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Skin Sensitivity Testing for Tea Tree Oil

A report for the Rural Industries Research and Development Corporation

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Foreword

Tea tree (*Melaleuca alternifolia*) oil has experienced renewed popularity in Australia in recent years and international markets for the product are expanding.

Evidence regarding many of the antimicrobial properties of the oil has been scientifically established, justifying claims of its medicinal uses that were previously based solely on anecdotal reports. Markets are likely to expand as a result of such information being available. However, as more people use the product, the possibility of adverse events occurring increases.

Until now, little safety information relating to the use of tea tree oil was available. Determining the prevalence of sensitivity to tea tree oil in the general population, and attempting to discern the cause of reactions, is essential for the long-term survival of the product. The aim of this project was to produce such safety information and, given favourable results, add strength to the argument for registration of the oil with national regulatory bodies.

This publication describes work that determines the prevalence of immediate and delayed sensitivity to tea tree oil, including both irritant and allergic skin reactions following extended exposure. It also provides an indication as to which components of the oil might be responsible for such reactions, and the circumstances in which the likelihood of reacting may be increased.

This report, an addition to RIRDC's diverse range of almost 400 research publications, forms part of our Tea Tree Oil Program, and provides information that will allow continued development of a safe and efficacious product by the Australian tea tree oil industry.

Most of these 400 research publications are available for viewing, downloading or purchasing online through our website:

- downloads at www.rirdc.gov.au/reports/Index.htm
- purchases at www.rirdc.gov.au/pub/cat/contents.html

Peter Core
Managing Director
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Thanks are also extended to the many volunteers who freely gave of their time to participate in the sensitivity testing, and without whom there would have been no project.

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Executive Summary

For almost 80 years, tea tree oil has been marketed as a safe and efficacious topical antimicrobial agent. Its reputation as an antimicrobial agent is now supported by sufficient scientific evidence, however safety data remain limited, fragmented and largely anecdotal. While adverse reactions appear to occur relatively infrequently, a number of cases of contact dermatitis to tea tree oil have been reported in the medical and scientific literature. These reports would be better viewed in the light of information regarding the frequency and reasons for these allergic or irritant reactions, but little is available. Therefore the aim of this project was to evaluate the prevalence of, and reasons for, skin sensitivity to tea tree oil and its major components.

Over 200 healthy volunteers were tested for sensitivity to 10 different samples of 100% tea tree oil using two different tests. The volunteers ranged in age from 18 to 82 years, with an average age of 40. Females constituted 61% of the group, and 63% of volunteers were certain of having used tea tree oil in the past.

An immediate allergic reaction is detected by the prick test, which is routinely used in the diagnosis of allergy to airborne substances such as grass and dust-mite. Standard allergens such as these were tested in addition to tea tree oil in order to determine if our volunteer population was more or less allergic than average. The volunteers were overly reactive, with the proportion of those responding to dust-mite and rye grass nearing 50%, where other work has given levels of less than 30%. Yet only 1.8% (4 of 219) displayed any reaction to tea tree oil, and reactions were to only one or two of 10 oils. It therefore appears that immediate allergic reaction to tea tree oil is very rare, and unlikely to be a cause for concern.

The patch test is used to diagnose allergic contact dermatitis, which is a delayed reaction. Irritant contact dermatitis may also be evident in some cases. The European Standard Series contains substances that commonly cause allergic contact dermatitis. We included this series when testing the volunteers in order to determine overall reactivity in this type of test. The volunteers appeared to be slightly more reactive to contact allergens than

might be expected, with ~20% reacting to nickel sulphate compared to 5-15% in most other studies, for example.

A total of 52 of the 219 volunteers displayed some reaction to tea tree oil, although in a number of these people this consisted only of a questionable reaction to 1 or 2 of the 10 oils. Besides those with only 1 questionable reaction (n = 8), volunteers exhibiting a reaction to tea tree oil on this initial test were asked to attend a secondary test where another patch test of 100% oil, 10% oil and oil components was applied. Eleven volunteers were unavailable to attend secondary testing for various reasons, leaving 33 on whom additional tests were performed. Results were classified as allergic contact dermatitis, mild or marked irritant contact dermatitis, indistinguishable between allergy and irritancy, or no response.

The prevalence of allergy in the whole group was 2.9% up to a possible 4.8% if indistinguishable reactions were included. An allergic reaction requires a previous exposure to the substance being tested, and the level of allergy amongst those who had previously used tea tree oil was 4.6% up to a possible 7.6% including the indistinguishable reactions. These figures are therefore perhaps more relevant to current consumer groups. The percentage range for the whole group is more applicable to the entire population, although it is probably lower in reality as it is unlikely that 63% of the population have used tea tree oil, even with its current widespread availability in products. The prevalence of allergy to tea tree oil compares quite reasonably with other topical antimicrobials in use such as neomycin sulfate (0-11.6% reaction rates reported elsewhere, with 2.8% of volunteers in this study responding), bacitracin ointment (0.9-9.1% reactivity) and thimerosal (3.4-6.2% reactivity).

