metabolic activation. Sister chromatid exchanges were induced in CHO cells only in the absence of metabolic activation at doses that induced cell cycle delay (Galloway, Armstrong et al. 1987). Sister chromatid exchanges induced by mitomycin C in CHO K-1 cells were not increased by posttreatment with eucalyptol (Sasaki, Imanishi et al. 1989). Cineole at concentrations ranging from 10 – 1000 μM did not increase the frequency of spontaneous sister-chromatid exchanges in Chinese hamster ovary cells (Sasaki, Imanishi et al. 1989). A number of in vitro studies have come to different conclusions in relation to mutagenicity, chromosomal damage. Taken together, it is the impression that 1,8-cineole is possibly a weak promoter. If 1,8-cineole acts as a promoter only and not by itself damages DNA, it may be defendable to calculate no-effect-levels.

Cineole did not induce primary lung tumors in male or female A/He mice following 24 ip injections during an eight week period with 24 weeks follow-up. The doses used were either MTD (maximal Tolerated Dose) or 20% of MTD (Stoner, Shimkin et al. 1973).

BIBRA in 1991 suggested a NOAEL for eucalyptol of 300 mg/kg. Using an uncertainty-factor of 100, this would give an estimated ADI of 3 mg/kg anticipating 100% dermal absorption in the absence of specific data on dermal absorption. Based on these estimates, the Norwegian Food Control Authorities in 1999 calculated, that exposure to eucalyptol using a 1 gram facial cosmetic TTO cream daily holding 3% TTO of which 15% was eucalyptol would cause an exposure of approximately 2% of the ADI (Acceptable Daily Intake, Norwegian Food Control Authorities, December 1999). Other exposure scenarios with other TTO products were presented, none of them causing an exceeded ADI for eucalyptol.

Currently eucalyptol is regarded as GRAS (generally recognised as safe) by FEMA (1965) and is approved by the US Food and Drug Administration (FDA) for food use. The FDA advisory review panels on over-the-counter drugs have concluded that eucalyptol is safe for a variety of products, such as lozenges taken every 0.5 - 1 hr at 0.2 – 15 mg or taken every 2 hrs at 1 –30 mg of eucalyptol (US Food and Drug Administration, 1976 – 1990).

Maximum concentrations of eucalyptol in cosmetic products have been reported to be 0.4% in soap, 0.04% in detergents, 0.1% in creams and lotions and 1.6% in perfume (Opdyke 1975).

Evaluation:

Irritancy:	Neither animal nor human evidence indicate that 1,8-cineole should have any significant potential as irritant.
Sensitisation:	Available information from animal as well as human exposure indicate that pure 1,8-cineole is not a sensitiser.
Mutagenicity:	1,8-cineole is not mutagenic in Ames test. 1,8-cineole is possibly a weak promoter, but not carcinogenic in mice tested at MTD.
Systemic toxicity:	Based on the studies on hepatic and renal toxicity a NOAEL might be estimated as 300 mg/kg body weight, which is in agreement with the BIBRA evaluation from 1991 and used by the Norwegian Food Control Authorities in 1999. As 1,8-cineole on average constitutes 5% of TTO, this NOAEL for

1,8-cineole corresponds to an oral NOAEL for TTO (based on liver and kidney toxicity of 1,8-cineole) of 6000 mg/kg.

6.5 Terpinolene

Terpinolene has low acute toxicity. Oral and dermal LD_{50}s are 3800 mg/kg in rats and mice, and >5000 mg/kg in rabbits (Opdyke 1988).

A study on the skin penetration from matrix-type transdermal systems demonstrate that close to 12% of the dose penetrate the epidermis within 8 hour, corresponding to 0.7 mg/cm^2 in this experimental setup (Cal, Janicki et al. 2001).

Terpinolene applied full strength to intact or abraded rabbit skin for 24 hr under occlusion was not irritating (Opdyke 1976). Tested at 20% in petrolatum it produced no irritation after a 48-hr closed-patch test on human subjects (Opdyke 1976).

Ten hours following application of 0.1% terpinolene on rat abdominal skin histopathology demonstrated effects on epidermis (liquefaction, desquamation) as well as dermis/hypodermis (collagen fiber swelling) (Kitahara, Ishiguro et al. 1993). The same authors also demonstrated that terpinolene at a concentration in cell cultures of 0.1% caused significant toxicity by affecting the cell survival of human keratinocytes (Kitahara, Ishiguro et al. 1993). The interpretation of this study is difficult considering the effects observed at very low concentrations of terpinolene compared to the concentrations applied to human skin in vivo with no irritant effects. To gain credibility, these observations on cell cultures need to be repeated.

In a maximization test on 24 volunteers terpinolene was tested at a concentration of 20% in petrolatum and produced no sensitization reactions (Opdyke 1976). In an older study on occupational skin disorders, terpinolene was found not to be a sensitizer for human skin (Woeber and Krombach 1969). A high fraction of TTO-sensitised patients with existing skin disease demonstrated positive patch tests against terpinolene when tested with 10% oil in ethanol (17 out of 18), whereas patch testing with terpinolene (1%) did not show any erythema (Knight and Hausen 1994; Hausen, Reichling et al. 1999). A 52-year-old man developed an acute contact dermatitis after application of undiluted TTO to his scalp. Patch tests revealed a specific hypersensitivity to TTO and to 6 of its constituents including terpinolene (Reindl, Gall et al. 2000).

Terpinolene was given the status of a “generally regarded as safe” (GRAS) direct food additive by the Flavor Extract Manufacturers Association (FEMA No. 3046) in 1965 and is approved by the FDA for use in foods (Opdyke, 1988). The Council of Europe included terpinolene in the list of artificial food flavouring substances that may be added to food without risk to human health in 1974 (Opdyke, 1988).

Significant dietary exposure to terpinolene occurs through ingestion of such foods as ice cream and ices (64 mg/kg), candies (0.12 - 48 mg/kg), non-alcoholic beverages (16 mg/kg), and baked goods (49 mg/kg). Dermal exposure can occur from such products as soaps (200 – 4000 mg/kg), lotions (100 –1000 mg/kg), perfumes (1200 – 5000 mg/kg), and detergents (20 – 400 mg/kg).

Evaluation:

Irritancy:	Human evidence indicates that terpinolene does not cause irritant reactions at exposures below 20%.
Sensitisation:	Available information from human exposure indicate that terpinolene is a weak sensitiser in pre-sensitised individuals (no effect at 1%, significant response at 10%).

Mutagenicity:	No mutagenicity, genotoxicity, or carcinogenicity studies were identified for terpinolene
Systemic toxicity:	No subchronic, chronic, or foeto- toxicity studies were identified for terpinolene, and a NOAEL for systemic toxicity can not be estimated. Based on irritancy and sensitization a NOAEL of 1% may be suggested. As terpinolene constitutes approximately 3.3% of TTO, this would equal a NOAEL of 30% for TTO regarding irritancy and sensitization.

6.6 p-Cymene

p-Cymene is formed through oxidation of α-terpinene or γ-terpinene (McGraw, Hemingway et al. 1999), and oxidised tea tree oil contains increased levels of p-cymene and decreased levels of α-terpinene, γ-terpinene (Brophy, Davies et al. 1989; Hausen, Reichling et al. 1999; Hausen 2004). Cumene is a different chemical than cymene, as cumene is lacking the methyl-group residing in the para-position on p-cymene. Studies on the metabolism of p-cymene in rabbits do not indicate that cumene is among the primary metabolites of p-cymene as metabolism appears to affect the isopropyl group (Matsumoto, Ishida et al. 1992). However, as studies on the further metabolism of cumene and cymene demonstrate considerable similarities, they are expected to have comparable toxicological profiles. Thus, several recent toxicological evaluations, including one by for US EPA (The Flavour and Fragrance High Production Volume Consortia - the Terpene Consortium 2002), have due to insufficient data on p-cymene used data on cumene to develop NOAEL values for cymene. This approach will also be used in this report with the exceptions of evaluations of sensitising and mutagenic effects where even minor chemical differences may have implications for the outcome.

The acute oral LD_{50} of p-cymene in rats was reported as 4750 mg/kg (Jenner, Hagan et al. 1964). The lethal dose by ip administration was 2162 mg/kg in the guinea-pigs and the acute dermal LD_{50} of p-cymene in rabbits was reported as > 5000 mg/kg (Opdyke 1974).

p-Cymene is well absorbed through the skin. In studies with ^{14}C-labelled p-cymene on mice and rats, the penetration observed was around 250 μg/cm^2 in 60 min (Wepierre 1963; Wepierre 1963). Likewise, cumene is rapidly absorbed by oral administration or inhalation exposure (The Flavour and Fragrance High Production Volume Consortia - the Terpene Consortium 2002). Following absorption, the ring substituent is oxidized to yield aromatic alcohol and carboxylic acid metabolites that are excreted free or conjugated in the urine. There is no evidence that cumene accumulates in the body even following high dose or repeat dose exposure (The Flavour and Fragrance High Production Volume Consortia - the Terpene Consortium 2002).

The effects of p-cymene on the brain chemistry of rats was studied by exposing male Long-Evans rats to 0, 50 or 250 ppm p-cymene by inhalation (Lam, Ladefoged et al. 1996). Rats were exposed for 6 hours per day, 5 days per week for four weeks and then had an 8 week wash-out period. No obvious toxicity was seen during the exposure period and body weights did not differ after the 12 week trial period. Levels of synaptosomal protein were significantly reduced in treated rats, whereas relative amounts of noradrenaline and dopamine were increased (Lam, Ladefoged et al. 1996). The doses used were, however, in excess of the occupational TLV's (Threshold Limit Values), and the relevance of the study is probably limited in relation to topical use of TTO oil with minor amounts of cymene.

