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

Status:	Draft Final Report for Panel Review
Release Date:	August 20, 2021
Panel Meeting Date:	September 13-14, 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 (CIR) Executive Director is Bart Heldreth, Ph.D. This safety assessment was prepared by Monice M. Fiume, Senior Director, CIR.

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Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Monice M. Fiume MMF
Senior Director, CIR
Date: August 20, 2021
Subject: Safety Assessment of *Melaleuca alternifolia* (Tea Tree)-Derived Ingredients as Used in Cosmetics

Enclosed is the Draft Final Report of the Safety Assessment of *Melaleuca alternifolia* (Tea Tree)-Derived Ingredients as Used in Cosmetics. (It is identified in this report package as *melalt092021rep*.) At the March 2021 meeting, the Panel issued a Tentative Report with a conclusion stating that the 8 *Melaleuca alternifolia* (tea tree)-derived ingredients are safe in cosmetics in the present practices of use and concentration described in this safety assessment when formulated to be non-sensitizing. Comments on the Tentative Report were received (*melalt092021pcpc*), and have been addressed.

Several recent studies have been published, and these have been added to the report. The results of these studies appear to be similar to information already included in the document. All new information is indicated by yellow highlighting.

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

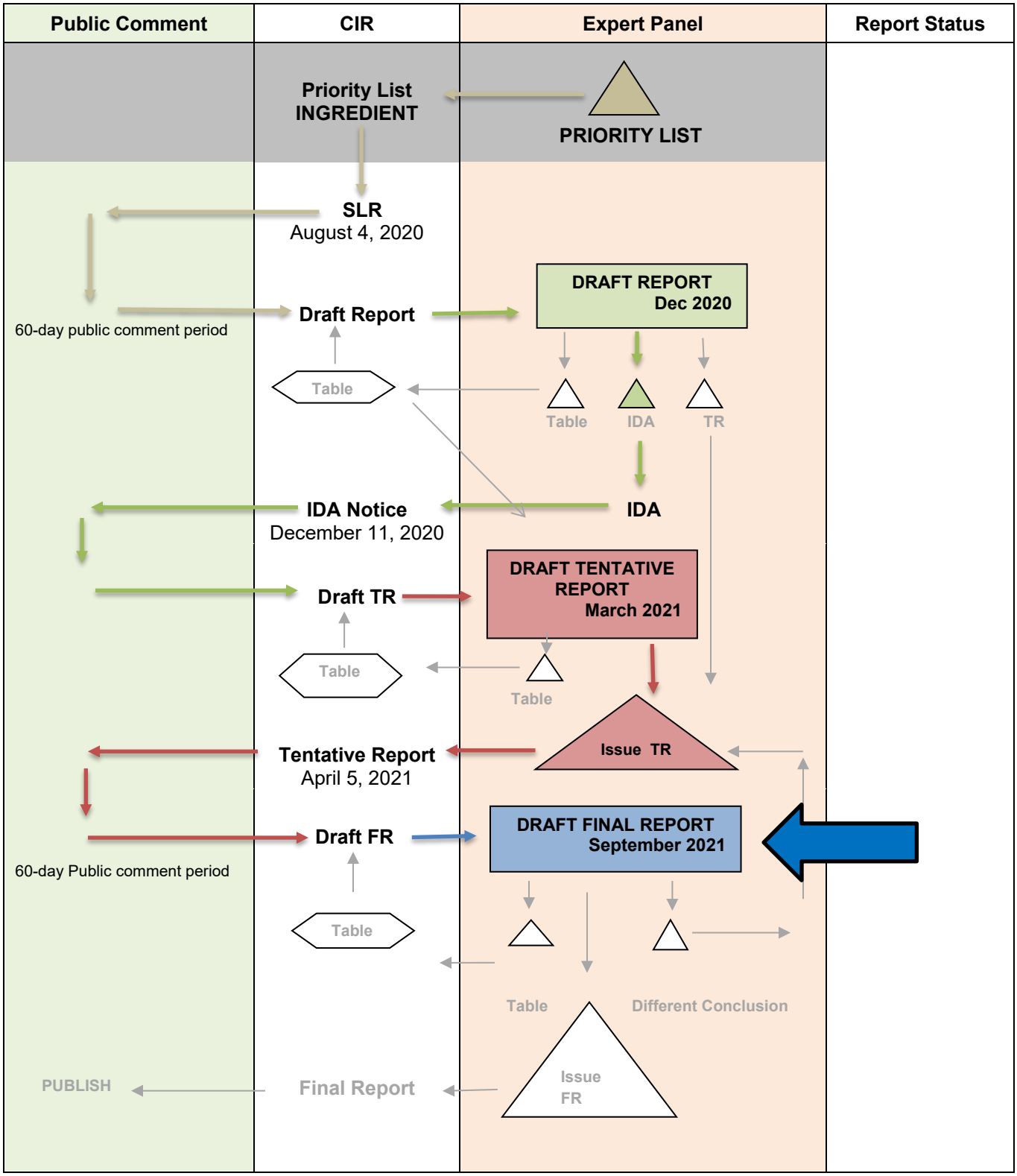
melalt092021flow: report flowchart
melalt092021hist: report history
melalt092021prof: data profile
melalt092021min: transcripts
melalt092021strat: search strategy
melalt092021FDA: 2021 VCRP data

The Panel should carefully consider the Abstract, Discussion, and Conclusion presented in this report. If these are satisfactory, the Panel should issue a Final Report.

SAFETY ASSESSMENT FLOW CHART

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

MEETING September 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.

At the meeting, the Panel issued a Tentative Report with the conclusion that the 8 *Melaleuca alternifolia* (tea tree)-derived ingredients included in the report are safe in cosmetics in the present practices of use and concentration described in this safety assessment when formulated to be non-sensitizing.

Draft Final Report: September 13-14, 2021
Comments\on the Tentative Report, received from PCPC, were addressed.

A few additional published papers were added to the document.

Melaleuca alternifolia (Tea Tree)-Derived Ingredients * – September 13-14, 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	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 REVIEW/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.

MARCH 2021 MEETING – 2ND REVIEW/DRAFT TENTATIVE REPORT

Belsito Team – March 11, 2021

DR. BELSITO: So then we move on to tea tree oil, all right? So at the December 2020 meeting, we noted that the data was good for the flower/leaf/stem oil and the leaf oil. And we weren't sure how relevant that data was to the six non-oil ingredients, the extracts of the various parts of the plant, essentially, and the leaf powder and the leaf water, and asked for method of manufacture, composition/impurities for non-oil ingredients. And if they were significantly different from the oils, then the following would be needed: irritation and sensitization, other toxicity datapoints.

We got a lot of data back on the leaf extract. And we got two sensitization studies on the flower/leaf/stem extract. (audio skip). And on the tea extract in a Draize human repeated insult, that 105 volunteers, and it wasn't a sensitizer. And then we got some data on other skin care products. And then the Australian tea tree industry commented in a wave. Was that Wave 2 where they commented?

DR. LIEBLER: Yes.

DR. BELSITO: Yeah. In Wave 2. So we have all of that new data and so just going down the list. So on page PDF 29, we have composition and impurities on leaf extract there. And I didn't think that it was very helpful because it was like what kind of flavonoids? 20 to 50 percent of leaf extract doesn't help, at least to me, but may be to you. I don't know. And then the second paragraph, it's less than a certain amount of tocopherol -- the fragrance -- the 26 fragrance allergens weren't in it. It's just not really telling us very much.

But do you think that that's adequate for composition and impurities in the leaf extract, and does it allow us to say whether that's similar to the oils? Which it probably is not going to be, right?

DR. LIEBLER: So actually, I looked at the new data, and you're right that it's not highly specific in terms of listing amounts of the different classes of organics or potential substances of concern. But I did note that the descriptions of the tea tree leaf extract indicated things that were relatively polar organics, like phenolics and flavonoids. Whereas the tea tree leaf oil indicates also phenolics and flavonoids, but also those analyses have data indicating the presence of various terpenes.

And then I coupled that with the information that Tony Larkman provided. And I know that Tony was on the call when we started this morning. It's the middle of the night for him. I see him logged in, but I don't know if he's on the call right now.

But I found that very helpful. This is a Wave 2 submission. It's the third item under the tea tree oil where it discusses of the items -- I'm looking at page 114 of the Wave 2 submissions, where we have the items numbered -- the tea tree extract, flower/leaf/stem extract, flower/leaf/stem oil, leaf, leaf extract, leaf oil, et cetera. And he said items one, two, three, and six are all identical. So what he's saying is those extracts and six, which is the leaf oil, are all identical. They are described in various ways the essential oil is steam distilled from the plant.