The prevalence of marked irritancy to 100% tea tree oil ranged from 2.4 to 4.3% (without or with the indistinguishable reactions). Any level of irritancy (mild and marked) ranged from 7.2 to 10.1%. Irritant reactions do not require previous exposure, and therefore these figures should be applicable to the wider population. Irritancy is particularly concentration dependent, which means that many of these reactions should be avoidable if a lower concentration of oil is used. Indeed, none of the individuals who displayed irritant contact dermatitis to 100% tea tree oil were found to react to 10% oil.

Very few reactions were elicited by the components of tea tree oil which were tested at concentrations approximating those in whole oil meeting the ISO 4730 standard. Both viridiflorene and limonene caused a mild reaction in one subject each. The component *p*-cymene elicited mild to marked reactions in four volunteers (including the subject who reacted to limonene). Whether other minor components of tea tree oil and combinations of components are able to elicit reactions is unknown and warrants further investigation.

While the prevalence of irritant or allergic reactions to tea tree oil is low, it is clear that a proportion of people may experience adverse effects, especially if 100% tea tree oil is used. Application of products containing a lower concentration of oil should still provide therapeutic benefits whilst avoiding the risk of most irritant reactions.

The information this project has provided will allow appropriate marketing of the product for safe usage, and strengthens the case for registration of tea tree oil as a safe topical antimicrobial agent with regulatory bodies such as the FDA in the USA.

1 Introduction

For almost 80 years, tea tree oil has been marketed as a safe and efficacious topical antimicrobial agent. In recent years it has experienced renewed popularity in Australia, and effective marketing of this unique oil has increased its international profile. For tea tree oil to realise its full potential on the world market, it will require approval by various regulatory bodies such as the Food and Drug Administration (FDA) in the USA. To obtain such registration requires not only scientific evidence of the claims of therapeutic properties, but also sound safety information.

While there is now sufficient scientific evidence to justify tea tree oil's reputation as an antimicrobial agent [1-10], data supporting its safety remain limited, fragmented and largely anecdotal. Further information is necessary to secure the position of tea tree oil as a safe topical antimicrobial agent. The relative infrequency with which adverse reactions to tea tree oil appear to have occurred during its "long history of use" bears this reputation out to some extent. However, there are reports of allergic contact dermatitis to tea tree oil and in some cases, its components [11-23], and as markets expand the possibility of further reactions being reported increases. These reports of adverse reactions would be some of the first information on tea tree oil obtained by health professionals searching the medical and scientific literature, and would be better viewed in the light of sound information regarding the frequency and reasons for allergic or irritant reactions.

Citing the prevalence of sensitivity and adverse reactions to tea tree oil as "low" without producing scientific data will not satisfy regulatory authorities such as the American FDA. Furthermore, lack of safety information leaves the industry in a precarious position in the event of a serious adverse reaction, jeopardising the reputation and unrestricted availability of tea tree oil on world markets.

2 Objectives

There are 20 previous reports of adverse events associated with tea tree oil found in widely consulted medical and scientific literature databases [11-30], including some reports of contact dermatitis to tea tree oil and its components. From these reports it is clear that tea tree oil has the capacity to elicit cutaneous reactions. However, because many are case reports about small numbers of individuals, there is little information regarding the prevalence of reactions. There have been some recent investigations [19, 20] into sensitivity to tea tree oil in non-allergic individuals, including more detailed studies on individuals displaying allergic reactions. Unfortunately these studies are not found on the most widely consulted medical literature databases.

Thus the objective of this project was to evaluate the skin sensitivity of tea tree oil and its major components in the general population, and to make this information available to both the industry and wider biomedical community by publication in peer-reviewed medical journals.

3 Methodology

3.1 Subjects

Volunteers for this study were recruited from the general population by various means, including:

- recruitment posters placed at the Queen Elizabeth II Medical Centre (including Sir Charles Gairdner Hospital (SCGH)), Hollywood Private Hospital, The University of Western Australia (UWA), and various local shops.
- articles in local print media – the Claremont Nedlands POST, the Community Newspapers News Chronicle, the UWA Leader, the SCGH Newsletter
- word of mouth

The criteria for inclusion in the study were that the individual was a healthy adult able to give informed consent for participation. Factors which precluded participation in the study included severe skin conditions, immunosuppressant treatment or the use of various medications within a specified time period (in particular corticosteroids, antihistamines and antidepressants).