Repeat dose toxicity studies have been performed with cumene (Wolf, Rowe et al. 1956; Cushman, Norris et al. 1995). In the only oral toxicity study on cumene, rats were gavaged with cumene up to 769 mg/kg bw/day, 5 days/week for a period of 6 months (Wolf, Rowe et al. 1956). Following necropsy and hematological examination, the only effect reported was an increase in average kidney weight (not specified if absolute or relative weight) in the 2 highest dose groups (no statistical analysis). This finding was not accompanied by histopathological renal changes. In all probability the kidney weight changes may be early indications of

species and sex specific *alpha*-2μ-globulin-induced nephrotoxicity. Other terpene hydrocarbons including limonene and camphene have been reported to produce *alpha*-2μ-globulin-induced nephrotoxicity in male Fisher 344 rats. This phenomenon is specific to Fisher 344 male rats and has neither been observed in other sexes or strains of rats, other rodents, nor in humans.

A recent well-conducted developmental toxicity study was conducted with cumene in CD rats and New Zealand white rabbits. Rats and rabbits were used to assess the potential developmental toxicity of cumene (Darmer Jr, Neeper-Bradley et al. 1997). Pregnant rats were exposed to atmospheres containing up to 1,200 ppm of cumene inhalation, 6 hours/day during gestation days 6-15 and pregnant rabbits were exposed at up to 2,300 ppm of cumene 6 hours/day during gestation days 6-18. In rats, reported effects included reduced food consumption, reduced body weight gain, perioral wetness, encrustation, and increased relative maternal liver weight. No statistically significant effects were reported in the fetuses. In rabbits, the reported effects included, death of 2 does at the highest concentration, reduced body weight gain, reduced food consumption, increased incidence of perioral wetness, lung color changes in 33% of high-dose does, and increased relative maternal liver weight. No statistically significant effects were reported in the fetuses. There was a significant increase in the incidence of skeletal and visceral variations; however, they were not exposure related. In reviewing this study, EPA (EPA 1997) set the maternal NOAEL at 488 ppm in rats based on the significant decrease in body weight gain during exposure and increased relative liver weight. Even at maternally toxic concentrations, exposure to cumene vapor did not produce developmental toxicity in rats. In further review of this study, EPA determined that the changes in gestational parameters of the rabbits, though not significant, were consistent in indicating possible developmental effects and therefore set the NOAEL in rabbits for both developmental and maternal effects at 1,206 ppm and the LOAEL at 2,297 ppm, respectively (EPA 1997).

p-cymene was not irritating when assessed in vitro using the HET-CAM assay (Demirci, Paper et al. 2004). Moderate irritation was seen when neat p-cymene was applied to intact or abraded rabbit skin for 24 h with occlusion (Opdyke 1974). Using 48 h closed patch tests and human volunteers p-cymene (4%) in petrolatum applied daily for 10 days to the same spot on the backs of volunteers did not produce irritation (Opdyke 1974). Patch testing of 10 volunteers with 1% p-cymene did not show any irritant reactions (Knight and Hausen 1994). Eye irritation thresholds in humans between 100 ppm and 1000 ppm has been determined for p-cymene (Cometto-Muñiz, Cain et al. 1998; Cometto-Muñiz, Cain et al. 1998).

p-Cymene (4%) was not sensitising using an open epicutaneous test in guinea pigs (Klecak 1985). Using a maximisation test and 25 human volunteers p-cymene (4%) did not cause positive responses (Opdyke 1974). The results of the patch testing of 21 TTO-sensitised individuals with TTO components (Knight and Hausen 1994; Southwell, Freeman et al. 1997 Knight, 1994 #362; Hausen, Reichling et al. 1999) demonstrated that one patient gave a positive response to p-cymene at 1%.

An extensive review on the toxicity of cymene and cumene has recently (2002) been submitted to the US EPA under the HPV (high production volume) Challenge Program by The Flavor and Fragrance High Production Volume Chemical Consortia (The Flavour and Fragrance High Production Volume Consortia - the Terpene Consortium 2002). This review includes detailed descriptions of the available literature including data on metabolism, accumulation, elimination, and potential for systemic toxicity as well as mutagenicity/genotoxicity. Regarding possible mutagenic/genotoxic effects, they conclude: that p-Cymene produced no increase in the frequency of mutations when tested in Sd-4-73 *Escherichia coli*.

Concentrations up to 2,000 µg/plate of cumene did not increase the number of revertants in *Salmonella typhimurium* strains (TA97, TA98, TA100, TA1535, and TA1537) in the Ames preincubation assay with or without metabolic activation (NTP unpublished results). In cultured mammalian cells, cumene showed no consistent evidence of mutagenicity or genotoxicity at non-cytotoxic concentrations. Cumene did not increase mutations in the CHO/HGPRT test with or without metabolic activation at concentrations of up to 175 µg cumene/plate. Cultured rat hepatocytes treated with cumene up to 5,000 µg/ml showed cytotoxicity at concentrations of 128 µg/ml and higher and unscheduled DNA synthesis was reported at 16 µg/ml. However, the authors note that the results between triplicates were highly variable and inconsistent (The Flavour and Fragrance High Production Volume Consortia - the Terpene Consortium 2002).

Cymene was found to be non-mutagenic using bacterial assays such as the Ames test (Rockwell and Raw 1979). The US EPA has concluded that cumene does not appear to metabolize to highly reactive chemical species and in terms of metabolism, cumene is analogous to methyl benzene for which a 2-year inhalation study was conducted by NTP in 1990, and no evidence of carcinogenic activity was reported in either rats or mice (EPA 1997). Overall, the EPA concluded "there is not much suspicion that cumene would pose a significant carcinogenic hazard."

Evaluation:

Irritancy:	p-cymene is a moderate irritant to rabbits at high concentrations. Tested up to 4%, p-cymene was not an irritant to humans.
Sensitisation:	Based on the available information from human exposure, p-cymene is not expected to be a sensitiser.
Mutagenicity:	Neither p-cymene nor cumene appear to be mutagenic or genotoxic at non-cytotoxic concentrations. There is not much suspicion that cumene would pose a significant carcinogenic hazard to humans.
Systemic toxicity:	A limited number of relevant repeat-dose studies are available and the inhalation route is often used for cumene. A NOAEL of 488 ppm based on inhalation might be suggested as might also a LOAEL of 769 mg based on the only study with oral exposure. Based on the oral study and using an uncertainty factor of 10, a NOAEL for cumene/p-cymene of 75 mg/kg body weight is suggested. As p-cymene on average constitutes 6% of TTO, this NOAEL for p-cymene corresponds to an oral NOAEL for TTO (based on possible renal effects of p-cymene) of 1200 mg/kg body weight.

6.7 α-Pinene

In a memorandum from April 2005 (EPA 2005), the US EPA (registration division, office of prevention, pesticides and toxic substances) has assessed the toxicity of α- and β-pinene and conclude:

α-and β-pinene are the major components of turpentine. The two chemicals are closely related, having the same empirical formula of $C_{10}H_{16}$ and the same basic ring structure. The predominant uses of the pure forms of α- and β-pinene are as fragrances.

Dermal LD_{50} (rabbit) for α- as well as β-pinene was larger than 5000 mg/kg bw (Opdyke 1978; Opdyke 1978).

A patient attempting suicide ingested 400-500 ml pine oil and was admitted to the clinic. Since more than the lethal dose had been ingested hemoperfusions with activated charcoal and amberlite and hemodialysis were performed. The composition of the ingested pine oil was determined by gas chromatography/mass spectrometry. Four monoterpenes were identified: 57% α-pinene, 8% β-pinene, 26% carene, 6% limonene and 3% other hydrocarbons. The blood and urine monoterpene concentrations were continuously monitored. The data suggest that monoterpenes are poorly resorbed in the gastrointestinal tract. The resorbed portion of the hydrocarbons cumulates in the lipophilic body compartments and is slowly metabolized and then excreted by the kidneys. The main metabolic pathways are hydration, hydroxylation, and rearrangement, and acetylation. Five metabolites were identified (Koppel, Tenczer et al. 1981).

α-Pinene is well-absorbed via the skin, lungs, and gastro-intestinal tract (EPA 2005).

In a 3-month oral toxicity study, rats were fed an α-pinene resin or pinene polymer made predominantly from α-pinene. (The ratio of α-and β-pinene was 10:1.) The dose levels were 0, 1, 3 or 5% in the diet. Effects seen at 5% (3967 mg/kg/day) included an increase in relative liver weight in both sexes, and absolute liver weight in females only. Increased relative thyroid weights in males were noted at the 3 and 5% dose levels. In the absence of histopathological alterations, these changes were not considered treatment related. No effects were noted at 1%, which corresponds to roughly 800 mg/kg/day (EPA 2005).

The effect of oral pretreatment with α-pinene on the hexobarbital sleeping time was examined in healthy female rats and rats rendered cirrhotic with thioacetamide. After pretreatment with α-pinene the sleeping time of both healthy and cirrhotic rats was significantly shortened. This is attributed to microsomal enzyme induction (Marosi, Pap et al. 1973).

A mixture of α- and β-pinene (and other terpene hydrocarbons) was tested in three developmental toxicity studies. Summaries of the results of these studies report that no maternal or developmental effects were noted in mice, hamsters, or rats at the highest dose levels, 560, 600, or 260 mg/kg/day, respectively. α- and β-pinene are not structurally related to any known developmental or reproductive toxicants (EPA 2005).

Undiluted α-pinene applied to the backs of hairless mice and swine was not irritating. However, once applied to intact or abraded rabbit skin for 24 hr under occlusion it was a moderate irritant**.** When tested at 10% in petroleum it produced

no irritation after a 48 hr closed patch test on two different panels of human subjects. Beta pinene applied full strength to intact or abraded rabbit skin for 24 hr under occlusion was a moderate irritant. When tested at 12% in petroleum it produced no irritation after a 48 hr closed patch test on human subjects (EPA 2005). Too few human subjects in an in vivo exposure chamber reported eye-irritation for α-pinene and α-terpineol to allow estimates of thresholds of these compounds which therefore have much less irritative potency than n-butanol, 3-carene, and limonene (Mølhave, Kjaergaard et al. 2000).