So if you recall the discussion in our last meeting, I made the point that these steam distillations probably will include a mixture of all the potential substances of concern and that I was feeling that I could group the extracts in with the leaf oil and the tea tree oil, which had much better data packages. And I was proposing that we could read across. In the discussion, I

think, from the Cohen team, Lisa and Ron focused on whether or not those steam distillates include just the sort of the aqueous soluble or the aqueous inorganic layers of a steam distillate. And it wasn't resolved.

But I think with this follow up memo from Tony Larkman and the additional data I was able to infer that the extracts are lighter on the terpenes than the oil, but they contain the flavonoids and the phenolics. So in a way, the leaf oil is kind of a more -- in the RIFM read-across we often say more reactive to less reactive read-across. This is kind of a mixture that has more substances of potential concern than the extracts do but in many ways is substantially similar, as Tony Larkman's memo indicated. So I think this supports the position that I originally took at the last meeting that we can use the tea tree oil data to support the extracts.

DR. BELSITO: I would agree, Dan. Certainly in terms of sensitization and irritation it's not even tea tree oil. It's oxidized tea tree oil that causes the issue.

DR. LIEBLER: Yeah. I see that Tony Larkman has unmuted his microphone. Tony, I hope by naming you I didn't wake you up but if you have a comment?

MR. LARKMAN: Good morning. It's nearly tomorrow, so I'm already awake.

DR. LIEBLER: Yeah. Okay. Did I mischaracterize anything that you said in your memo, Tony?

MR. LARKMAN: I don't think so, no. I'd just like to point out that the leaf extract will hold a considerable number of other products outside of the oil because of the way it's extracted from the leaf itself. And I think leaning towards -- I would lean personally towards a little bit more caution with the read-across. There's no doubt that all the terpenes will be in there and in the same ratios. Terpenes, sesquiterpenes, and monoterpenes are roughly 1 to 2 percent of the leaf.

DR. LIEBLER: Okay.

MR. LARKMAN: When you extract, you're going to get -- you're absolutely right -- an unknown, who knows what of flavonoids, all sorts of good gear. I don't know what there is in there. I don't think anyone has really looked. So I don't think there's anything that's going to be sensitizing. I'm sorry to butt in.

DR. LIEBLER: Oh, no. That's okay. Thank you. That's very helpful. I think we tend to think about the terpenoids as at the top of the list when it comes to sensitization concern.

MR. LARKMAN: Yes. I think that's probably the case, although you've seen my multiple responses on that one -- very, very low levels of sensitization, as you've rightly pointed out, if the product is correctly stored, correctly used, correctly formulated.

DR. LIEBLER: Right.

DR. BELSITO: Yeah. Even with oxidized tea tree oil, which is what we use for patch testing, the incidence is extremely low among patients who are referred for presumed allergic contact dermatitis, not the general population. So yeah, I'm fine with using the oil data as read-across for the extract because I actually wouldn't expect that -- I expect that the sensitizers are terpenes, and they're more likely to be in a higher concentration in the oil than the extract.

DR. LIEBLER: I think that's reasonable. So where I'm going with this is that this will allow us to use the tea tree oil data to help clear the extracts. And then I think we'd be heading towards the safe as used when formulated to be non-sensitizing, for the entire group.

DR. BELSITO: Yeah.

MR. LARKMAN: There is one area I'd like to raise if you can indulge me?

DR. BELSITO: Sure.

DR. LIEBLER: You've earned it.

MR. LARKMAN: I'm sorry?

DR. LIEBLER: I said you've earned it by staying up so late.

MR. LARKMAN: That's all right. I've got all tomorrow to sleep. Adulteration, it's something that I don't know whether it is of concern to you guys. But it's of deep concern to me, and I've done a lot of work around this. Do you think that adulteration is a critical topic?

DR. BELSITO: Of course.

DR. LIEBLER: Say that again, Don.

DR. BELSITO: I think adulteration is important, no?

DR. LIEBLER: Well, is it something that we're likely to encounter with these ingredients? Is it a -- we normally don't talk about it. Is it a problem here?

MR. LARKMAN: Yes.

DR. BELSITO: I don't know.

DR. LIEBLER: Tony, can you elaborate?

MR. LARKMAN: 2012 is when I started. I've been gathering and testing samples since 2012, roughly 350 samples. They're all commercial samples of tea tree oil that I've purchased. In the United States and Canada combined, the incidence of adulteration in tea tree oil has dropped from 51 percent to around 30 percent.

I found some pretty interesting stuff in some of it. My favorite has to be hashishene at 0.9 percent. One of the problems of adulteration is what you find in it because every single sample is different. And when you go GC-MS and you do a deep dive into it, you just sit there and shake your head at the stuff that you find in there. It's industrial waste, basically. And it is a prime concern in my opinion. It's a safety factor. And the best place to hide adulterated oil is in a formulation -- very, very easy to do. So that's just something I wanted to draw your attention to. Thank you.

DR. LIEBLER: Thanks, Tony. We normally proceed in our evaluations on the basis of the expectation of good faith in industry in providing an ingredient that is what they say it is under the specifications that are submitted for our review. And so I understand that what you're saying could represent a problem of -- adulterated could include adulteration-derived ingredients that have no health effect and others that could have a health effect.

MR. LARKMAN: Yes.

DR. LIEBLER: So we often do not have the benefit of having somebody who is actually done the analytical deep dive on complex natural substances that you have done and that you're talking about here. So for many of the ingredients for other botanical-derived things, we have no idea. And we are sort of reliant on industry to act in good faith in providing what they say they're providing and controlling the quality of their products.

So I don't know if this is something we specifically raise in the report because what we have is your account. If there are data that would support a problem with ingredients provided to the cosmetic ingredient industry -- or the cosmetics industry by cosmetic ingredients suppliers, that a different kettle of fish.

DR. BELSITO: But we now have that information, Dan. He just told us that it's 31 percent in Canada and the U.S. So someone out there is selling to the CPG companies who are using tea tree oil adulterated tea tree oil.

DR. LIEBLER: I'm trying to remember now. In Tony's memo -- I think you mentioned it.

DR. BELSITO: And we could put that in the discussion if you sent us that information, Tony, to be released.

DR. LIEBLER: Yeah. We need a little something more to hang our hat on, Tony.

MR. LARKMAN: That's fine. I've got so much stuff it almost frightens me, never mind you guys.

DR. LIEBLER: Well, if you could condense it into some reasonably data-rich, data-focused document and provide to CIR, it would be very helpful to us.

MR. LARKMAN: Yep. That's no problem at all. I can do that. One of the reasons why I approached this the way I did is I fully understand that, doing what you're doing, you have to assume that everybody's doing this in good faith. It's a major problem with all essential oils. There's no doubt in my mind about that. I've got a lot more proof than just tea tree oil because it fascinates me. So I understand where you're coming from. So what I wanted to do is I wanted to flag it with the group because it's something I've been working on for 12 years now, and I'll continue to work on it.

I'll never get it to zero. I accepted that right back at the beginning. But we've done a really, really good job because we've got Europe down from 70 percent to 30 percent as well. So I'll do what I can, and I'll put something together. And I'll shoot it through to Bart.

DR. LIEBLER: Excellent.

DR. BELSITO: Okay. So basically, I think with that in mind I agree, Dan, we can go safe as used for all of them. And I think we need to craft something in the discussion pointing out that it's been brought to our attention that an estimated 30 percent of products containing tea tree oil in Europe and Canada and the United States have a variety of adulterants. And it's the expectation --

MR. LARKMAN: I'm sorry. I'd like to correct that statement, please.

DR. BELSITO: Okay.

MR. LARKMAN: It's not products containing tea tree oil. It's the little bottles of tea tree oil that you buy from the discount store through to Walmart or wherever it is you buy them in the States.

DR. BELSITO: Okay. So they were not made by consumer product companies like Procter & Gamble putting tea tree oil in?

MR. LARKMAN: No. They're not.

DR. BELSITO: Okay.

MR. LARKMAN: Those are, nevertheless, the people that buy the tea tree oil.

DR. BELSITO: Okay. Well, again, we can put that in that what presumably is pure tea tree oil being sold, 31 percent, 30 percent in Europe, the U.S. and Canada -- a variety of adulterants are found. And it's our expectation that the tea tree oil that is used in the manufacturing of cosmetic products be unadulterated.

MR. LARKMAN: That would be a fantastic statement. Thank you.