Each person who enquired about the study was provided with a thorough explanation of what participation would involve. The vast majority of people willingly agreed to participate. A letter providing a written explanation was forwarded to each person with details of their first appointment. At this appointment it was ensured that they understood the procedures that would be performed, and a form indicating their consent was then signed, as required by the Committee for Human Rights of The University of Western Australia.

3.2 Tea Tree Oil

Ten oils were selected for evaluation in the study. Nine tea tree oils were selected randomly from amongst those previously submitted to our laboratory for antimicrobial activity testing. All oils had been submitted via the Australian Tea Tree Industry Association (ATTIA) and forwarded to us with code numbers only. In this way we remain unaware of the source of individual oil samples. Australian Plantations oil was

chosen as the tenth oil as this company supplies approximately 20% of the tea tree oil produced in Australia.

Each oil sample was thoroughly analysed, including gas chromatography (GC) (NSW Agriculture Essential Oils Chemistry, Wollongbar NSW), pesticide analysis (NSW Agriculture Chemical Residue Laboratories, Lismore NSW), and metal analysis (Australian Environmental Laboratories, Welshpool WA and Becquerel Laboratories Pty Ltd, Lucas Heights NSW). In addition, the tea tree oil suppliers were sent a questionnaire via ATTIA covering various aspects of the oil production such as pest and weed control, distillation method and the age of the oil at time of submission. At the conclusion of testing, samples of the original oils which had been in storage were submitted again for GC analysis, together with samples of the oil that had been used for the duration of the project and kept at 4°C in brown glass dropper bottles with rubber tops.

Immediately prior to commencement of secondary phase testing, preparations of 10% tea tree oil and tea tree oil components at various concentrations were made in petrolatum (white paraffin) at the SCGH pharmacy. These were stored in brown glass bottles with plastic screw caps. The preparation consistency was quite firm and a small metal spatula was sterilised and used for addition of the substances to patch chambers. The preparations were made using "stored oils" and unopened or newly purchased bottles of the components. Aromadendrene (+), 1.8-cineole (eucalyptol), limonene R (+), α-phellandrene, viridiflorene (ledene) were purchased from Fluka, Switzerland; *p*-cymene, α-pinene R (±), terpinen-4-ol were purchased from Aldrich, Australia; α-terpinene, γ-terpinene, α-terpineol were purchased from Sigma, Australia.

3.3 Sensitivity tests

Tests to determine the prevalence of adverse cutaneous events to tea tree oil must take into consideration two types of immunologic sensitivity reactions: Type I (immediate) and Type IV (delayed) reactions.

3.3.1 Type I Reactions: The Prick Test

Type I reactions are mediated by IgE antibody and are generally immediate and accompanied by inflammation. This was assessed by skin prick tests, which are economical and quick to perform, virtually painless and offer high specificity. Testing can be finished on a single occasion and the risks of a serious adverse side-effect are very low.

A drop of the test substance was placed on the skin of the inner forearm. The most superficial layer of the skin was pricked through the drop with a lancet (Bayer Corporation, USA). Less than 5µl of the test substance is introduced into the skin. Positive and negative controls were included to ascertain if subjects were reacting normally. The liquid was blotted from the skin with a tissue, and after 15 minutes each site was inspected and if a wheal was present its diameter was measured.

All subjects were tested with 10 different samples of 100% tea tree oil and a series of standard allergens to determine whether each subject was generally atopic (i.e. allergic). The standard allergen series consisted of: histamine positive control (8mg/ml); glycerol negative control; cat hair; dog hair; feathers; house-dust mite; rye grass; 7 grass mix; *Alternaria*; *Aspergillus*; *Eucalyptus* pollen (Bayer Corporation, USA).

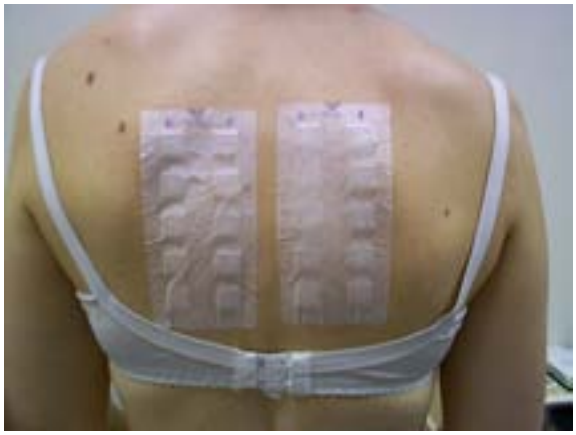
3.3.2 Type IV Reactions: The Patch Test

Type IV reactions are also called delayed type hypersensitivity reactions and symptoms of exposure to a sensitising agent begin to appear 24-48 hours after exposure [31]. These reactions are assessed by patch testing, in which a small occlusive chamber impregnated with the test substance is applied to the skin.