In a dermal human sensitization study, α- and β-pinene produced no dermal sensitization when tested at concentration of 10% and 12% in petroleum, respectively (EPA 2005). In experiments with oil of turpentine and α-pinene it was shown that only the autoxidation products of oil of turpentine and not the terpenes themselves were eczematogenic. Autoxidation of a-pinene in the presence of air and light was sufficient to produce the eczematogenic agent, but its formation could be prevented by addition of inhibitors such as hydroquinone and pyrogallol (Opdyke 1978).

No phototoxic effects were reported for undiluted α-pinene on hairless mice and swine (Opdyke 1978).

The mutagenicity of (+) and (-)-α-pinene was evaluated by the Salmonella/microsome assay (TA100, TA98, TA97a, TA1535, and TA1537 tester strains), without and with addition of an extrinsic metabolic activation system (rat liver S9 fraction induced by Aroclor 1254). Results indicated that (+) and (-)-α-pinene are not mutagenic in the Ames test (Florin, Rutberg et al. 1980; Gomes-Carneiro, Viana et al. 2005). β-pinene at concentrations ranging from 10 – 1000 μM did not increase the frequency of spontaneous sister-chromatid exchanges in Chinese hamster ovary cells (Sasaki, Imanishi et al. 1989). No chronic or carcinogenicity studies were identified; however, α- and β-pinene are not structurally related to any known carcinogens (EPA 2005).

Evaluation:

Irritancy:	Neat α- and β-pinene are moderate irritants to rabbits but not to mice and swine. Tested at 10-12% in petroleum neither α- nor β-pinene were irritants to humans.
Sensitisation:	Based on the available information from human exposure, the pinenes are not expected to be sensitisers. The oxidation product may be eczematogenic.
Mutagenicity:	Neither α- nor β-pinene appear to be mutagenic or genotoxic.
Systemic toxicity:	Given the low acute toxicity by the oral, dermal and inhalation routes, the low subchronic toxicity, the lack of reproductive or developmental effects at high dose levels, and the extensive naturally-occurring (primarily inhalation and oral) exposures, the US EPA concluded that a quantitative approach was not needed. If a NOAEL had to be developed it could be based on the study on developmental toxicity in the most susceptible animal species and result in a NOAEL for pinene of 260 mg/kg/day. As α-pinene on average constitutes 3.5% of TTO, this NOAEL for α-pinene corresponds to an oral NOAEL for TTO (based on possible developmental effects of α-pinene) above 7000 mg/kg body weight.

6.8 α-Terpineol

The acute toxicity of α-terpineol is limited with an oral LD_{50} value in rats above 4000 mg/kg and a dermal LD_{50} value in rabbits above 3000 mg/kg (Opdyke 1974). However, human cases of intoxication include several cases of accidental ingestion of large amounts of α-terpineol (400-500 mL) with fatal as well as non-fatal outcome and including young as well as older individuals (Hill, Barer et al. 1975; Welker and Zaloga 1999; Cording, Vallaro et al. 2000).

In his review on terpineol Opdyke (Opdyke 1974) describe that terpineol is rapidly absorbed through the intact shaved abdominal skin of the mouse.

Ten (10) male and 10 female weanling Osborne-Mendel rats were fed alpha-terpineol acetate in the diet for 20 weeks at concentrations of 0, 1000, 2500 or 10,000 ppm (Hagan, Hansen et al. 1967). These dietary levels were calculated by the US FDA to result in daily intakes of 0, 50, 125 and 500 mg/kg bw, respectively. All animals were examined for growth, hematology, and macroscopic changes in the tissues. Microscopic examination was performed on 6-8 male and female animals in the high dose and control groups. No statistically significant adverse effects were reported (Hagan, Hansen et al. 1967).

Terpineol has been demonstrated in vitro (liver microsomes prepared from phenobarbital-treated rats) to dose-dependently inhibit the liver enzyme CYP2B1 (De-Oliveira, Ribeiro-Pinto et al. 1997; De-Oliveira, Fidalgo-Neto et al. 1999). An inhibited isoenzyme will affect the metabolism of those chemicals.that depend on CYP2B1, though IC_{50}-values between 0.1μM and 15μM in microsomal preparations will be expected to require a substantial in vivo dose to reach significant target organ concentrations.

Ten hours following in vitro application, 0.1% α-terpineol caused significant cytotoxicity by affecting the cell survival of human keratinocytes and fibroblasts in vitro (Kitahara, Ishiguro et al. 1993).

Moderate irritation was seen when terpineol was applied to intact or abraded rabbit skin for 24 h with occlusion (Opdyke 1974). Investigation of the irritant capacity of several terpenes by transepidermal water loss (TEWL) and histological observations suggested that α-terpineol is potentially irritating (Fang, Hung et al. 2003). Evaluation of skin damage and cytotoxicity of a range of terpenes on rat abdominal skin showed no irritation for α-terpineol (Kitahara, Ishiguro et al. 1993). Patch testing of 10 volunteers with 1% α-terpineol did not show any irritant reactions (Knight and Hausen 1994). Using 48 h closed patch tests and human volunteers, terpineol (12%) in petrolatum was non-irritating (Opdyke 1974).

In a larger multicenter study, a set of 5 to 10 fragrances at 2 concentrations was patch tested in each centre on a minimum of 100 consecutive patients seen in the patch test clinic. These patients were also patch tested to a standard series with the 8% fragrance mix and its 8 constituents. In patients with a positive reaction to any of 48 food fragrancies, a careful history with regard to past or present reactions to perfumed products was taken. A total of 1323 patients were patch tested in 11 centres and none of them demonstrated irritancy or allergic response to α-terpineol (Frosch, Pilz et al. 1995). An earlier study by six of the same dermatological departments demonstrated that among 18 fragrances tested in 1606 consecutive patients from these dermatological clinics, the lowest reactivity was observed with α-terpineol, yelding only 1 positive (<0.1%) and 11 doubtful reactions in a patch

test with 5% α-terpineol (Frosch, Johansen et al. 2002) The results of the patch testing of 10 TTO-sensitised individuals with α-terpineol (1.1-1.3%) did not demonstrate any positive response (Knight and Hausen 1994; Southwell, Freeman et al. 1997).

Terpineol was negative using the *Bacillus subtilis* rec- assay (Oda, Hamano et al. 1978). α-Terpineol was not mutagenic when assayed for mutagenicity towards four *Salmonella*-strains (TA 98, TA 100, TA 1535, TA 1537) with and without metabolic activation (Florin, Rutberg et al. 1980). Terpineol caused a slight but dose-related increase in the number of hisq revertants with TA102 tester strain both without and with addition of S9 mixture, but results were negative with the TA97a, TA98, and TA100 tester strains (Gomes-Carneiro, Felzenszwalb et al. 1998).

Terpineol did not induce primary lung tumors in male or female A/He mice following 24 ip injections during an eight week period with 24 weeks follow-up. The doses used were either MTD or 20% of MTD (Stoner, Shimkin et al. 1973).

α-Terpineol was included in a study on the male rat specific renal toxicity/carcinogeniciy mediated through formation of hyaline droplets (this species-specific toxic effect is also discussed in relation to limonene). The authors evaluated a new ligand displacement model, and α-terpineol as well as its metabolites demonstrated minimal ligand displacement (Lehman-McKeeman and Caudill 1999). Thus, α-terpineol had a binding affinity for the ligand that was 175 times lower than the positive control and seven times lower than limonene, and the binding affinity for the metabolites were lower (Lehman-McKeeman and Caudill 1999). The data demonstrates that α-terpineol does not induce this kind of renal toxicity.

Evaluation:

Irritancy:	An in vitro study and a study on rabbits indicate that α-terpineol is an irritant at high concentrations. Human dermal exposure to up to 12% α-terpineol in petroleum does not appear to induce irritancy
Sensitisation:	Based on the available information from human exposure, α-terpineol is not expected to be a sensitiser.
Mutagenicity:	α-Terpineol was negative in 5 out of six salmonella strains. However, the result from the last strain (TA102) can not be ignored as a false positive finding because of the dose-related toxicity. To exclude mutagenicity, another study based on the TA102 tester strain is needed. However, in support of a lack of genotoxic potential, α-terpineol did not induce lung tumors in mice following repeated intraperitoneal administrations.
Systemic toxicity:	Based on the study using dietary exposure of rats, a NOAEL for α-terpineol of 500 mg/kg bw can be suggested. As α-terpineol on average constitutes 5% of TTO, this NOAEL for α-terpineol corresponds to an oral NOAEL for TTO (based on the only available study on systemic toxicity for α-terpineol) of 10,000 mg/kg body weight might be suggested.

6.9 Aromadendrene

Aromadendrene is one in a row of minor constituents of TTO for which published scientific literature on potential toxicity is absent or limited.

No data on acute, sub-chronic, or chronic toxicity is available. No information on mutagenicity or potential genotoxicity is available.

Following topical application, patch testing of 10 volunteers with 1% aromadendrene did not show any irritant reactions (Knight and Hausen 1994).

A high fraction of TTO-sensitised patients (5 out of 7) demonstrated positive patch tests against 1% aromadendrene (Knight and Hausen 1994). On the other hand, none of the 11 patients tested in the Hausen et al. study from 1999 demonstrated any positive reaction to aromadendrene when tested at 5% (Hausen, Reichling et al. 1999). Differences do occur between dermal reactions recorded in different studies, but these differences are often explained by presense of impurities or oxidative product in test oils.