DR. LIEBLER: Yeah. This is very helpful, Tony. So if you can provide -- if there are any publications, we'd definitely like those. And if there are, in addition, any kinds of reports or anything that's been submitted to anybody and sort of document in that respect and then, of course, anything that you could provide as your own data summary that doesn't fall into a publication or having been submitted as a report to anyone is still of interest to us. The more documentation we can have for that the better.

MR. LARKMAN: Yeah. Good. There's a 2015 paper that I co-authored, and there's a follow up on methodology. And, like I said, there's this vast swimming pool of data which I'll try and summarize for you. I've done that for a number of different people, so I'm really familiar. It's copy paste, something that's fairly close to my heart.

DR. LIEBLER: Great. All right, us MS guys got to stick together, Tony. Thank you.

MR. LARKMAN: Love it. Thank you very much indeed, guys. It's been somewhat interesting listening to you. I must admit, when you were doing silicas, I slept a bit.

DR. LIEBLER: Good for you.

MR. LARKMAN: So look, I don't know if you've got any more questions, but it looks to me like it's starting to come together well. And I'd just like to report to you that I'm beginning another journey now.

In 2008 the SCCP, now the SCCS, in the EU put out an opinion on tea tree oil. And they came to the conclusion that they couldn't state whether it was safe or not. And this is, again, for cosmetics. So we've addressed, now, all of their concerns. And I still don't have the final draft document, but we've got some really, really good permeation data -- percutaneous absorption data with tea tree oil for cosmetic and two bespoke creams at 90th percentile and maximum, which is 5 percent. We're going to put all that together, and that will be published before the end of this year.

That will make a significant difference when we submit it to the SCCS, and eventually we're expecting to get a better opinion on it. My target is to get it generally approved as safe up to 5 percent in cosmetic, and I believe we have the data to do that. But it's a panel just like you guys, and you've got to step back. And you've got to look at these things. So that's what I'm hoping will come out of that before the end of 2022 because that's how long it takes them to move.

And the other thing I want to point out is, in the SCCS when they did their SEDs, they made a wee small mistake with their math. They didn't take into account the estimate 3 percent percutaneous dermal penetration. So all of their SEDs are 33 times higher than they should be, which means the NOAELs are technically lower than they should be. And we're expecting to see significantly better SEDs and NOAELs coming out of this. But we have to wait for the data, and we have to wait for the numbers to be crunched by the statisticians. But I just wanted to flag you that. And when we do finally get all that data together, I'll be contacting you guys.

DR. LIEBLER: Thank you.

MR. LARKMAN: All right. I'll listen a little bit longer, and I'll sign off. And if you've got any more questions, just sing out. Thank you very much indeed.

DR. LIEBLER: Thanks again, Tony.

DR. BELSITO: Okay. So basically, I think -- so in the discussion we have review of all the ingredients. Oxidized tea tree oil is a sensitizer. Multiple botanicals, we've got that. The botanical boilerplate we got. We dealt with the DART, the penetration, the inhalation.

And then at some point we can say that the panel has been made aware that presumably pure tea tree oil sold in Europe -- that approximately 30 percent of supposedly pure tea tree oil sold in Europe, the United States, and Canada has been adulterated with various -- or contains adulterants. And the expectation is that manufacturers of cosmetic products will use good

manufacturing processes to mitigate this or to assure that there are no adulterants in the tea tree oil they're using. Does that make sense?

DR. KLAASSEN: Yes.

DR. BELSITO: Okay. And then our conclusion is safe as used.

DR. SNYDER: Don, one last thing probably should be addressed in the discussion is that we did ask for genotox data on the extracts. But I think we can say that that's mitigated by the fact that we got composition data -- that it's less of a concern. And we have lots of data on the oil, the composition, oil -- there's less concern about the composition of leaf extract with regards to impurities compared to the oil. And we have lots of genotox data on the oil, all negative.

DR. BELSITO: Yeah. Okay. Negated by the oil data?

DR. SNYDER: Correct.

DR. BELSITO: All right. Okay. Anything else?

DR. LIEBLER: Looks good.

Cohen Team – March 11, 2021

DR. COHEN: Okay. All right. It doesn't -- we still have more naturals to go. Our next one is tea tree. And this is Monice's, right?

MS. FIUME: Yes.

DR. COHEN: And this is a draft tentative report. In December of 2020, we issued an IDA looking for method of manufacture and composition and impurity for the non-oil ingredients. And based on those findings we would ask for further information. We wanted irritation and sensitization for the extract at expected max use concentration and other toxicity endpoints like genotox.

At the last meeting, we had a lengthy conversation about oxidation of tea tree oil. We received some stability data on the oil since then. We've received information about composition and impurity of the leaf extract where the SCCNFP allergens were not detected.

We have max concentration of 0.001 on the leaf extract but not the leaf powder and the leaf oil. In the late-breaking information that came, we have some sensitization at 0.0078 for the leaf extract. And we didn't see sensitization, and we had a letter from the Australian tea tree industry.

DR. BERGFELD: Are we still on?

DR. SHANK: Yeah.

DR. BERGFELD: Okay. Just nobody's speaking?

DR. SHANK: Yeah.

DR. BERGFELD: Okay.

DR. SHANK: This is Ron Shank. I think the oils are safe as long as they're formulated to be non-sensitizing. That would be the tea tree flower, leaf stem oil, and the tea tree leaf oil, safe. And then the others are insufficient. But the letter from the ATTIA, the Australian group says that these are all the same. But in our report, we have different compositions for the extract, the leaf, the leaf extract, the powder, and the water. So I'm confused. If we can take the Australian information, we can put those all together, but if we don't accept that, then we don't know very much about the non-oil ingredients.

DR. PETERSON: So I --

DR. SHANK: So I don't know how --

DR. PETERSON: Yeah. I --

DR. SHANK: I don't know what to do.

DR. PETERSON: Yeah. I kind of agreed with you, Ron. I thought that all the oils were safe.

DR. SHANK: Okay.

DR. PETERSON: That maybe the leaf powder was --that the leaf powder was okay but that the water might be insufficient. And this was going off of what -- after reading the Australian comment about how to pull them together.

DR. SHANK: Okay.

DR. PETERSON: Because I thought that the leaf could still have some things in it that the oil might not, and we don't really know what's in the water. So it's hard -- and because it's what's left after the distillate according to that memo, I felt we needed more information on the composition to be able to read -- you know, to judge whether we could use the oil information or not for safety.

DR. SHANK: Okay.

DR. PETERSON: So that's how I had it split out. Anything that had oil in it was basically safe as long as it was formulated in a non-irritating, sensitizing manner. And that the other two, you know, maybe the leaf is okay, but again, there could be things in the leaf that aren't in the oil, so I would go for insufficient and the water, too, because we don't really know the full composition. If the -- if we found the composition of the water, then I would say the leaf is probably fine, you know, if they're similar -- you know, if we knew what was in them.

DR. SHANK: Okay. Can we accept the Australian statement that the extract -- the oils are the same? It's on a hundred -- page 114 in Wave 2.

MS. FIUME: So --

DR. SHANK: Can we -- do -- can we accept that the Australian position represents all of these cosmetic ingredients?

MS. FIUME: So I'll speak first, and then I don't know if Jay wants to jump in. But first, did we lose David?

DR. COHEN: I'm on the phone, but I just got back on. I'm trying -- I don't know what happened. I literally just went -- cut out, dead. And I'm trying to get into the Teams meeting, but all I get is a spinning circle.

MS. FIUME: Okay. We'll watch for you to pop up there so that you can get on through the computer. But as long as you can hear us. So --

DR. COHEN: Yeah.

MS. FIUME: -- procedurally -- so the panel is welcome to make whatever, you know, decisions they want based on the information provided to them. But historically and procedurally, generally, for our reports, the definitions are based on the INCI dictionary. That's the source that we have for all of our ingredients, and that's what we rely on. That's -- those -- and Jay, correct me if I'm not saying it right.

The members submit forms to the INCI committee who reviews them and then approves them for the dictionary. So those definitions have come from some supplier, and that's how they have gotten into the dictionary. So Table 1 are the industry submitted definitions of those ingredients. And Jay, I don't know if you have any input on it or anything different.

DR. ANSELL: No, no. I mean, not -- I'm not exactly sure what point we're trying to make, but certainly, that's the process.

MS. FIUME: Yeah.

DR. ANSELL: I mean, is that someone applies for an INCI name. They provide sufficient data for the assignment of the name, but yeah.