IQ chamber patch test units (Chemotechnique Diagnostics, Sweden) were utilised and consist of chambers made from inert material attached to hypoallergenic adhesive tape. The pre-prepared chambers were usually applied to the mid-section of the upper back (Figure 1). The site of each patch was indelibly marked and they were left in place for 48 hours. During this time the subject was requested to keep the site dry during washing, and to avoid sunbathing and strenuous activity that might lead to excessive sweating. Subjects were instructed to remove any section of the patches that caused

excessive discomfort due to a reaction, and were provided with contact details in case they had any concerns during the test time. These instructions were included on an information sheet that also listed subsequent appointment times.

Figure 1: Placement of patch test units.



After removal of the patch, the appearance of the skin at each test site was graded according to two similar scales. The scale in the Manual for Clinical Laboratory Immunology (MCLI) may be considered more stringent than that recommended by the International Contact Dermatitis Research Group (ICDRG). Therefore the MCLI scale was used in order to obtain more detailed results. The ICDRG scale was used since it is the internationally recognised standard, and facilitates comparison to other published work. The greater stringency of the MCLI scale was particularly useful in primary testing when selecting subjects for further tests. The two scales follow, and reactions are illustrated in Figure 2 using the MCLI grading:

Reaction characteristics	MCLI scale	ICDRG scale
no reaction	0	0
abnormal appearance, but less than half the test area affected	±	0
erythema	1	? or IR*
erythema and oedema	2	+ (or 1)
vesiculation	3	++ (or 2)
bulla formation	4	+++ (or 3)

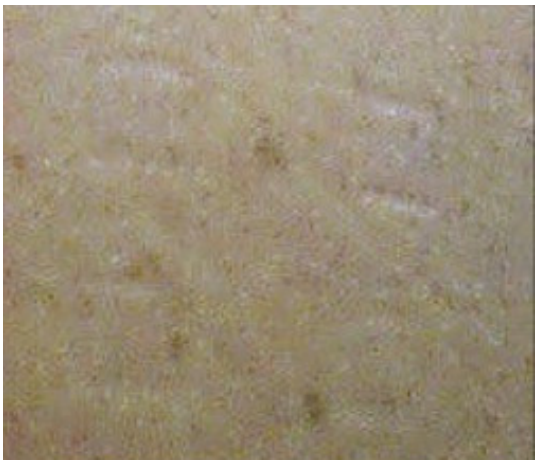
*IR = irritant reaction

The site was inspected again 2-5 days later. This second reading is particularly important to confirm the status of questionable reactions, because allergic reactions may become more apparent, whereas irritant reactions usually fade once the substance is

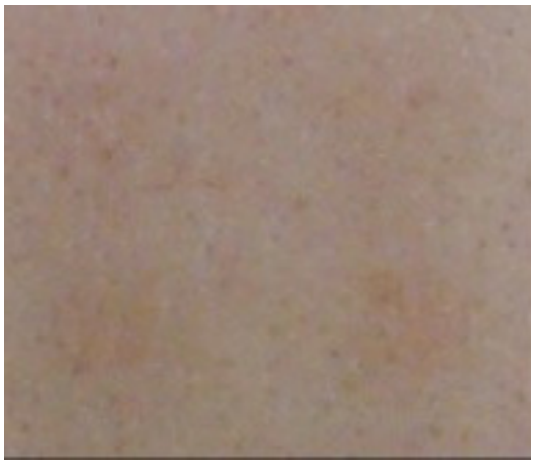
removed. While the patch test is not considered diagnostic for irritant contact dermatitis, such reactions are often detectable, and may be distinguishable from allergic contact dermatitis by some of the characteristics unique to each type of reaction. These include the changes after removal of the substance mentioned above, together with a sharply defined area in the case of irritancy compared to a spreading response in many allergic reactions.

As with the prick tests, 10 different samples of 100% tea tree oil were tested, together with the European standard series of contact allergens (obtained from Chemotechnique Diagnostics, Sweden). This series of 23 standardised preparations is widely used in contact dermatitis testing as it contains the substances most often implicated in contact allergy, and thus provided an indication of the overall reactivity of each test subject.

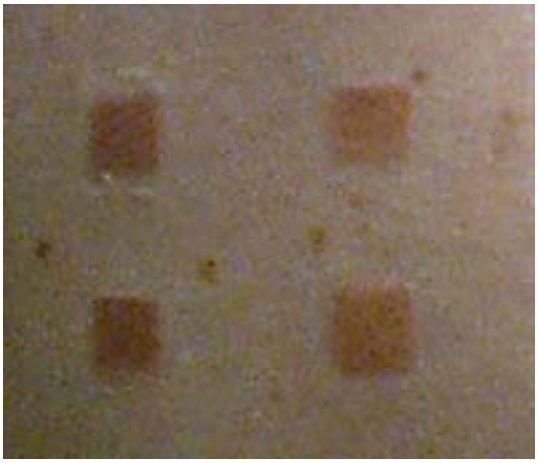
Figure 2: Examples of the appearance of reactions at different levels on the grading scale