Evaluation:

Irritancy:	One study in humans is available in which aromadendrene did not demonstrate irritancy at a concentration of 1%.
Sensitisation:	Two studies in humans by the same group of authors are published. The most recent, with the highest concentration of aromadendrene (5%) including 11 patients did not demonstrate a sensitisation potential.
Mutagenicity:	No published data are available.
Systemic toxicity:	No published data are available, and no NOAEL can be suggested. Aromadendrene is a minor constituent of TTO (3.5%). Comparison with other chemicals of close chemical resemblance does not indicate that aromadendrene exposure following topical use of TTO products should pose a significant risk for systemic toxicity.

6.10 δ-Cadinene

Cadinene is the principle component of *Juniperus oxycedrus* tar, and some of the available information on the toxicity of cadinene is limited to studies using oils derived from the various varieties of juniper.

The acute oral LD_{50} of cadinene was reported to be higher than 5 g/kg in the rat and the acute dermal LD_{50} in the rabbit was likewise above 5 g/kg (Opdyke 1973).

The activities of testosterone hydroxylation and the levels of P4502B1 and 3A2 were increased following experimental exposure to cadinene. The P450 isoform induced by cadinene is similar to that induced by phenobarbital. However, the magnitude of induction by cadinene was less than that by phenobarbital at the dose levels studied (Hiroi, Miyazaki et al. 1995).

When cadinene was tested at a concentration of 10 % in petroleum it produced no irritation in a 48-hr closed-patch test in 25 human subjects (Opdyke 1973).

The oil from *Juniperus communis* was not phototoxic in animal tests (Anonymous 2001).

Juniperus oxycedrus Tar was genotoxic in several assays (Anonymous 2001). However, no genotoxicity data were available for any of the extracts which means that cadinene was not tested alone but only as part of the tar.

Evaluation:

Irritancy:	One study in humans is available in which cadinene did not demonstrate irritancy at a concentration of 10%.
Sensitisation:	A single study on 25 volunteers has been published. Cadinene at a concentration of 10% inpetroleum did not demonstrate a sensitisation potential.
Mutagenicity:	No published data are available on cadinene.
Systemic toxicity:	No published data are available; except for a study demonstrating that cadinene induce liver enzymes in animal experiments, which is insufficient for suggesting a NOAEL. Cadinene is a minor constituent of TTO (4%). Comparison with other chemicals of close chemical resemblance together with the low acute toxicity does not indicate that cadinene exposure following topical use of TTO products should pose a significant risk for systemic toxicity.

6.11 Limonene

Acute toxicity
d-Limonene is rated as moderately toxic (with a probable lethal dose in humans of 0.5-5.0 g/kg (between 40 and 400 gram for a 80-kg adult) (Gosselin, Hodge et al. 1976). No toxicity was reported after humans were given a single dose of 20 g d-limonene in an attempt to dissolve gallstones (Igimi 1976). Both the acute oral LD_{50} in rats and the acute dermal LD_{50} in rabbits exceeded 5 g/kg (Opdyke 1975).

Toxicokinetics
Lemonade prepared with whole lemon (Mediterranean-style lemonade) contains high levels of d-limonene. In humans drinking 800-1200 mL of lemonade (containing 447-596 mg limonene), no toxicity was observed and maximal concentration of the primary metabolite, perillic acid, was reached after one hour and declined rapidly with a terminal elimination half-life ranging from 1-2 hours (Chow, Salazar et al. 2002).

Limonene was well absorbed on to the skin of rats (Opdyke 1975).

The toxicokinetics of d-limonene was studied in Sprague-Dawley rats following intravenous and oral administration at 200 mg/kg each. Blood concentration–time profiles after intravenous administration showed a biphasic decline with a mean initial $t_{½}$ of 12.4 min and a terminal $t_{½}$ of 280 min. The plasma/red blood cell partition was found to be 0.84. The plasma protein binding of d-limonene was found to be 55.3% at 20 mg/ml. The mean total clearance was 49.6 ml/min/kg, the volume of distribution at steady-state was 11.7 l/kg, and median residence time was 263 min. The blood concentration–time decline following oral administration also showed a biphasic decline with a mean initial t½ of 34 min and terminal t½ of 337 min. The oral bioavailability of d-limonene was 43.0 % (Chen, Chan et al. 1998).

Carvone and carveol are oxidation/degradation products of limonene (Anandaraman and Reineccius 1986)

Systemic toxicity
After intraperitoneal administration of high doses (100 or 200 mg/kg bw) of limonene to mice, sedative as well as motor relaxant effects were observed (Gurgel do Vale, Couto Furtado et al. 2002).

d-Limonene given orally to rats (250, 500, 1000 mg/kg/d) for 8 consecutive days resulted in a marked increase in both the number and the phagocytic activity of alveolar macrophages compared to the controls. These results suggest that d-limonene taken up from the thoracic duct lymph moves to the lung and directly activates the immune response of alveolar macrophages there, or indirectly activates it through activated lymphocytes (Hamada, Uezu et al. 2002).

In vitro studies using the L929 cell line demonstrated cytotoxicity at concentrations as low as 0.25% in the tissue medium (Vajrabhaya and Suwannawong 2004). The susceptibility in specified cell lines and general problems related to dose transferal between in vitro studies and the in vivo situation do, however, complicate quantitative use of in vitro data for the risk evaluations.

Renal toxicity
Renal toxicity following exposure to limonene has received special focus as this is one of the cases where one gender of a specific strain of a specified species (male rats of the Fisher 344 strain) develops a characteristic toxic response. Thus,

limonene produces renal tumors in male F344 rats (Turner, Tinwell et al. 2001; Sekihashi, Yamamoto et al. 2002). Under the conditions of 2-year gavage studies, there was clear evidence of carcinogenic activity of d-limonene for male F344/N rats, as shown by increased incidences of tubular cell hyperplasia, adenomas, and adenocarcinomas of the kidney. However, there was no evidence of carcinogenic activity of d-limonene for female F344/N rats that received 300 or 600 mg/kg. There was no evidence of carcinogenic activity of d-limonene for male B6C3F1 mice that received 250 or 500 mg/kg. There was no evidence of carcinogenic activity of d-limonene for female B6C3F1-mice that received 500 or 1,000 mg/kg. A range of studies using different strains and species have been published in order to support the hazard evaluation regarding renal toxicity following exposure to limonene.

d-Limonene given to male Fischer 344 rats at 75, 150 or 300 mg/kg body weight 5 days per week for up to 4 weeks resulted in hyaline droplet formation by the 6th day (Kanerva, Ridder et al. 1987). In another study by the same group of researchers limonene administered by oral gavage at 150-2400 mg/kg/day in a subchronic (91-day) study induced renal alterations in male rats at all dose levels, whereas kidneys of male mice, female rats and female mice were unaffected (Kanerva and Alden 1987).

In a separate subchronic study, groups of 5-wk-old male Fischer 344 rats were administered d-limonene in a corn oil vehicle at 0 (control), 2, 5, 10, 30 or 75 mg/kg body weight by single daily gavage (5 days/wk) for 13 wk. It is concluded that treatment with d-limonene caused an increase in the formation of hyaline droplets in male rats only, that this increase was associated with an accumulation of $\alpha_{2\mu}$-globulin , that d-limonene (or its metabolite) accumulated significantly in male rat kidney compared with that in females and that subchronic dosing produced a triad of morphological changes in the male rat kidney. These observations suggest that d-limonene caused nephrotoxicity specific to the male rat and that this toxicity may not be predictive of a similar response in humans (Webb, Ridder et al. 1989). In a study to assess the presence or absence of this response in a non-rodent species, adult beagle dogs were gavaged twice daily for 6 months with 100 or 1000 mg d-limonene/kg body weight per day. Limonene ingestion did not affect feed consumption or body weight and there were no evidence of hyaline droplet accumulation nor of any other sign of hydrocarbon-induced nephropathy typical of those seen in male rats treated with d-limonene. Thus, dogs are refractory to the hyaline droplet nephropathy observed in male rats, thereby providing additional evidence that the male rat kidney is uniquely sensitive to hydrocarbons like d-limonene, and that this specific male rat nephropathic response may be inappropriate for interspecies extrapolation and human risk assessment (Webb, Kanerva et al. 1990).

d-Limonene administered to 10-wk-old Wistar rats for 4 weeks (125, 500 and 4000 ppm) caused damage to the epithelial cells of the proximal tubes. The dosage of 4000 ppm reduced growth slightly in males whereas 500 ppm did not. Other changes in males included slightly increased kidney weights, and/or slight histopathological changes in the kidneys and epithelial cells in the urine (Jonker, Woutersen et al. 1993).

d-Limonene produces tumors only in the kidneys of male rats in association with hyaline droplet nephropathy, which is due to the accumulation of the rat-specific, low molecular weight protein $\alpha_{2\mu}$-globulin in the P2 segment cells of renal proximal tubules. Human urine contains no $\alpha_{2\mu}$-globulin and, compared with the male rat, much less protein and almost no low molecular weight protein. Genotoxicity tests for d-limonene are negative, and the mechanism of

tumorigenesis involves tumor promotion and enhanced cell proliferation. There is no risk of cancer for humans from d-limonene, since the binding of d-limonene to $\alpha_{2\mu}$-globulin would not occur (Whysner and Williams 1996).