DR. SHANK: Okay. Well, it makes a big difference because if the Australian version is representative of all of these ingredients that makes our job a lot easier because basically, these are all oils. But if that's not correct, then we have to ask for a lot more data. So I guess we have to go by the dictionary, and we're going to need a lot of safety data on the water-soluble ingredients, and that would be a 28-day dermal toxicity test, possibly genotoxicity, developmental and reproductive toxicity, skin irritation and sensitization would be needed on the water-soluble ingredients.

DR. COHEN: So, I'm sorry. I'm just playing a little catch up here. So we're going to issue safe for the oil components?

DR. SHANK: Yes.

DR. COHEN: Are we putting in not to be sensitizing or irritating?

DR. SHANK: Yes.

DR. BERGFELD: Do you think that irritation needs to be added there?

DR. SLAGA: I thought we were only doing sensitization.

DR. BERGFELD: Sensitization.

DR. COHEN: Just --

DR. SHANK: Yes. Sensitization.

DR. COHEN: -- sensitization. Not to be sensitizing. Again, I -- with the technical issues, did the conversation about adulteration come up? That was another --

DR. SHANK: No. Not yet. Not yet. Should we --

MS. FIUME: David?

DR. SHANK: -- put that into the report about adulteration?

MS. FIUME: So as a point of clarification, it's my understanding adulteration comes down to the product versus the ingredient. Is that correct?

DR. SHANK: Yes. I think so.

DR. COHEN: Yes. That sounds -- makes sense.

MS. FIUME: Okay. Typically, the panel doesn't comment on the adulteration of a product that can occur because I think it can occur with probably very many products. The panel tries to keep it to the ingredient. So it's up to how you want that handled or not handled, but generally in the discussion they typically refer to the ingredient versus the product, historically.

DR. SHANK: Okay. I think that's the better way to go because, if you get into adulterated products, I don't know how to handle that.

DR. BERGFELD: Well, it's never ending.

DR. SLAGA: And that opens up a black box.

DR. COHEN: It's a very sober look at it, Monice. Thank you because that makes it clearer.

DR. ANSELL: Yeah. The adulteration is actually a regulatory term, and an adulterated cosmetic would itself be illegal.

DR. SHANK: Right.

DR. COHEN: I had another comment about phototox. In the November 2005 report by Jesper Bo Nielsen, there's a description of no phototoxicity in dermal studies in mice. But in our report, there's a description in mice that swelling was significantly increased when tea tree oil was applied before UVB radiation.

DR. SHANK: Can you give us the page number?

DR. COHEN: I'll try to direct you there.

DR. BERGFELD: It's right after irritation and sensitization.

DR. SHANK: Do you have that page number?

DR. COHEN: Right. Their animal dermal. It's under animal dermal, and it's the second to last paragraph, last line of that paragraph. The paragraph starts, "Researchers also examined whether tea tree oil alleviated swelling." Do you see that paragraph? The last sent- --

DR. SHANK: What page? Can you tell us the page? The PDF page?

MS. FIUME: Yes. PDF page 37.

DR. SHANK: 37. Thank you.

MS. FIUME: The fourth paragraph in that subsection under immunological effects.

DR. BERGFELD: That was undiluted, David.

DR. COHEN: Say that again.

DR. BERGFELD: It was undiluted tea tree oil.

DR. COHEN: Yeah. Yeah. I think that's a very important point, Wilma. That's right. Yeah. Okay.

DR. PETERSON: So again, we were talking about this on a previous thing that if -- that, you know, it was tea tree oil but not a cosmetic ingredient, do we have to -- I mean, we have a lot of information about the tea tree oil but not on the tea tree oil. I guess there's overlap with some of the cosmetic ingredients, and I'm comfortable reading across for those. But it does look like it might have some phototoxicity. But it's undiluted.

DR. COHEN: So --

DR. BERGFELD: That's quite a difference.

DR. COHEN: Yeah.

DR. PETERSON: Yeah.

DR. COHEN: It is. And I'm just looking back to our max use is 0.63 percent in a cuticle softener. So we really are pretty far off there. Thank you, Wilma. That's reassuring. Okay. So we're going to go as formulated to be non-sensitizing for the oil

components and for the water-based components, which are the leaf water -- and what else? Specifically which components on the table?

DR. SHANK: It would be the flower/leaf/stem extract, leaf, leaf powder, and water would be insufficient.

DR. COHEN: Got it.

DR. BERGFELD: Now, Lisa said the leaf powder she could accept. Is that real or not real? Are we putting the leaf powder in the insufficient?

DR. SHANK: Well, if you accept the leaf powder, then I would think you could accept the leaf.

DR. BERGFELD: Lisa?

DR. PETERSON: Well, I guess I was looking at if you could look at the components of it, you know, what it's made up. I was on the fence on the leaf. I defer to Ron because under that argument you could also say then the water's safe. I think that we don't really have -- I would say insufficient. Just like --

DR. BERGFELD: Okay.

DR. PETERSON: -- was spoken.

DR. BERGFELD: Okay.

DR. SLAGA: The rest, yeah.

MS. FIUME: So just to clarify --

DR. SHANK: I would think these --

MS. FIUME: -- is it insufficient for all of the ingredients except for the two oils? Do I have that correct?

DR. SHANK: Correct.

DR. COHEN: Oh, wait --

DR. SHANK: Yes.

DR. COHEN: -- that is on --

DR. SHANK: Yes.

MS. FIUME: Okay. Is that correct?

DR. SHANK: The oils are safe when formulated to be non-sensitizing. And the others, insufficient.

DR. COHEN: So the two oils are going as safe, non-sensitizing, but all the rest of them are going as an IDA with 28-day dermal tox, skin irritation and sensitization, possible genotox, and DART?

DR. SHANK: Yes.

DR. COHEN: Okay. Okay. Anything else?

MS. FIUME: I was just --

DR. COHEN: Tom, any comments?

DR. SLAGA: No.

MS. FIUME: And then, can I ask -- I tried to form a discussion based on the last conversations in December. Are there any changes that need to be made to the draft discussion? Anything that needs to brought in or taken out?

DR. COHEN: I liked the draft discussion.

DR. PETERSON: Yeah. I thought it captured the discussion that we had last time well.

MS. FIUME: Great.

DR. BERGFELD: I liked the inclusion of the oxidation paragraph.

DR. PETERSON: Yeah.

MS. FIUME: Great. Thank you. I just wanted to make sure that I did capture it correctly.

DR. BERGFELD: You did great.

MS. FIUME: Thank you.

DR. PETERSON: As usual.

DR. COHEN: Okay. So can we move on from tea tree now?

DR. SHANK: Yeah.

Full Panel – March 12, 2021

DR. BELSITO: Tea Tree, at the December panel meeting we issued an insufficient data announcement for the six non-oil ingredients, which are the Melaleuca Alternifolia (Tea Tree) Extract, the Tea Tree Flower/Leaf/Stem Extract the Tea Tree Leaf, the Tea Tree Leaf Extract, the Tea Tree Leaf Powder and the Tea Tree Leaf Water.

We got a lot of data back -- the insufficiencies were method of manufacture, composition and impurities and if they were significantly different from the oils then we wanted irritation and sensitization for the extract at maximum reported concentration of use and other toxicity endpoints might be needed.

We got a lot of information, we got a lot of feedback from the Australian Tea Tree Industry Association. They were also on the phone with us yesterday. And, based upon what we received and really looking at the composition of these, vis-à-vis the composition of the oil, we felt that we could go safe as used for all of them.

DR. BERGFELD: And that's a motion?

DR. BELSITO: Yes.

DR. BERGFELD: Is there a second?

DR. COHEN: Don, you felt that there was enough information on the hydrophilic components with skin irritation, sensitization and tox from the oil only?

DR. BELSITO: Yeah, so, I mean, in terms of sensitization the terpenes really are going to be highest in the oil. So, I mean, that's where you're going to see your sensitization. But also looking at some of the composition data we have from the extracts, there really were no signal chemicals in them to then raise any red flags for us.

And, I'll let Dan comment, but he felt that the ingredients in various parts of the Tree Tea Leaf -- well, various parts of the (audio distorted) plant would be in the oils.

DR. LIEBLER: So last time we talked about this the issue was whether or not the extracts were really comparable to the oils such that you could use the oil data to clear the extracts. I had suggested you could do that. I think there was some hesitation on the part of the Cohen team in taking that approach.