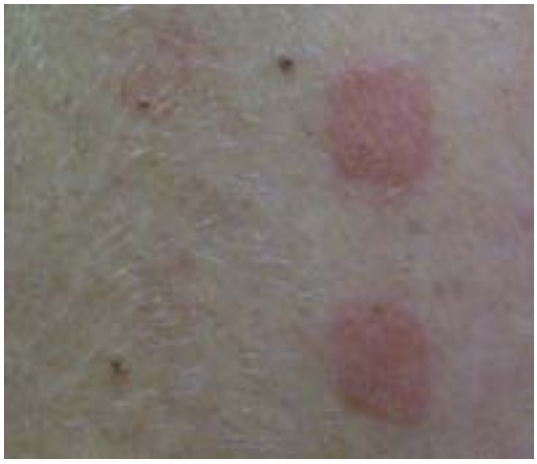
0 = no reaction
Skin appears normal at test site (although indents from the chambers still visible).



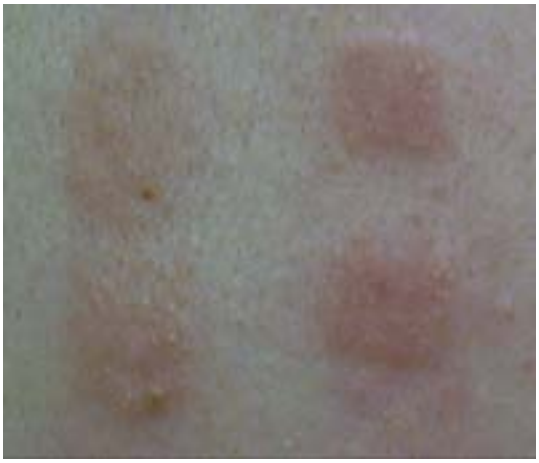
± = abnormal appearance, but less than half the test area affected
Some alteration visible, but reaction questionable.



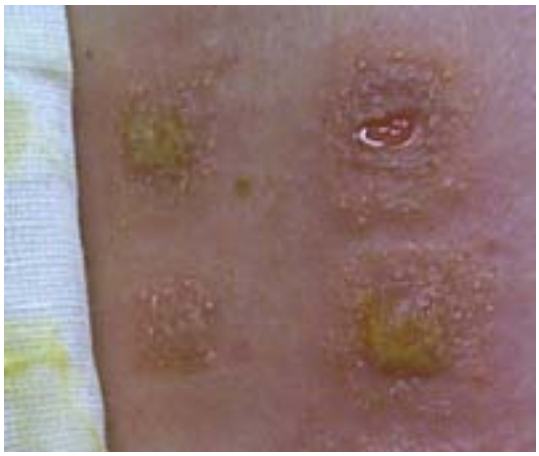
1 = erythema
Inflammation at the test site.
Note the sharp demarcation of these squares, indicating that the nature of the response is probably irritant contact dermatitis.



2 = erythema and oedema
The test site is red and raised due to fluid accumulation.



3 = vesiculation
In addition to inflammation and fluid accumulation, many tiny blisters form.



4 = bulla formation
In addition to vesicles, blisters cover the entire site of substance contact.
Note the spreading nature of the response, implying allergic contact dermatitis.

3.3.3 Secondary Patch Testing of Reactive Individuals

Subjects who exhibited any reaction to oil were requested to attend a further series of appointments. Their contact sensitivity to tea tree oil was further characterised by the application of another set of patches containing the 10 oils at concentrations of 100% and 10%, and the major components of tea tree oil at concentrations approximating those found in oil meeting the ISO 4730 standard.

Individuals experiencing marked reactions were instructed to soothe the site with a cold compress, and given corticosteroid cream to hasten resolution.

All tests were performed and read by Dr Jane Greig, with advice from Dr Martin Stuckey in some cases, to ensure that there was consistency in reaction assessments. Photographic records were made of some primary test responses, and all secondary patch test results.

3.4 Statistical Analysis

Where necessary, two-tailed t-tests or ANOVA were employed to compare various sets of data. GraphPad PRISM software was used for statistical analysis.

4 Results

4.1 Subjects

A total of 219 volunteers completed the primary phase of sensitivity testing. Subjects ranged in age from 18 to 82 years, with an average age of 40. Female volunteers outnumbered males, constituting 61% of the subjects. This was considered an acceptable ratio. Of the sample population, 63% were certain of having previously come into contact with tea tree oil in some form. Pre-exposure rates could have been higher, as some subjects were unsure and hence classified as not exposed (Table 1).