In line with this argument, Flamm and Lehman-McKeeman states: The three major lines of evidence supporting the human safety of d-limonene are (1) the male rat specificity of the nephrotoxicity and carcinogenicity; (2) the pivotal role that $\alpha_{2\mu}$-globulin plays in the toxicity, as evidenced by the complete lack of toxicity in other species despite the presence of structurally similar proteins; and (3) the lack of genotoxicity of both d-limonene and d-limonene-1,2-oxide, supporting the concept of a nongenotoxic mechanism, namely, sustained renal cell proliferation. Collectively, the evidence that the renal effects of d-limonene are confined to male rats because of the unique presence of $\alpha_{2\mu}$-globulin is quite compelling. In this regard, d-limonene is readily distinguished from classical renal carcinogens and should, therefore, not be subjected to traditional interspecies extrapolation and quantitative risk assessment. As d-limonene shows no toxicity or carcinogenicity in female rats or male and female mice when administered over a lifetime, it is considered safe for human consumption (Flamm and Lehman-McKeeman 1991).

Dermal toxicity

In an in vivo study in rats on penetration enhancing effects and skin irritation, 1% d-limonene was demonstrated to significantly enhance the percutaneous penetration of the test substance ketoprofen (Okabe, Obata et al. 1990). The same study also demonstrated that limonene at a 5-10% concentration on the skin did not induce skin irritation (edema or erthema) during 72 hours observation period following application (Okabe, Obata et al. 1990). Moderate irritation was seen when neat *d*-limonene was applied to intact or abraded rabbit skin for 24 h with occlusion (Opdyke 1975). Evaluation of skin damage and cytotoxicity on rat abdominal skin showed histopathological changes and cytotoxicity against human keratinocytes after exposure to limonene (Kitahara, Ishiguro et al. 1993). Using 48 h closed patch tests and human volunteers, d,l-limonene (dipentene) (20%) in petrolatum was non-irritating (Opdyke 1974). Patch testing of 10 volunteers with 1% d-limonene did not show any irritant reactions (Knight and Hausen 1994).

Limonene was not a respiratory irritant when tested in humans at concentrations of 10, 225, and 450 mg/m^3. At the highest exposure level a temporary decrease in lung capacity was observed (Falk-Filipsson, Lof et al. 1993).

d-Limonene (8%) was not sensitising using an open epicutaneous test in guinea pigs (Klecak 1985). Limonene at 25% and 50% did not produce a response in the local lymph node assay, but 100% did (Warbrick, Dearman et al. 2001). This was regarded as being a weak response. d-Limonene did not produce sensitisation reactions when applied to guinea pigs whereas oxidised d-limonene did (Karlberg, Boman et al. 1991). Using a maximisation test and 25 human volunteers, d-limonene (8%) did not produce sensitisation reactions (Opdyke 1975).

Limonene cause skin reactions in six out of seven participants in the Knight and Hausen study from 1994 when applied in 1% as compared to only one in eleven subjects exposed to 5% limonene (Knight and Hausen 1994; Hausen, Reichling et al. 1999). Whether the positive reactions observed in the 1994 study on limonene were caused by impurities or oxidative products is not to say, but positive patch test reactions to oxidised limonene are common amongst dermatology patients (Karlberg, Dooms-Goossens et al. 1997; Matura, Goossens et al. 2002; Matura, Karlberg et al. 2003). Patch testing with limonene (1%) produced one irritant or doubtful positive reaction in 192 participants, whereas 0.1% limonene produced no reactions (Frosch, Pilz et al. 1995). Further, patch testing with 3% limonene

produced only 7 positive in 1606 dermatology patients (Frosch, Johansen et al. 2002).

Autoxidation of d-limonene readily occurs to give a variety of oxygenated monocyclic terpenes that are strong contact allergens (Karlberg, Dooms-Goossens et al. 1997). Thus, patch testing with oxidized R-(+)-limonene was performed on 2273 patients at 4 dermatology clinics in Europe, and a total of 63 patients (2.8%) showed positive reactions (Matura, Goossens et al. 2002). Oxidation products of *d*-limonene, (R)-(-)-carvone, (+)-limonene oxide, along with air oxidized *d*-limonene, were found to be potent sensitizers in the Freund complete adjuvant test and in the guinea pig maximization test (Haneke 2002).

Reproductive toxicity

Pregnant rabbit were administered oral doses of 250 or 1,000 mg/kg d-limonene (Kodama, Okubo et al. 1977). Decrements in feed intake and body weight gain and deaths in 6/21 animals were observed in the high dose group. These effects were not seen at 250 mg/kg d-limonene; no teratogenic effects were observed. Pregnant rats were given 2,869 mg/kg d-limonene orally from day 9 to 15 of gestation (Tsuji, Fujisaki et al. 1975). Body weight gain of the dams was decreased, and a prolongation of the ossification of metacarpals and proximal phalanges was observed in the foetuses. Oral administration of 2,363 mg/kg d-limonene to mice between days 7 and 12 of gestation also caused maternal body weight decrements and increased incidences of abnormal bone formation in the foetuses (Kodama, Okubo et al. 1977).

Mutagenicity and genotoxicity

d-(+)-Limonene at concentrations ranging from 10 – 1000 μM did not increase the frequency of spontaneous sister-chromatid exchanges in Chinese hamster ovary cells (Sasaki, Imanishi et al. 1989). *d*-Limonene exposures failed to result in observable mutations either *in vitro* or *in vivo* (Haneke 2002). Limonene was not mutagenic when assayed for mutagenicity towards four salmonella-strains (TA 98, TA 100, TA 1535, TA 1537) with and without metabolic activation (Florin, Rutberg et al. 1980). Limonene was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 when tested in a pre-incubation protocol in both the presence and absence of Aroclor 1254-induced male Sprague Dawley rat or Syrian hamster liver S9 (Haworth, Lawlor et al. 1983). Watabe et al. investigated the mutagenicity, with and without induced S9, of d-limonene and two presumed intermediate metabolites (the 1,2- and 8,9-epoxides, which are in turn converted to the corresponding glycols) in *Salmonella typhimurium*, and they also observed no increase in revertants (Watabe, Hiratsuka et al. 1980).

Carcinogenicity

d-Limonene is classified as a group 3 carcinogen by the IARC (evidence of carcinogenicity is inadequate in humans or limited in experimental animals. Limonene did not induce primary lung tumors in male or female A/He mice following 24 ip injections during an eight week period with 24 weeks follow-up. The doses used were either MTD or 20% of MTD (Stoner, Shimkin et al. 1973). Elegbede et al. (1986) compared orange peel oil and DMBA in a two-stage skin carcinogenesis model with female CD-I mice and confirmed that topically applied orange peel oil was a very weak promoter of both skin papillomas and carcinomas but that minor terpene components, and not topically applied d-limonene, possessed the promoter activity (Elegbede, Maltzman et al. 1986).

Evaluation:

Irritancy:	Neat limonene is a moderate irritant to rabbits. Limonene does not induce irritancy in humans when tested up to a concentration of 20%
Sensitisation:	Neat limonene induces a response in the LNNA, but concentrations of 50% and below did not. In the absence of oxidation/degradation products the published literature describes limonene as a non-sensitiser. However, autooxidation of limonene has repeatedly been demonstrated to generate potent sensitisers.
Mutagenicity:	There is no support in the literature that limonene is mutagenic. Limonene is potentially a very weak promoter and the evidence for carcinogenicity is rated as limited in experimental animals by IARC.
Systemic toxicity:	Limonene is generally of limited acute toxicity and a natural ingredient in many soft drinks and lemon juice products. Renal carcinogenicity and toxicity in humans following topical application of limonene is not seen as relevant. Based on the study on reproduction, 250 mg/kg orally is suggested as a NOAEL value for limonene. As limonene on average constitutes 2,5% of TTO, this NOAEL for limonene corresponds to an oral NOAEL for TTO (based on the study on reproductive toxicity for limonene) of 10,000 mg/kg body weight.

6.12 Sabinene

Sabinene constitutes on average below 2% of TTO.

The only published literature on sabinene describe that sabinene (1%) has an anti-inflammatory effect when tested against experimentally induced eye inflammation in rabbits. No sign of eye irritation due to sabinene at this concentration is reported (Yao and Chiou 1993).

Based on the information that no eye irritation was observed at 1% sabinene and that sabinene constitutes below 2% of TTO, it can be anticipated that an irritant response due to sabinene in a TTO product is unlikely.

There is no available published literature describing mutagenic, genotoxic, or systemic effects in humans, in experimental animals, or in vitro.

6.13 Globulol

Globulol constitutes on average 1.5% of TTO.
Globulol has been evaluated and topical treatment with globulol is found useful in controlling Th2-type inflammatory cutaneous disorders. These disorders may include atopic dermatitis (Hori, Nonomura et al. 2001).

No published literature on toxicity of globulol is available and a toxicological profile can not be developed. The chemical nature of this Sesquiterpene alcohol and the amounts present in TTO product does, however, not indicate that acute or systemic toxicity can be expected.

6.14 Viridiflorol

Viridiflorol constitutes on average below 1% of TTO.

The only available information on viridiflorol is that it inhibits acetylcholinesterase (Miyazawa, Watanabe et al. 1998).

Thus, there is no information available for a judgement of the potential toxicity of this very minor ingredient in TTO.

6.15 Degradation products

Like most natural oils several constituents of TTO may undergo oxidation. This is a natural process that occurs over time and primary depends on storage conditions related to temperature, access to oxygen, and presence of antioxidants. Photooxidation may also be an issue for some chemicals. A few descriptions of degradation of TTO with time are described in the literature. It has been stated that the oxidation products formed may increase the irritancy capacity of the TTO. In one experiment TTO was stored on a window sill to study the influence of light, oxygen and warmth. GC analysis demonstrated an increase in peroxides within 4 days from 50 ppm to more than 500 ppm. Peroxides, epoxides and endoperoxides were formed (Hausen et al. 1999). Chemicals of this type are well recognized as demonstrating a range of toxic effects including skin irritation, irritation of mucous membranes, formation of lipid peroxides (membrane damage), mutagenicity, and adduct formation with DNA.