I think that the information that we've gotten now, particularly the memo from Tony Larkman, you know, Australia -- who also was, I think, on the meeting with both teams yesterday -- was helpful. I know the problem is that the extracts, particularly the characteristic features of these extracts were not that well defined. And, so, it was not clear how you could compare them to the oil . Tony's memo clarifies that the extracts and the oils are really all, as he put it, the same thing. And, so, that I think helps support the idea that the oil data could help clear the extracts.

The oil data -- I realize in PDF Page 29 under method of manufacture, and then the follow up data under composition and impurities -- is kind of a high-levels relatively superficial description of what's in there. But you'll notice in the extracts there's mention of phenolics and flavonoids but not the terpenes. Whereas the oils describe a lot of data on the terpenes.

So, I think of the oils as being heavier on the terpenes and also containing some flavonoids and phenolics. So it contains a broader range of the material of the potential substances of concern for sensitization. So, based on that, I think, I still suggest that the oil can help clear the extracts. And (audio distorted) (audio fades) this additional information I think proves it.

DR. COHEN: That's pretty compelling. Before I go to the team, Don, you had it safe as used? Did you have it safe but formulated to be non-sensitizing?

DR. BELSITO: Yeah, our usual botanical, because of the terpenes in the discussion that, you know, you could have other terpenes being added in as botanical products, yes. So, safe as used formulated to be non-sensitizing. Because it could be mixed with other botanicals containing the same sensitizers.

DR. COHEN: Well, I think just from the tea tree oil itself you have some opportunity there for sensitization.

DR. BELSITO: Right. But we know that is really due to oxidize and that can also go into the discussion about proper store, perhaps, addition of antioxidants, etcetera, etcetera to reduce the chances of developing oxidized tea tree oil.

DR. COHEN: Yeah, I agree with that and we had some other late breaking data about oxidants.

DR. BERGFELD: Are you going to second that motion, David?

DR. COHEN: Oh, yes, I'll second the motion but I wanted input from the team to get their feedback because we had a discussion about the two phases of this extracts.

DR. BERGFELD: Okay.

DR. COHEN: Are you guys okay with that?

DR. SLAGA: I am. Yeah.

DR. PETERSON: I am too, I just want to say I started there and then -- I forget who was on the call yesterday told us that we could not take just one company's judgement about, you know, that we could equate these different things, but I actually agree with you, Dan. But I started out where you guys are, and then got talked back from it based on the discussion yesterday.

DR. BERGFELD: Ron?

DR. SHANK: Yeah, I had the same approach as Dr. Peterson. If the water extracts are very similar to the oil, as stated by the Australian contributions, I say I agree with the proposed motion they're all safe.

There was some questions is the Australian product typical of all of the products used in cosmetics. If we accept that then I think they're all safe.

DR. BERGFELD: I wonder if you can respond to that, Don, since we do not hear (audio skip).

DR. BELSITO: Yeah, I mean, we usually take what the information is in the document. Obviously, we're going to (audio distorted) information that there are a, you know, a major (audio distorted). And, you know, that will be in the report. And, we base our safety off the data that we have in the report. So I'm fine with it.

DR. BERGFELD: Okay. Are you fine with it too, David, now?

DR. COHEN: Yeah, we were going --

DR. BERGFELD: Can't hear you, David.

DR. COHEN: How about now?

DR. BERGFELD: Okay.

DR. COHEN: We're persuaded by Don and Dan and we'll follow their motion.

DR. BERGFELD: Okay.

DR. COHEN: It's formulated to be non-sensitizing. I don't know if that was in the initial motion.

DR. BELSITO: Correct, I failed to mention that, thank you, David.

DR. BERGFELD: Okay, so it's been moved and second to go safe, non-sensitizing. Okay, anyone opposing? Abstaining? So this ingredient is approved. Any further discussion? We've already had discussant points mentioned when the motion was proposed, anything else?

DR. BERGFELD: Yeah, so, actually we were told that in Europe, the United State and Canada, that tea tree oil sold as tea tree (audio skip) and not the cosmetic. When analyzed, up to about 30 percent of what was called (audio skip) oil had a variety of adulterants. So, I think, in the discussion, Tony said he would send us that data. That information should be included under manufacturing and impurities, so that we can mention in the discussion that manufactures should assure that the tea tree oil that they're blending into cosmetics is pure and lacking adulterants.

DR. BERGFELD: Is that okay?

DR. COHEN: We don't disagree with that, Don. Isn't that true for every item that we review?

DR. BELSITO: I couldn't hear what you said, David.

DR. COHEN: I said, we agree with that statement about the adulterants, but isn't that true for any item we review?

DR. BELSITO: You're absolutely right. It's never been brought to (audio skip).

DR. COHEN: (Audio skip).

DR. BELSITO: And probably that is what cosmetic companies are doing. You know, they're running whatever assay to assure that what their tea tree oil -- or their tea tree product is, is a certain amount of purity and to certain (inaudible), as opposed to these, you know, companies that are just selling pure tea tree oil. They are all little, small companies that do aroma therapy and all sort of other homoeopathic treatments with these materials.

So, I just, I think, I personally was a little shock that the numbers were that high. Apparently in Europe, at one point, it was up to 70 percent of samples analyzed had adulterants. So, we don't necessarily have to put that in, but, I mean, it's just some information that we got.

DR. BERGFELD: Bart wants to make a statement.

DR. HELDRETH: I was thinking about this issue and it made me have a question to pose to see if the panel felt that this was appropriate. We already have a sentence in the draft discussion that I wondered if maybe covered this issue. Where it states, "They stressed that the cosmetics industry should continue to use current good manufacturing practices (cGMPs) to limit impurities."

DR. COHEN: I think that's good.

DR. BELSITO: Then that takes care of my suggestion. So that's perfect, Bart, thank you.

DR. BERGFELD: Okay. Then we'll delete that portion of the (audio skip) ingredients. Okay, so, we're going to call the vote. I forgot if I did or not, but we're going to do it again. Those opposed to this motion, please indicate with name. Abstaining? Approved. Okay, this document is now approved. And, we have had discussion, but do we need more discussion? Seeing none, moving to the other items, and the first one up is hair dyes.

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

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ABBREVIATIONS

ACC	allergic contact cheilitis
ACD	atopic contact dermatitis
AD	atopic dermatitis
ADR	adriamicin-resistant
aq	aqueous
AR	androgen receptor
ATTIA	Australian Tea Tree Industry Association
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
CMC	carboxymethylcellulose sodium
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
GEI-DAC	Spanish Group for the Investigation of Contact Dermatitis and Skin Allergy
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

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. 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 these ingredients are safe in cosmetics in the present practices of use and concentration described in this safety assessment when formulated to be non-sensitizing.

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 some of these reported functions (i.e., anti-acne, antifungal, and antimicrobial agents) are not considered cosmetic functions 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.

According to correspondence received from a representative of the Australian Tea Tree Industry Association (ATTIA), he is of the opinion that several of the *Melaleuca alternifolia*-derived ingredients (i.e., the Extract, Flower/Leaf/Stem Extract, Flower/Leaf/Stem Oil, and Leaf Oil) are essentially identical because the definitions for these ingredients describe, in various ways, the essential oil that is steam distilled from the plant (personal communication; T. Larkman, Feb 17, 2021). Additionally, the representative of ATTIA stated that the Melaleuca Alternifolia (Tea Tree) Leaf and Melaleuca Alternifolia (Tea Tree) Leaf Powder both describe the dried leaf.

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] According to this submission, as well as certificates of analysis provided by another source,[33] 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%). 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 tea tree oil 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

ECHA provided estimates of absorption via various routes[7] Oral, dermal, and inhalation absorption rates were estimated 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] In one study in ICR mice, the oral LD_{50}s of tea tree oil and nano-tea tree oil were estimated to be 0.854 g/kg and 1.565 g/kg, respectively.[85] In another study, the LD_{50} of tea tree oil 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 10 ICR mice were used in a 28-d oral toxicity study, in accordance with Organisation for Economic Co-operation and Development (OECD) test guideline (TG) 407, to determine the toxicity of a nano-tea tree oil.[85] The test article was prepared using ultrasonic emulsification, and comprised the oil (4% w/w), Tween 80 (2% w/w), carboxymethyl-cellulose sodium (CMC; 0.2% w/w), and water; the mean droplet diameter was 161.80 nm. The animals were dosed by gavage with 0, 50, 100, or 200 mg/kg bw of the test article, once a day, for 28 d. No effects on food or water consumption, body weights, or mortality were observed. Additionally, there were no physical signs of toxicity during the study, and no gross findings, effects on organs, or microscopic effects observed at necropsy. No differences in hematology parameters were reported. Serum alanine aminotransferase levels showed a dose-related increasing trend, and this value was statistically significantly increased in the high-dose group compared to controls; no other statistically significant differences in serum biochemistry values were noted. The no-observable-adverse-effect-level (NOAEL) of this nano-tea tree oil in mice was > 200 mg/kg bw.