Table 1: Average age and relative distribution of study volunteers by gender and prior exposure to tea tree oil

Prior exposure	**Female**		**Male**		**Total**	
to tea tree oil	n (%)	Av. age (yrs)	n (%)	Av. age (yrs)	n (%)	Av. age (yrs)
Yes	103 (47)	35.7	36 (16)	43.3	139 (63)	37.7
No or unsure	31 (14)	44.9	49 (22)	43.8	80 (37)	44.2
Total	134 (61)	37.9	85 (39)	43.6	219 (100)	40.1

4.2 Tea Tree Oil

All tea tree oil samples were shown by GC analysis to comply with the ISO 4730 standard for Oil of *Melaleuca alternifolia* (Table 2). No organochlorine, organophosphate or synthetic pyrethroid pesticides were detected in any samples (refer to Appendix for list of pesticides tested). Low levels of copper (0.6-2.1 mg/kg) and zinc (<0.5-7.3 mg/kg) were detected in most oils, together with a few other metals in some oils (refer to Appendix for results). All 10 producer questionnaires were returned.

Table 2: Initial GC analysis showing the % of each component of the 10 tea tree oil samples used for sensitivity testing

Component	ISO 4730	Tea tree oil										*mean*	min	max
	range %	A	B	C	D	E	F	G	H	I	J			
a-pinene	1-6	2.7	2.5	1.2	3.0	1.7	1.6	1.8	2.4	2.4	3.2	*2.3*	1.2	3.2
sabinene	tr-3.5	0.7	0.7	1.7	0.6	0.3	0.8	0.6	0.4	0.4	0.6	*0.7*	0.3	1.7
a-terpinene	5-13	8.7	9.9	9.4	8.4	9.4	8.1	9.7	8.7	11.3	11.6	*9.5*	8.1	11.6
limonene	0.5-4	1.0	1.0	1.3	1.1	1.2	1.0	1.1	1.0	1.4	1.8	*1.2*	1.0	1.8
p-cymene	0.5-12	4.2	1.4	5.0	2.0	3.0	4.0	2.8	3.2	3.7	4.0	*3.3*	1.4	5.0
1,8-cineole	0-15	1.9	3.5	3.4	0.9	3.8	3.1	3.4	2.3	4.6	8.7	*3.6*	0.9	8.7
g-terpinene	10-28	19.9	19.9	22.4	20.1	21.5	19.6	21.4	20.3	23.7	25.7	*21.5*	19.6	25.7
terpinolene	1.5-5	3.1	3.4	3.9	3.3	3.4	3.2	3.5	3.3	3.9	3.9	*3.5*	3.1	3.9
terpinen-4-ol	30->	36.2	39.6	40.4	35.2	37.6	41.6	39.0	39.0	36.3	30.3	*37.5*	30.3	41.6
a-terpineol	1.5-8	2.6	3.1	2.7	4.4	2.7	3.2	2.9	2.8	2.3	1.6	*2.8*	1.6	4.4
aromadendrene	tr-7	2.1	1.3	0.8	2.5	1.6	1.1	1.3	1.9	1.0	0.7	*1.4*	0.7	2.5
ledene	0.5-6.5	1.7	1.2	0.5	2.2	1.4	1.0	1.1	1.4	0.7	0.4	*1.2*	0.4	2.2
d-cadinene	tr-8	1.7	1.2	0.5	2.1	1.3	1.0	1.1	1.5	0.6	0.5	*1.2*	0.5	2.1
globulol	tr-3	0.7	0.3	0.2	0.8	0.4	0.6	0.4	0.5	0.2	0.1	*0.4*	0.1	0.8
viridiflorol	tr-1.5	0.3	0.1	0.1	0.3	0.2	0.2	0.1	0.2	0.1	0.0	*0.2*	0.0	0.3

4.3 Sensitivity tests

4.3.1 Type I Reactions: The Prick Test

Results of the prick test for Type I allergic reactions are shown in Table 3. Average wheal size for each allergen, the number of subjects who responded and the percentage of the total sample population that they constituted are presented. Only three volunteers responded to tea tree oil sample B, and two of these also responded to sample A. No other tea tree oil samples elicited a response.

Table 3: Prick test results for 219 subjects

Allergen	Mean wheal size (mm)	Reactors (n)	Reactors (%)
100% tea tree oil			
A	2.5	2	0.9
B	2.7	3	1.4
C	0.0	0	0.0
D	0.0	0	0.0
E	0.0	0	0.0
F	0.0	0	0.0
G	0.0	0	0.0
H	0.0	0	0.0
I	0.0	0	0.0
J	0.0	0	0.0
histamine (+ control)	5.3	219	100.0
glycerol (- control)	0.0	0	0.0
cat pelt	4.1	57	26.0
dog pelt	3.0	22	10.0
feathers	2.1	4	1.8
dust mite	5.2	108	49.3
rye grass	6.5	104	47.5
grass mix	6.5	90	41.1
Alternaria	3.7	79	36.1
Aspergillus	2.7	35	16.0

4.3.2 Type IV Reactions: The Patch Test

Primary patch testing involved 48 hour occluded dermal exposure of subjects to 10 different samples of 100% tea tree oil and a set of 23 common contact allergens (the European standard series) in order to detect allergic contact dermatitis. Irritant contact dermatitis may also occur. Irritant reactions may or may not be distinguishable from allergic reactions, and such distinctions are better made after confirmatory tests. Therefore results for primary screening tests are classified as contact dermatitis, and only after secondary testing are listed as irritant, allergic, or indistinguishable reactions.