Besides these peroxides, degradation products from the original constituents of TTO may be formed, which may potentially exert a different degree of toxicity than their parent TTO constituent. Oxidised tea tree oil contains increased levels of ρ-cymene and decreased levels of α-terpinene, γ-terpinene and terpinolene (Brophy, Davies et al. 1989; Hausen, Reichling et al. 1999; Hausen 2004). The amount and rate of transformation may be illustrated by the observation, that during a 4 day period the p-cymene content in a TTO sample increased from 2% to 11.5%, while the contents of α-terpinene, γ-terpinene as well as terpinolene were reduced to one half of their original concentrations during the same period (Hausen et al. 1999). Not all constituents do, however undergo degradation to the same extent. Thus, a detailed study on the autoxidation of terpenes in cell-free nutrient medium demonstrated that alpha-pinene and beta-pinene were both autoxidized to a certain extent, while limonene remained unaffected (Lindmark-Henriksson, Isaksson et al. 2004).

These natural processes related to the ageing of a product have led regulatory bodies to focus not only on parent constituents of a product, but also on degradation products formed during storage. Thus, Directive 2003/15/EC from February 2003 states that limitations and restrictions as to the use of certain ingredients in cosmetics will be implemented besides a general requirement for information on minimum durability (‘best used before the end of ‘ on the label followed by a date or details of where it appears on the packaging). Among ingredients present in TTO, d-limonene is mentioned specifically: The presence of d-limonene in the product must be indicated in the list of ingredients when the concentration exceeds 0.001 % in leave-on products and 0.01 % in rinse-off products.

Further to d-limonene; limolene and natural products containing substantial (??) amounts of it, should only be used when the level of peroxides is kept to the lowest practical level, for instance by adding antioxidants at the time of production. Such products should have a peroxide value of less than 20 millimoles peroxide per liter (IFRA guidelines) (SCCNFP/0392/00, final adopted September 2001).

Degradation products derived from individual components found in tea tree oil are listed below (Table 4). These have been derived by autoxidation from exposure to heat, air or oxygen, rather than by biotransformation or metabolic processes. An important notion was that the rate of the autoxidation was more than one order of magnitude slower than that of the biotransformation. Moreover, different products

were formed by autoxidation than by biotransformation (Lindmark-Henriksson, Isaksson et al. 2004).

Some of the degradation products occur already in newly distilled TTO and are part of the ISO list of the 14 main constituents of TTO. The toxicological profiles of these compounds have already been described in the above section, but the most important of the remaining known degradation products will be described in the detail that the available literature allows.

Table 4. Oxidation products of tea tree oil components

Component	Oxidation/degradation product(s)	References(s)
α-Terpinene	ρ-cymene	(McGraw, Hemingway et al. 1999)
	Thymol	(McGraw, Hemingway et al. 1999)
	Carvacrol	(McGraw, Hemingway et al. 1999)
	1,8-cineole	(McGraw, Hemingway et al. 1999)
	Ascaridol	(Karapire, Kus et al. 2005)
	1,2,4-trihydroxymenthane	(Hausen, Reichling et al. 1999)
γ-Terpinene	ρ-cymene	(Foti, Sortino et al. 2005)
Limonene	(+)-limonene oxide	(Haneke 2002; Marine and Clemons 2003)
	(R)-(-)-carvone	(Anandaraman and Reineccius 1986; Haneke 2002)
	Carveol	(Anandaraman and Reineccius 1986)
	Limonene-(1,2)-epoxide	(Anandaraman and Reineccius 1986)
α-Pinene	Sobrerol	(Haneke 2002)
	Verbenone	(Lindmark-Henriksson, Isaksson et al. 2004)
β-Pinene	α-terpineol	(Lindmark-Henriksson, Isaksson et al. 2004)
	Pinocarvone	(Lindmark-Henriksson, Isaksson et al. 2004)
	1,8-cineole	(Lindmark-Henriksson, Isaksson et al. 2004)

Degradation of α-terpinene caused formation of mainly p-cymene, but degradation products also included 1,8-cineole (already described above), thymol, ascaridol, iso-ascaridol, and 1,2,4-trihydroxymethane (Hausen, Reichling et al. 1999). Especially peroxides, epoxides (e.g. iso-ascaridol) and endoperoxides (e.g. ascaridol) generated through photooxidation seems to be toxicologically important products (Hausen, Reichling et al. 1999).

6.15.1 Thymol and carvone

The oral LD_{50} of carvone and thymol in rats were found to be 1640 mg/kg bw and 980 mg/kg bw, respectively (Jenner, Hagan et al. 1964).

The metabolism of thymol and carvacrol in rats was studied using gas chromatographic-mass spectrometric methods. The urinary excretion of metabolites was rapid. Only very small amounts were excreted after 24 hrs. Although large quantities of carvacrol and, especially, thymol were excreted unchanged (or as their glucuronide and sulphate conjugates), extensive oxidation of the methyl and isopropyl groups also occurred (Austgulen, Solheim et al. 1987).

Thymol and carvone did not induce any subacute or chronic toxicity following dietary exposure of rats to 2500 ppm in the feed (Hagan, Hansen et al. 1967).

l-Carvone (1%) was not sensitising using an open epicutaneous test in guinea pigs (Klecak 1985). In accordance with this observation in guinea pigs, carvone (5%) was not positive in two separate studies with patch tests of 18 humans presensitized to TTO (Knight and Hausen 1994; Hausen, Reichling et al. 1999).

Neither carvone nor thymol were mutagenic when assayed for mutagenicity towards four salmonella-strains (TA 98, TA 100, TA 1535, TA 1537) with and without metabolic activation (Florin, Rutberg et al. 1980; Stammati, Bonsi et al. 1999). Likewise, DNA-repair tests of thymol and carvone were negative at exposure-relevant concentrations, though inhibition of DNA-repair was observed at high doses of carvone (Stammati, Bonsi et al. 1999).

The genotoxic potential of major compounds of thyme oil, i.e. thymol and carvacrol, were investigated in human lymphocytes by single-cell gel electrophoresis. Also, the effects of these substances on the induction of DNA damage by 2-amino-3-methylimidazo[4,5-f]-quinoline (IQ) and mitomycin C (MMC) were evaluated. No increase in DNA strand breakage was observed at thymol concentrations below 0.1 mM, but at the higher concentration of 0.2 mM significant increases in DNA damage were seen. Thymol significantly reduced the DNA strand breakage induced by IQ and MMC at the lower concentrations studied. Carvacrol, which is an isomer of thymol, seemed to protect lymphocytes from the genotoxic effects of IQ and MMC at non-toxic concentrations below 0.05 mM, but at the higher concentration of 0.1 mM carvacrol itself induced DNA damage (Aydin, Basaran et al. 2005). Thus, these data indicate that thymol and carvacrol protect against DNA damage at concentrations below 0.1 mM, but cause DNA damage themselves at higher concentrations. Interpreting these findings in a human risk assessment is complicated, but as the DNA damage only emerges at the millimolar range, this kind of toxicity will not be expected to occur during topical use of TTO products containing thymol and/or carvacrol as degradation products.

Thymol and d/l-carvone did not induce primary lung tumors in male or female A/He mice following 24 ip injections during an eight week period with 24 weeks follow-up. The doses used were either MTD or 20% of MTD (Stoner, Shimkin et al. 1973).

Evaluation:

Based on the available published literature and the fact that thymol as well and carvone are minor degradative constituents of TTO, acute or systemic toxicity from these compounds will not be expected. Likewise, none of the two compounds appears to pose and mutagenic or carcinogenic risk to humans.

6.15.2 Ascaridol / isoascaridol and 1,2,4-trihydroxymenthane

A high fraction of TTO-sensitised patients demonstrated positive patch tests against 5% ascaridol (9 out of 11) (Knight and Hausen 1994; Hausen, Reichling et al. 1999). Positive patch test results were also recorded for 1,2,4-Trihydroxymenthane at a concentration of 5% (4 out of 11 patients) (Knight and Hausen 1994; Hausen, Reichling et al. 1999).

By 2003 close to 7000 patients at German dermatological clinics had been tested epicutaneously with a 5% dilution of oxidised TTO containing the original constituents as well as oxidation products (Hausen 2004). Seventy patients (1%) had a positive reaction to TTO (Hausen 2004). The most important allergens of TTO appear to be terpinolene, ascaridol, α-terpinene, and 1,2,4-trihydroxy menthane for which the prevalence of allergic responses among patients visiting dermatological clinics varies between 0.4% and 0.6% (Hausen 2004).

Evaluation:

Sensitisation:	These degradation products of TTO have clear allergic potencies demonstrated repeatedly in independent studies in humans.

6.16 Other components of interest

Besides the known major and minor constituents of TTO and known degradation products, for which the toxicological profiles have been described above, a few other terpenes of close familiarity to the TTO constituents have been evaluated because toxicological information on these structurally comparable terpenes/terpenoids were available that could supplement the available information on the more important constituents of TTO. These compounds include myrcene, phellandrene, and caryophyllene.

6.16.1 Myrcene

Myrcene can be found in TTO in concentrations up to 0.5%, and is the only TTO constituent without a ring structure. Myrcene has a low acute toxicity (Opdyke 1976) with oral as well as dermal LD_{50} values above 5000 mg/kg bw. Myrcene is not mutagenic in Ames test (Gomes-Carneiro, Viana et al. 2005) and systemic toxicity related to this constituent is not expected. Myrcene is included in this section because three relevant studies on reproductive toxicity are available for this minor constituent of TTO. Therefore, only the reproductive data are described to any length in this section. Data on acute toxicity, induction of hepatic enzymes, allergy, and mutagenicity are available, but not considered to add to the review of the toxicity of TTO and constituents.

In a study on the embryo-foetotoxic potential of beta-myrcene in the rat beta-myrcene (250, 500 and 1200 mg/kg) in corn oil was given orally to Wistar rats from day 6 to 15 of pregnancy. From the data presented the NOAEL for embryo-foetotoxicity could be set at 500 mg beta-myrcene/kg body weight (Delgado, Carvalho et al. 1993).