Groups of 5 male and 5 female Sprague-Dawley rats were dosed for 28 d with tea tree oil in corn oil by gavage at doses of 0, 5, 15, and 45 mg/kg/d, in accordance with OECD TG 407.[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 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.[86] 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,[7,87,88] in chromosomal assays using Chinese hamster lung fibroblasts (V79) cells (≤ 58.6 µg/ml)[7] or human lymphocytes (≤ 365µg/ml),[89] 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%).[90] 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,[91] it impaired the growth of human M14 melanoma cells,[92,93] and it induced apoptosis in human malignant melanoma (A-375) and squamous cell carcinoma (Hep-2) cells.[94] Tea tree oil also exhibited anti-proliferative activity against human lung carcinoma (H1299, A549) cells; however, in this study, tea tree oil did not have significant effect on the proliferation of breast (MDA-MB-231)) or colon carcinoma (HCT116) cell lines.[93] In a different study using human MCF-7 and murine 4T1 breast cancer

cells, tea tree oil exhibited an antitumor effect by decreasing cell viability and modulating apoptotic pathways.[95] 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.[96] 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).[97] The IC_{50} in several other cancer cell lines ranged from 12.5 µg/ml (24 h) in human HT29 colon cancer cells,[98] to 2800 µg/ml (4 h) in epithelioid carcinomic (HeLa), hepatocellular carcinomic (Hep G2), and human chronic myelogenous leukemia (K-562) cells.[99] In immunocompetent C57BL/6 mice, tea tree oil inhibited the growth of subcutaneous tumors; effectiveness was carrier-dependent.[100] 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%).[101,102] 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.[103] 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).[101] 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.[102] 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%.[101] 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,[102] 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.[104] 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.[105] 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.[106] 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. [107] 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 (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.[108] 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.[109] (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.[109] 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.[110]

Human

Dermal

The effect of tea tree oil on a histamine-induced wheal and flare reaction was examined.[111] 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.[112] 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.[113] 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,[114] and after a single 24-h occlusive application[82,115] 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[116] 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.[115,117] 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.[118-120] 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");[121] 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.[3,122] 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.[121] In clinical studies, a formulation containing 0.001% Melaleuca Alternifolia (Tea Tree) Flower/Leaf/Stem Extract (25 subjects; maximization test),[123] a formulation containing 0.0078% Melaleuca Alternifolia (Tea Tree) Leaf Extract (105 subjects; modified Draize human repeated insult patch test (HRIPT)),[124] and Melaleuca Alternifolia (Tea Tree) Leaf Oil at 1% in pet (22 subjects; maximization test)[115,117] and at 10% in caprylic/capric triglycerides (102 subjects; modified HRIPT),[125] were not sensitizers. 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.[126] 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.[118-120]

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.[115,127] Thirty min after application, the skin was treated with a combination of psoralen and long-wave ultraviolet 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.[128] 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.[129] 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%).[130] 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).[128,131-135] 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.[136] 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*.[137] 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.[138] Testing at the Northwestern Medicine patch-testing clinic with 5% Melaleuca Alternifolia (Tea Tree) Leaf Oil (oxidized, in pet) found no difference in positive results between patients with or without atopic dermatitis.[139]

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.[140] 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%).[141] Three of 60 patients (5%) with lip allergic contact cheilitis (ACC) (2001 - 2004) had positive reactions to oxidized tea tree oil.[142] 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.[143] 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).[144] 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.[145]

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;[146] in June – August 2003, 5/160 subjects had irritant reactions to lotions containing 5% tea tree oil.[146] In Sweden (prior to 2004), 2.7% of 1075 patients tested had a positive reaction to 5% tea tree oil in alcohol.[147] 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),[4,6,148] and testing at 5% (oxidized) in pet (1998 - 2003) produced positive results in 0.9%-1.0% of the patients tested.[149] 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.[150] 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.[151] 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.[152,153] 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.[154] 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,[6,155] and in Spain (prior to 2015), 0.4% of patients had positive reactions to testing with 5% tea tree oil in pet.[156] 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,[157] 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,[158,159] 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.[129]

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);[160] 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.[161] Additionally, positive reaction rates of up to 4.8% have been reported with 10% tea tree oil.[160]

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.[162] 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.[6,155] 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.[148] However, in another study, no correlation was reported between positive reactions to tea tree oil and to colophony.[147] 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.[163] In several case reports of reactions to tea tree oil (described later in this report), reactions were also noted with eucalyptol,[49] colophony,[164,165] and ascaridole.[166]

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,[49,121,122,156,166-168] other direct skin applications,[121,164-166,169-178] and from use of hand wash or shampoos[121,179,180] 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.[181] Positive reactions were also reported in a patient with hand eczema following inhalation of tea tree oil vapors.[182]

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.[183] 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.[184] 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; species not specified) 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, and flavoring ingredient.

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.

The estimated rates pf oral, dermal, and inhalation absorption of tea tree oil were 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 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. In one study, the oral LD_{50}s of tea tree oil and a nano-tea tree oil were estimated to be 0.854 g/kg and 1.565 g/kg, respectively. In another study, the LD_{50} of tea tree oil 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) in which groups of 10 male ICR mice were dosed with up to 200 mg/kg bw of a nano-tea tree oil (comprising the oil (4% w/w), Tween 80 (2% w/w), CMC (0.2% w/w), and water), the NOAEL was determined to be > 200 mg/kg bw. In a 28-d gavage study in which male and female Sprague-Dawley rats were given 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 mg/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. Tea tree oil also exhibited anti-proliferative activity against human lung carcinoma (H1299, A549) cells; however, in this study, tea tree oil did not have significant effect on the proliferation of breast (MDA-MB-231) or colon carcinoma (HCT116) cell lines. In a different study using 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 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. 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, a formulation containing 0.001% Melaleuca Alternifolia (Tea Tree) Flower/Leaf/Stem Extract (25 subjects; maximization test), a formulation containing 0.0078% Melaleuca Alternifolia (Tea Tree) Leaf Extract (105 subjects; modified Draize HRIPT),and Melaleuca Alternifolia (Tea Tree) Leaf Oil at 1% in pet (22 subjects; maximization test) and at 10% in caprylic/capric triglycerides (102 subjects; modified HRIPT), were not sensitizers. 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 (oxidized); 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 with 5% Melaleuca Alternifolia (Tea Tree) Leaf Oil (oxidized, in pet) 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.[147] In Germany, testing with 5% tea tree oil (standardized) in diethyl phthalate produced positive results in 1.1% of the patients tested (1999 - 2000), and testing at 5% (oxidized) in pet (1998 - 2003) produced positive results in 0.9% - 1.0% of the patients tested. Testing performed in the Netherlands (2012 - 2013) reported positive results in 0.9% of patients patch-tested with 5% tea tree oil (oxidized, in pet). However, when this group and an additional 29 patients from a different study were patch-tested with the 5% oxidized tea tree oil and up to 5% ascaridole (a possible contaminant in aged tea tree oil), 6 of 30 patients (20%) that had positive reactions to any concentration of ascaridole also tested positive with tea tree oil; in the 220 patients that did not react to any concentration of ascaridole, none reacted to tea tree oil. In Belgium, 10.5% of patients had positive reactions to 1 and 5% oxidized tea tree oil in pet; these patients were a sub-group of 15,980 patients that were tested (1990 - 2016) and identified as being allergic to herbal medicines and/or botanical ingredients. Additional studies performed in Belgium (2000 - 2010) with fragrance and non-fragrance allergens reported positive reactions in skin care products containing tea tree oil, but not in the other cosmetic product categories. In testing in Italy with 19 patients that had positive reactions to a botanical integrative series, 2 (10.5%) reacted to 5% tea tree oil in pet. In a Swiss clinic (1997), positive reactions were reported in 0.6% of patients tested with 5 – 100% tea tree oil in arachis oil, and in Spain (prior to 2015), 0.4% of patients had positive reactions to testing with 5% tea tree oil in pet. In the UK (1996 - 1997), 7 of 29 patients (24%) thought to have a cosmetic dermatitis had positive patch test reactions to tea tree oil, applied neat, and in 2001, 2.4% of 550 patients tested with neat, oxidized tea tree oil had positive reactions. Between 2008 and 2016, positive reactions from testing with 5% tea tree oil in pet ranged from 0.1 – 0.29% in the UK, and in 2016 - 2017, 0.45% of 4224 patients in the UK and Ireland that were patch-tested with 5% tea tree oil (oxidized) in pet had positive reactions.