The patch tests results for the European standard series for all subjects using both the MCLI and ICDRG scales, are presented in Table 4. As this series of allergens are in standard usage internationally, all further discussion of these reactions will refer to results in Table 4

from the internationally recognised ICDRG scale, so that comparisons can be directly made to other published studies.

Table 4: Results of patch tests with the European standard series for all subjects (219), with results from the MCLI (level 1-4) and ICDRG (level 1-3) scales

Allergen	**MCLI scale**		**ICDRG scale**	
	Reactors (n)	**(%)**	**Reactors (n)**	**(%)**
Potassium dichromate	49	22.4	20	9.1
4-phenylenediamine base	0	0.0	0	0.0
Thiuram mix	3	1.4	2	0.9
Neomycin sulfate	6	2.7	4	1.8
Cobalt chloride (total)	116	53.0	13	5.9
** *macular erythema reactions*	*102*	46.6		
Benzocaine	2	0.9	2	0.9
Nickel sulfate	54	24.7	45	20.5
Quinolone mix	0	0.0	0	0.0
Colophony	4	1.8	2	0.9
Parabens	4	1.8	0	0.0
N-isopropyl-N-phenyl-4-phenylenediamine	0	0.0	0	0.0
Wool alcohols	2	0.9	1	0.5
Mercapto mix	0	0.0	0	0.0
Epoxy resin	3	1.4	2	0.9
Balsam of Peru	8	3.7	5	2.3
4-tert-butylphenol formaldehyde resin	2	0.9	2	0.9
Mercaptobenzothiazole	1	0.5	1	0.5
Formaldehyde	3	1.4	3	1.4
Fragrance mix	15	6.8	9	4.1
Sesquiterpene lactone mix	0	0.0	0	0.0
Quaternium 15	2	0.9	2	0.9
Primin	1	0.5	1	0.5
Cl+Me-isothiazolinone	0	0.0	0	0.0
negative control	0	0.0	0	0.0

** *macular erythema reactions* refers to the different reactions observed in response to this substance – most reactions were evident as “pin-prick” red dots (macular erythema) as opposed to the more uniform erythema responses elicited by all other allergens, and therefore were included in the total, but also listed separately (NB as erythema only, not recorded on the ICDRG scale).

The overall MCLI results of primary patch testing with tea tree oil, separated by gender and prior exposure to tea tree oil, are provided in Table 5. There was a total of 52 individuals who exhibited some response, including questionable (±) reactions, to one or more samples of tea tree oil. Their individual results are presented in Tables 6 and 7 (MCLI and ICDRG scales respectively) as the total score (not including ±) for each site from both patch

assessments. There was no significant difference in total MCLI reaction score between the 10 oils (ANOVA, p = 0.8883).

4.3.3 Secondary Patch Testing of Reactive Individuals

Of the 52 individuals listed in Tables 6 and 7 who experienced some response to tea tree oil, 33 attended a second series of appointments. Some subjects were uncontactable, unavailable or unwilling to be retested, and individuals who had displayed only a single questionable (MCLI ±) response (n = 8) were not recalled. The individual's total scores (>0) on the secondary tests to 100% tea tree oil are presented in Tables 8 and 9, listed in order corresponding with Tables 6 and 7 for ease of comparison. The list is separated into two sections based on the oils used for secondary testing. The first group of subjects underwent secondary testing using the oils that had been used throughout the primary phase of the trial, and are referred to as "dropper bottle oils" because of the containers in which they were stored for the duration of the study. The second group of subjects were tested with oil retrieved from the original samples. Original oil samples had been stored undisturbed in the dark until this time. These samples are referred to as "stored oils".

The total score for each oil in the two sections was calculated, and then divided by the number of subjects in that section to give a proportional score. There was no significant difference in the total MCLI scores of the 10 oils for dropper bottle oils (n = 15; p = 0.9988), for stored oils (n = 18; p = 0.9997), or for the entire group (n = 33; p = 0.9988).