Another study by the same authors with the aim to provide data on the peri- and postnatal developmental toxicity of beta-myrcene used doses of beta-myrcene (250, 500, 1000 and 1500 mg/kg) in corn oil and given by gavage to female Wistar rats from day 15 of pregnancy, parturition and throughout the period of lactation up to weaning (postnatal day 21). From the data presented in this paper the NOAEL for peri- and postnatal developmental toxicity was set at 250 mg beta-myrcene/kg body weight (Delgado, De Almeida Nogueira et al. 1993).

The effects of myrcene on fertility and general reproductive performance were studied in the rat (Paumgartten, De-Carvalho et al. 1998). Myrcene (0, 100, 300 and 500 mg/kg) in peanut oil was given by gavage to male Wistar rats (15 per dose group) for 91 days prior to mating and during the mating period, as well as to females (45 per dose group) continuously for 21 days before mating, during mating and pregnancy, and throughout the period of lactation up to postnatal day 21. Myrcene did not affect the mating index (proportion of females impregnated by males) or the pregnancy index (ratio of pregnant to sperm-positive females). No sign of maternal toxicity and no increase in externally visible malformations were observed at any dose level. Only at the highest dose tested (500 mg/kg) did myrcene induce an increase in the resorption rate and a higher frequency of fetal skeleton anomalies. No adverse effect of myrcene on postnatal weight gain was noted but time of appearance of primary coat, incisor eruption and eye opening were slightly delayed in the exposed offspring. On the basis of the data presented in this paper the NOAEL for toxic effects on fertility and general reproductive performance was set at 300 mg of ß-myrcene/kg body weight by the oral route (Paumgartten, De-Carvalho et al. 1998).

Reproductive toxicity from myrcene is not expected to be relevant for exposures related to TTO due to the low amount of myrcene present in TTO. However, the data may serve as a supplement to the limited data on reproductive toxicity available on more dominant constituents of TTO.

6.16.2 Phellandrene

The available newer literature on the toxicological profile of phellandrene is limited. The older literature is covered in a review by Opdyke (Opdyke 1978).

The acute oral LD_{50} in rats was reported as 5.7 g/kg (4.7-6.7 g/kg) and the acute dermal LD_{50} in rabbits exceeded 5 g/kg (Opdyke 1978).

Phellandrene was readily absorbed through the skin of rats (Opdyke 1978). In sheep, α-phellandrene apparently undergoes reduction of one double bond and oxidation of the methyl group to give phellandral, which is further oxidized to phellandric acid; conjugation with glycine gives rise to phellanduric acid, which is then excreted in the urine (Opdyke 1978).

α-Phellandrene applied full strength to intact or abraded rabbit skin for 24 hr under occlusion was moderately irritating (Opdyke 1978). Tested at 4% and 8% in petrolatum, it produced no irritation after a 48-hr closed-patch test on two separate panels of human subjects. An older study by Valette and Cavier from 1954 cited by Opdyke (Opdyke 1978) states that α-Phellandrene is readily absorbed through the skin of rats.

A maximization test was carried out on 25 volunteers. The material (RIFM no. 71-68) was tested at a concentration of 4% in petrolatum and produced one sensitization reaction (Opdyke 1978). In view of the autoxidation problems, it was decided that the maximization procedure should be repeated on α-phellandrene using a freshly distilled sample processed under a blanket of nitrogen and containing butylated hydroxyanisole as an antioxidant. The same maximization test was carried out on another 25 volunteers using this freshly processed sample (RIFM no. 72-76). The material was tested at a concentration of 8% in petrolatum and produced no sensitization reactions (Opdyke 1978). However, phellandrene was identified as a sensitizer in another study on the sensitizing potential of some essential oils and their constituents (Woeber and Krombach 1969), and α-phellandrene induced a positive patch test in four of the eleven patients included in a study on patients from a dermatological department (Hausen, Reichling et al. 1999). To what extent the positive findings in the two latter studies are caused by oxidative degradation products of phellandrene is not clear.

l-Phellandrene at concentrations ranging from 10 – 1000 μM did not increase the frequency of spontaneous sister-chromatid exchanges in Chinese hamster ovary cells (Sasaki, Imanishi et al. 1989).

Phellandrene has in a study by Roe and Field in 1965 (reviewed by Opdyke in 1978) been reported to promote tumour formation on the skin of mice treated with the primary carcinogen 7,12-dimethylbenz[a]anthracene (Opdyke 1978).

Evaluation:

Irritancy:	Neat phellandrene is a moderate irritant to rabbits. α-Phellandrene does not induce irritancy in humans when tested up to a concentration of 8%

Sensitisation:	In the absence of oxidation/degradation products the published literature describes α-phellandrene as a non-sensitiser. However, autooxidation of α-phellandrene has been demonstrated to generate sensitisers and two studies on humans have demonstrated positive patch tests after exposure to products based on α-phellandrene.
Mutagenicity:	The only study available does not support that α-phellandrene is mutagenic. α-Phellandrene is potentially a weak promoter.
Systemic toxicity:	There are no studies available on systemic toxicity of α-phellandrene. However, the high LD_{50} value, the chemical familiarity with other terpenes, and the expected quantitative occurrence in TTO products do not indicate that systemic toxicity caused by α-phellandrene would be likely.

6.16.3 Caryophyllene

Consecutive testing of 1606 patients attending the patch test clinic of 6 European departments of dermatology was performed. The standard fragrance mix produced the highest reactivity in all centres (mean 11.4%; range 9.3–17.9%), whereas caryophyllene caused positive reactions in 0.6% of the patients (Frosch, Johansen et al. 2002).

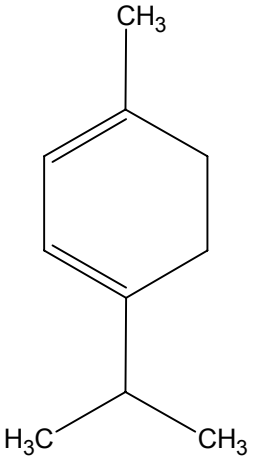
In a more recent study, 1511 consecutive dermatitis patients in 6 European dermatology centres were patch tested with oxidized fragrance terpenes and some oxidation fractions and compounds. About 0.5% of the patients reacted to oxidized caryophyllene (Matura, Sköld et al. 2005).

Evaluation:

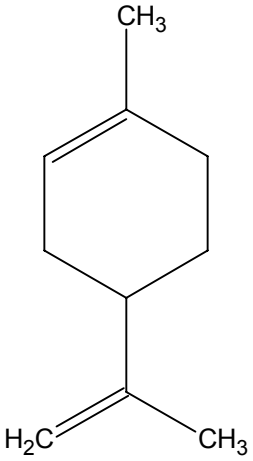
Caryophyllene induced positive allergic response in 0.5% of approximately 3000 dermatological patients participating in two independent European studies.

7 Structural formulas

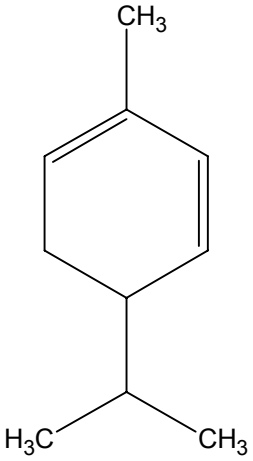
This section includes the structural formulas and chemical constituents of the most important TTO constituents and their metabolites.



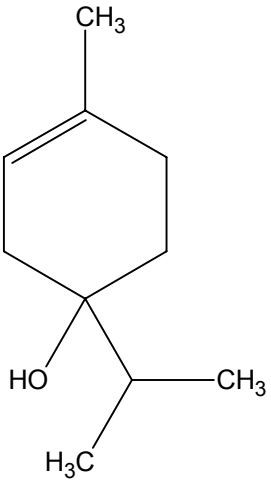
α-Terpinene ($C_{10}H_{16}$)



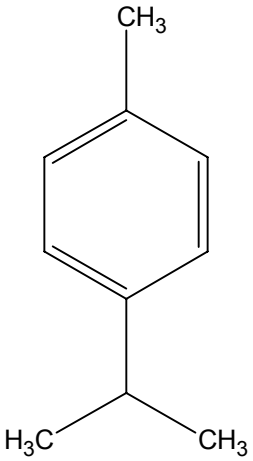
d-Limonene ($C_{10}H_{16}$)



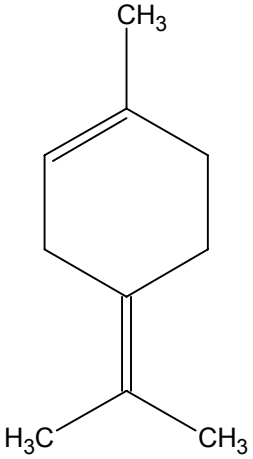
α-phellandrene ($C_{10}H_{16}$)



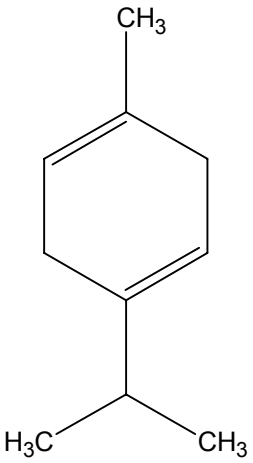
Terpinen-4-ol ($C_{10}H_{18}O$)



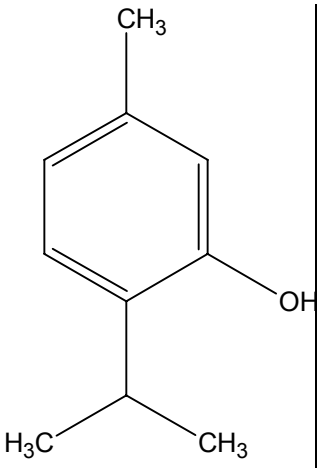
p-Cymene ($C_{10}H_{14}$)