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

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

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

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

DISCUSSION

This assessment reviews the safety of 8 *Melaleuca alternifolia* (tea tree)-derived ingredients as used in cosmetic formulations. The Panel concluded that the data included in this review are sufficient for determining the safety of these ingredients as reportedly used in cosmetics.

The majority of the data included in the report is 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 all of the cosmetic ingredients named in this report because most constituents of concern are present at the highest levels in oil-derived ingredients, and no signals for additional constituents of concern were noted in the extracts.

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, examples of the constituents the Panel was concerned about include 1,8-cineole (also known as eucalyptol), a possible allergen, and terpinolene, α-terpinene, α-phellandrene, and limonene, which are possible sensitizers. Additionally, the Panel is aware that variances in the composition of tea tree oil, based on geographical or geological differences 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. Additionally, the Panel was made aware that some of the *Melaleuca alternifolia* (tea tree)-derived ingredients could be supplied as adulterated products; the Panel acknowledged this could always be a concern. For these reasons, it was 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 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; for example, Melaleuca Alternifolia (Tea Tree) Leaf Oil is reported to be used at up to 0.5% in aerosol deodorant formulations, and Melaleuca Alternifolia (Tea Tree) Leaf Oil and Melaleuca Alternifolia (Tea Tree) Leaf Water are reported to be used in face powders. Therefore, the Panel discussed the issue of potential inhalation toxicity. Little inhalation toxicity data (acute studies in 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

The Expert Panel for Cosmetic Ingredient Safety concluded that the following 8 *Melaleuca alternifolia* (tea tree)-derived ingredients are safe in cosmetics in the present practices of use and concentration described in this safety assessment when formulated to be non-sensitizing.

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

* *Not reported to be in current use. Were ingredients in this group not in current use to be used in the future, the expectation is that they would be used in product categories and at concentrations comparable to others in this group.*

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)	185
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	185
	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	185
optical rotation	+7° to +12°	19
	+5º to + 15º	185
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 Water**	
Totals*	**23**	**0.0001-0.001**	**536**	**0.003-0.63**	**10**	**NR**
Duration of Use						
Leave-On	*18*	*0.0001*	*300*	*0.003-0.63*	*9*	*NR*
Rinse-Off	*5*	*0.001*	*221*	*0.0003-0.3*	*1*	*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]	4[a]; 3[b]	NR
Incidental Inhalation-Powder	14[b]	NR	4; 84[b]; 3[c]	0.03[b]	2; 3[b]	NR
Dermal Contact	22	0.0001-0.001	409	0.0003-0.5	9	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	1	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

*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	ICR mice	# males/group: 5 – 0.5 g/kg 7 – 0.75 g/kg 9 – 0.875 g/kg 3 – 1.0 g/kg 3 – 1.25 g/kg	0.5- 1.25 g/kg	Acute oral toxicity was evaluated using a 4-level up-and-down procedure, starting with 3 mice given a dose of 1g/kg. The number of animals was increased with each consecutive dose; each dose level decreased if half the animals died, and increased if half the animals survived.	LD_{50} = 0.854 g/kg (estimated) Mortality (in order of dosing): 1.0 g/kg – 3/3 0.5 g/kg – 0/5 0.75 g/kg – 2/7 0.875 g/kg – 4/9 1.25 – 3/3	85
a nano-tea tree oil (comprising the oil (4% w/w), Tween 80 (2% w/w), CMC (0.2% w/w) , and water; prepared using ultrasonic emulsification; mean droplet diameter of 161.80 nm)	ICR mice	# males/group: 3 – 1.0 g/kg 5 – 1.5 g/kg 9 – 1.625 g/kg 7 – 1.75 g/kg 5 – 1.875 g/kg	1.0 – 1.875 g/kg	Same procedure as described above.	LD_{50} = 1.656 g/kg (estimated) Mortality (in order of dosing): 1.0 g/kg – 0/3 1.5 g/kg – 1/5 1.75 g/kg – 4/7 1.625 g/kg – 4/9 1.875 – 5/5	85

Table 12. Acute toxicity studies

Ingredient	Animals	No./Group	Vehicle	Concentration/Dose	Protocol	LD_{50} or LC_{50}/Results	Reference
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]
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	[87]
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	[88]
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	[89]
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	[89]
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	[90]
			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.	[91]
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	[92]
tea tree oil	10 - 50 µg/ml	human melanoma (M14) cells	Analysis of proliferation/viability in an MTT assay; cells were treated for 24 – 72 h. The effect of 24 h treatment with tea tree oil , followed by 48 h of dabrafenib and/or trametinib (used in treatment of melanoma patients with BRAF mutations), was also examined	After 24 h, a dose-dependent reduction of cell proliferation/viability was observed ; however, between 48 and 72 h of treatment, no significant difference in the IC_{50} was observed. After 72 h with 50 µg/ml tea tree oil, the proliferation/ viability, reported as the cell proliferation-viability of treated cells/cell proliferation-viability of control cells ×100, was 20; the IC_{50} value was approximately 50 µg/ml When combined with dabrafenib and/or trametinib, a synergistic reduction in the viability of melanoma cells through activation of apoptosis was observed.	[93]
		lung (H1299, A549) carcinoma cells		cell proliferation/viability inhibition ranged from 67% to 82% for both cell lines	
		colon (HCT116) and breast (MDA-MB-231) carcinoma cells		proliferation/viability was not reduced in these cell lines	

Table 14. Anti-carcinogenicity studies

Test Article	Concentration/Dose	Test System	Procedure	Results	Reference
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	[94]
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	[95]
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	[97]
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	[96]
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	[98]
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)	[99]
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)	[99]
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)	[99]
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)	[99]
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)	[99]

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.	[100]
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.	[96]

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	[101]
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.	[102]
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%.	[103]

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	[101]
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.	[101]
			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.	[102]
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%	[101]
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	[101]

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	114
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)	82,115
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	116
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	115,117
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')	118-120
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	6,7
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	6,7
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	3,122
tea tree oil (freshly distilled)	"pure" 30 mg for induction 0.05 ml for challenge	10 female Pirbright white guinea pig	modified FCA technique; the material was dissolved in 4 ml FCA, 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	121
oxidized tea tree oil (exposed to light, warmth, moisture, and oxygen)	"pure"	10 guinea pigs	challenge material; oxidized tea tree oil	mean response: 0.45 (24 h); 1.78 (48 h)	
		10 guinea pigs	challenge material: oil stored for 2 mo in a transparent flask challenge material: oil stored for 2 mo in a brown flask challenge material: oil stored for 2 mo in a closed flask challenge material: oil stored for 2 mo in an open flask	mean response: 0.8 (24 h); 1.0 (48 h) mean response: 0.55 (24 h); 1.1 (48 h) mean response: 0.62 (24 h); 0.65 (48 h) mean response: 1.0 (24 h); 1.58 (48 h)	
		10 guinea pigs	challenge material: monoterpene fraction	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					
formulation containing 0.001% Melaleuca Alternifolia (Tea Tree) Flower/Leaf/Stem Extract	neat; 0.05 ml	25 subjects	Maximization test 5 induction exposures to either the upper outer arm, volar forearm, or back of each subject consisted of pretreatment with an occlusive patch (15 mm disc of Webril cotton) containing 0.05 ml 0.25% aq. sodium lauryl sulfate (SLS) for 24 h, followed by application of an occlusive patch containing 0.05 ml of the test material (to the same site) for 24 or 72 h. Because the test formulation contained volatile ingredients, it was allowed to air-dry for ~30 min prior to application. After a 10-d non-treatment period, challenge was performed at a previously untreated site by first applying an occlusive patch containing 0.05 ml 5.0% aq. SLS for 1 h, followed by a 48-h occlusive patch containing the test material.	not a sensitizer no reactions were observed 48 or 72 h after application of the challenge patch	[123]
bubbling face mask containing 0.0078% Melaleuca Alternifolia (Tea Tree) Leaf Extract	neat	105 subjects	modified Draize HRIPT during induction, a total of nine 47- or 71-h occlusive patches were applied 3x/wk for 3 wk; after a 14-d non-treatment period, a 48-h challenge application was made, and challenge sites were scored 1 and 48 h	low potential for irritation and sensitization faint, mild erythematous reactions were observed during induction; no reactions were observed upon challenge	[124]
Melaleuca Alternifolia (Tea Tree) Leaf Oil	1% in pet	22 subjects	Kligman maximization test occlusive patch applied to the volar forearm for 5 alternate-day 48-h periods; patch site was pretreated for 24 h with 5% aq. SLS; for challenge, after a 10 – 14-d non-treatment period, an occlusive patch was applied to a previously untreated site; 5% SLS was applied to the test site for 30 min under occlusion on the left side of the back, and the test materials were applied without SLS treatment on the right side	not a sensitizer	[115,117]
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	[125]