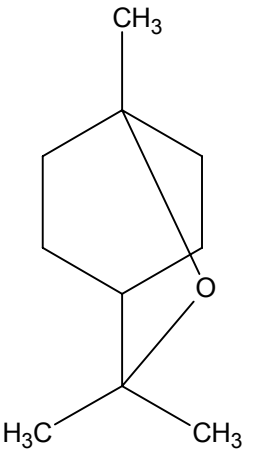
Terpinolene ($C_{10}H_{16}$)



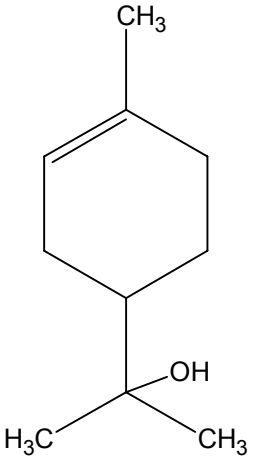
γ-Terpinene ($C_{10}H_{16}$)



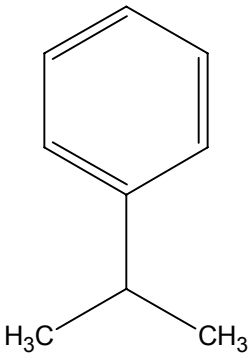
Thymol ($C_{10}H_{14}O$)



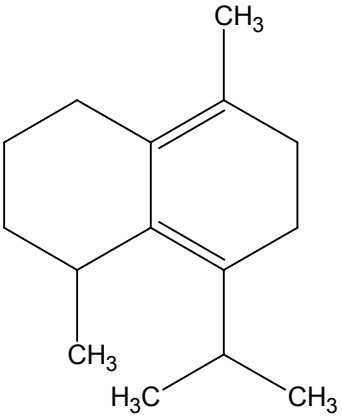
1,8-Cineole ($C_{10}H_{18}O$)



α-Terpineol ($C_{10}H_{18}O$)



Cumene (C_9H_{12})



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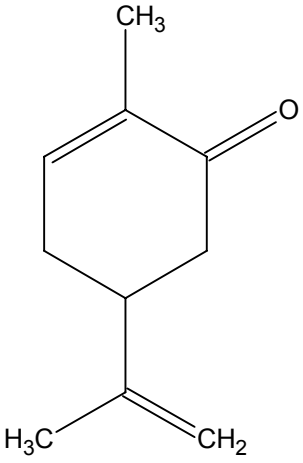

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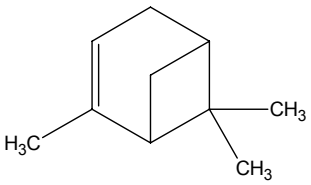

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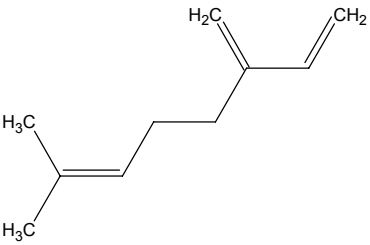
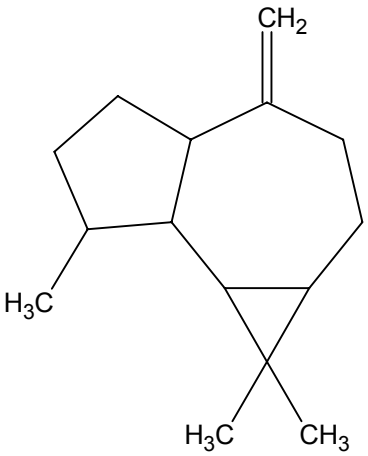
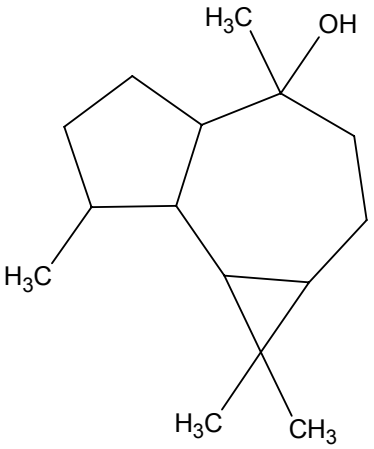


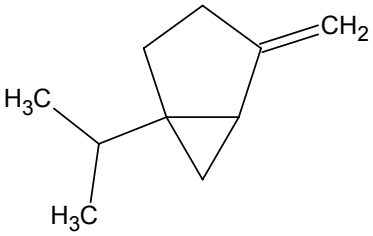
Myrcene ($C_{10}H_{16}$)



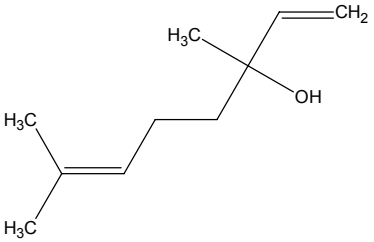
Aromadendrene ($C_{15}H_{24}$)



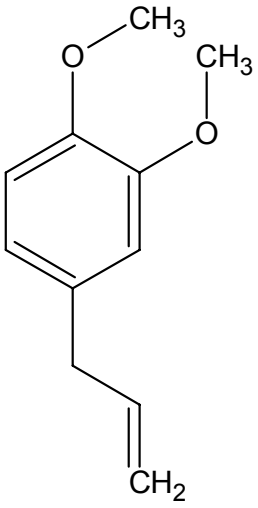
Viridiflorol ($C_{15}H_{26}O$)



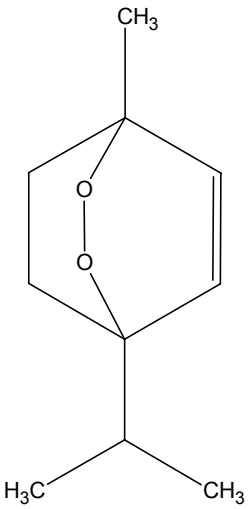
Sabinene ($C_{10}H_{16}$)



Linalool ($C_{10}H_{18}O$)



Methyleugenol ($C_{11}H_{14}O_2$)



Ascaridol ($C_{10}H_{16}O_2$)

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From: Tony Larkman [mailto:tlarkman@attia.org.au]
Sent: Tuesday, 8 December 2020 4:14 PM
To: 'Bart Heldreth' <heldrethb@cir-safety.org>; 'Monice Fiume' <fiumem@cir-safety.org>
Cc: Phillip Prather <phil@downunderenterprises.com>
Subject: CIR Expert Review for tea Tree Oil

Dear Bart & Monice,
First my apologies for being unable to attend the meeting last night; Phillip Prather, a Director of ATTIA Ltd, stood in for me and has provided me with a report on the discussion and his input.

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1. Phil has advised that the Panel wanted a copy of the ATTIA White Paper on the stability of TTO – I have attached a copy of this; please note that this is available, along with other information from this URL: https://teatree.org.au/teatree_about_packaging.php . I have also attached two reports commissioned by ATTIA and RIRDC in 2012 from which some of the Stability White Paper was derived.

2. Phil also advised that the Panel wants to know what oxidation rates might occur in a formulated product. I am unable to respond to this substantively as all of ATTIA's work focuses on the TTO itself rather than formulated products.
   There are an infinite number of formulations available and the rate of oxidation of TTO or its components will be governed both by the other ingredients present and of course the level of exposure to the atmosphere. As part of a comprehensive dossier prepared for the EU's SCCS (formerly SCCP) a report was prepared in 2006 titled "*Literature review on tea tree oil: Toxicity profiles for tea tree oil, constituents of tea tree oil and known oxidation products*" by JB Nielsen; I have attached a copy of this and while this is somewhat dated now it may assist you particularly in the areas raised (and noted by Phil) of *Tox and genal, dermal tox & skin sensitisation*.

Other comments:

- I am pleased that it was noted by the Panel that TTO has GRAS status; this is an important consideration.
- I was delighted that the Panel clearly noted the dichotomy between oxidised and fresh oil in the literature relating to dermal irritation and draw your attention to my specific comments on this in my submission to the Panel and ask you to note that the levels of reaction are close to non-existent when fresh, unoxidised TTO is deployed.
- Adulteration, which I raised repeatedly in my submission, was not addressed substantively. Again please refer to my submission comments on this area and the fact that oxidative products are often detected in these fraudulent samples along with a long list of extraneous products sourced from incomplete fractionation of other essential oils, principally pine, Eucalyptus and White Camphor oils. Some of these products have adverse effects when deployed on humans and animals.
- The Panel called for more toxicity data: these data exist as part of a REACH dossier submitted to the EU's ECHA in 2018; they are not available publically but it may be possible for the Panel to request summary evidence from the ECHA for specific data required.
- Concentrations of TTO have, as noted by the panel "*…come down from 15% to 3-4% over last 15+ years*". This is, in my opinion, largely driven by fears of dermatitis or other skin conditions and is almost wholly driven by the deliberate deployment of oxidised TTO in patch testing for TTO and strangely, turpentine. As you will have read in my emails on the subject researchers claim this is being done to obtain higher response rates because 'patients may therefore be exposed to oxidised fragrance chemicals and develop an allergy' per an email I shared with you earlier from Dr Sophie Rolls of Dermatology, ST4 - University Hospital Wales, Cardiff who stated on 30 Apr 2020:

  > *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.*

It remains beyond my comprehension why this is being recommended and done for TTO alone of all essential oils in the series and ask you to note that when challenged to explain this anomaly (cc to all others in the research group) no response whatsoever was forthcoming.

Please do not hesitate to contact me if you have any further questions.

Regards,

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