Table 16. Dermal irritation and sensitization studies

Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
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	126
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	118-120
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%)	130
2003 - 2004; NACDG	oxidized, 5% pet	5137	45 (0.9%)	not stated		128
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)	136
2003 - 2007; NACDG	oxidized, 5% pet	11,649 (ages 19 – 64)	35 (0.3%)	22 (0.2%)		143
2005 - 2006; NACDG	oxidized, 5% pet	4435	1.4%	definite - 8.2% probable - 27.9% possible - 36.1%		131
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	132
2009 - 2010; NACDG	oxidized, 5% pet	4299	1.0%	definite - 14.3% probable - 35.7% possible - 21.4%	SPIN – 45 (rank 36)	133
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)	134
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)	135
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	137
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	138
2014 - 2017; Northwestern Medicine patch-testing clinic; 48-h patch	oxidized, 5% pet (Melaleuca Alternifolia (Tea Tree) Leaf Oil)	502 (total) *current atopic dermatitis (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		139

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

Years/Testing Group	Concentration/Vehicle	# patients	# Positive (%)	Relevance	Comments	Reference
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%)	140
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	141
		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	142
age specific - children						
2003 - 2007; NACDG***	oxidized, 5% pet	1007 ≤18 yr	4 (0.4%)	4 (0.4%)		143
2003 – 2004, NACDG***	oxidized, 5% pet	age 0 – 5 y (n not specified)	14.3%	14.3%		144
		age 0 – 18 yr (n not specified)	1.1%	1.1%		
2005 – 2012, NACDG	oxidized, 5% pet	n = 40, age 0 – 5 yr	0%	0%		145
		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%)		143
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)	146

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

Years/Testing Group	Concentration/Vehicle	# patients	# Positive (%)	Relevance	Comments	Reference
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	146
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	147
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	4,6,148
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-trihydroxymenthane (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	149
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-trihydroxymenthane (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	149
2012, Feb – 2013, Mar; Netherlands	5% oxidized tea tree oil	221	2 (0.9%; +)		no irritant reactions reported	150
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	151
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	152
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	153

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

Years/Testing Group	Concentration/Vehicle	# patients	# Positive (%)	Relevance	Comments	Reference
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	154
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	6,155
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	156
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	157
2001, UK	neat, oxidized	550	13 (2.4%)	definite: 4 (30%) possibly: 5 (38.5%)	irritant reactions – 38%	4
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	158
2016, UK	5% pet	1019	0.29%	0.29%		159
2016-2017, UK/Ireland	oxidized, 5% pet	4224	0.45%			129
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	160
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	160
2001-2010; Skin and Cancer Foundation	oxidized, 5% pet**	794	28 (3.5%)	43%		161
	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[128]

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	6,155
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		148
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		147
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	163
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		164
tea tree oil		patient with pruritic ery-thematous rash	positive reactions to tea tree oil and colophony		165
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	166
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	166

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.	[121]
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	[122]
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	[156]
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	[166]
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	[167]
tea tree oil	used to treat psoriasis vulgaris		subject became sensitized within 3 mo; also reacted to fragrance mix, balsam of Peru, and turpentine	[121]
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	[168]
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%	[169]
tea tree oil	patient treated warts on his hands		became sensitized in 3 mo	[121]

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 days 2 and 4.	The patient showed positive reactions to the test product (50% pet; ++ on days 2 and 4) and to the patch with 5% oxidized tea tree oil (+day 2/++day 4), as well as nickel (++ days 2 and 4)	170
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	171
tea tree oil	female subject with tinea pedis developed allergic contact dermatitis after treatment with tea tree oil	the standard battery of the Spanish Group for the Investigation of Contact Dermatitis and Skin Allergy (GEI-DAC), the series of plants and cosmetics (Chemo-technique Diagnostic®), α-pinene, and limonene were tested; test sites were scored (ICDRG) on days 2 and 4	positive results with tea tree oil and rosin were reported negative results were reported with α-pinene or limonene	172
tea tree oil	male subject presented with eczematous lesions on the eyelids and legs for more than 1 yr; worsened after topical application of tea tree oil	the GEI-DAC standard battery and the series of plants and cosmetics (Chemotechnique Diagnostic®) were tested; test sites were scored (ICDRG) on days 2 and 4	positive results with tea tree oil and rosin were reported	172
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	164
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	173
tea tree oil, 5%	bullous eruption resulting from allergic contact dermatitis caused by application of Burnshield®, a tea tree oil-containing hydrogel, and a Burnshield® dressing	occlusive 48-h patch testing was conducted on the upper back using the British Contact Dermatitis Society baseline series, a cosmetic/facial series, a fragrances/ essential oils series, and the patient's own products, including the Burnshield® products	Positive reactions to tea tree oil were recorded on day 2 (+) and day 4 (++). Positive reactions (+++) also were observed at both time periods with both Burnshield® products. (Positive results were also reported with a number of other test substances.)	174
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 discontinu-ing application of the oil	175
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	121

Table 19. Case reports with tea tree oil

Test Substance	Subject(s)/Symptoms	Testing	Results/Comments	Reference
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	165
tea tree oil	used to treat sunburn		no reactions at site of application, but reacted to tea tree oil at patch testing	121
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. .	173
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	121
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.	176
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 (+)	177
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	166

Table 19. Case reports with tea tree oil

Test Substance	Subject(s)/Symptoms	Testing	Results/Comments	Reference
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	178
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).	170
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	179
shampoo containing tea tree oil	patient used the shampoo, and tea tree oil for blisters on his face	epicutaneous testing	patient became sensitized with use of the products reacted to tea tree oil only (other test substances were not identified)	121
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	121
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	180
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)	181
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	182

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: Alexandra Kowcz, MS, MBA
Industry Liaison to the CIR Expert Panel

DATE: April 14, 2021

SUBJECT: Tentative Report: Safety Assessment of *Melaleuca alternifolia* (Tea Tree)-Derived Ingredients as Used in Cosmetics (release date: April 5, 2021)

The Personal Care Products Council respectfully submits the following comments on the tentative report, Safety Assessment of *Melaleuca alternifolia* (Tea Tree)-Derived Ingredients as Used in Cosmetics.

Introduction – It is not clear why the Introduction just mentions that anti-acne agent is not a cosmetic function in the United States. Antifungal and antimicrobial agents are also considered drug functions in the United States.

Non-Cosmetic Use – The paragraph on the FDA final rule from April 2019 should focus just on tea tree oil. Rather than stating: "Drug products containing theses ineligible active ingredients", it should state "Drug products containing tea tree oil…".

Penetration Enhancement – Since one study did not see an increase in penetration with tea tree oil, perhaps this section should be called Effects on Drug Penetration.

ADME – It is not clear from the ECHA dossier (reference 7) that an ADME study in rats was completed. Rather than implying a study was done perhaps it would be better to state that ECHA provided estimates for oral, dermal and inhalation absorption.

Retrospective and Multicenter Studies – Please indicate the material that was used for testing at the Northwestern Medicine patch-testing clinic.

Summary – Please revise: "In an acute dermal toxicity tests in rabbits." (this sentence should either be singular or plural)

Discussion – Please revise: “this could always a concern” and “concern, concern for these effects”

Table 12 – The row for dogs and cats is not describing controlled acute toxicity studies. These are case reports that are in dogs and cats and should be presented in the case report section.

Table 16, Reference 119 – It is not clear what is meant by “FDA technique” and “dissolved in 4 ml FDA”. Maybe “FDA” should be “FCA”?

Table 16, Reference 121 – Please correct: “followed by application t of an occlusive patch”

Table 16, Reference 122 – Please add “hours” after “were scored 1 and 48”

Table 17, Reference 137 – What does “AD” mean as it is not defined in the list of abbreviations? Maybe “AD” should be “ACD”?

Table 17, Reference 147 (2 rows) – Please correct “1,2,4-treihydroxymenthane”

Table 19, reference 119 – Please revise “patient became sensitized use of the products”