Table 5: MCLI results of primary patch tests with 100% tea tree oil for all subjects, by gender and prior exposure to tea tree oil

Allergen	Female, Pre-exposed FP (n = 103)			Female, Not pre-exposed FN (n = 31)			Male, Pre-exposed MP (n = 36)			Male, Not pre-exposed MN (n = 49)			All subjects all (n = 219)	
Tea tree oil	*N*	*N*/FP (%)	*N*/all (%)	*N*	*N*/FN (%)	*N*/all (%)	*N*	*N*/MP (%)	*N*/all (%)	*N*	*N*/MN (%)	*N*/all (%)	*N*	*N*/all (%)
A	5	4.9	2.3	1	3.2	0.5	2	5.6	0.9	0	0.0	0.0	8	3.7
B	4	3.9	1.8	0	0.0	0.0	3	8.3	1.4	0	0.0	0.0	7	3.2
C	4	3.9	1.8	0	0.0	0.0	3	8.3	1.4	0	0.0	0.0	7	3.2
D	6	5.8	2.7	0	0.0	0.0	3	8.3	1.4	0	0.0	0.0	9	4.1
E	7	6.8	3.2	1	3.2	0.5	3	8.3	1.4	0	0.0	0.0	11	5.0
F	9	8.7	4.1	2	6.5	0.9	5	13.9	2.3	0	0.0	0.0	16	7.3
G	7	6.8	3.2	0	0.0	0.0	4	11.1	1.8	0	0.0	0.0	11	5.0
H	10	9.7	4.6	0	0.0	0.0	4	11.1	1.8	0	0.0	0.0	14	6.4
I	7	6.8	3.2	0	0.0	0.0	4	11.1	1.8	0	0.0	0.0	11	5.0
J	9	8.7	4.1	0	0.0	0.0	4	11.1	1.8	1	2.0	0.5	13	5.9

N = number of subjects who reacted (at level 1-4), where the MCLI reaction scale is as follows

0 = no reaction

± = abnormal appearance, but less than half the test area affected

1 = erythema

2 = erythema and oedema

3 = vesiculation

4 = bulla formation.

Table 6: Individual total MCLI scores (2 readings) to 100% tea tree oil in primary patch tests

Subject	Tea tree oil (100%)										*Oils reacted*
	A	B	C	D	E	F	G	H	I	J	*to at 1-4 (n)*
T014	±	±	±	±	±	1	±	1	±	±	***2***
T015					±	±					***0***
T052	1	±	±	±	1	1	1	1	1	1	***7***
T082	2	2	2	2	2	2	2	2	2	2	***10***
T088	2	±	1	2	2	2	±	±	±	±	***5***
T091						1					***1***
T092						1					***1***
T100						5					***1***
T101						2	1	1	2	2	***5***
T127				±	±	±	±	±	±	±	***0***
T128	±	±	±	±	±	±	±	±	±	±	***0***
T132										±	***0***
T134					±					1	***1***
T176	±	±			±					±	***0***
T198			±						±		***0***
T008			6								***1***
T017					±	±	±				***0***
T045	±		±	±	±						***0***
T066	3	3	±	3	3	3	3	3	3	3	***9***
T071	±	±			±	±	±	1	1	1	***3***
T074				±	±	±	±	±	±	±	***0***
T089	±	±	±	1	2	±	1	1	1	1	***6***
T115						3	2	2		3	***4***
T141					1						***1***
T146				1				1	1	1	***4***
T161	±	±	±	±	±	±	±	±	±	±	***0***
T183		1	1	2	2	1	2	2	1	1	***9***
T184		2			±						***1***
T196		±								±	***0***
T210									±	1	***1***
T211	1	1	1	1	1	1	±	1	1	1	***9***
T216	5	5	5	5	5	5	5	5	5	5	***10***
T218	1	±			1	1				±	***3***
T030					±						***0***
T050						±					***0***
T051						±					***0***
T053				±		±	±	±	±		***0***
T063					±						***0***
T123										±	***0***
T150				±							***0***
T162						±					***0***
T169	1	1	1	1	1	1	1	1	1	1	***10***
T175						±					***0***
T178						1					***1***
T191							4	4			***2***
T192	±					±					***0***
T204	±	±	±	±	±	±	±	±	±	±	***0***
T207							±			±	***0***
T209							±	±			***0***
T214							±			±	***0***
T217				±			±	±	±	±	***0***
T219	±	±	±	±	±	±	±	±	±	±	***0***
# reactors	**8**	**7**	**7**	**9**	**11**	**16**	**10**	**14**	**11**	**14**	
total score	**16**	**15**	**17**	**18**	**21**	**31**	**22**	**26**	**19**	**24**	

Table 7: Individual total ICDRG scores (2 readings) to 100% tea tree oil in primary patch tests

Subject	Tea tree oil (100%)										*Oils reacted*
	A	B	C	D	E	F	G	H	I	J	*to at 1-3 (n)*
T014						?		?			***0***
T015											***0***
T052	?				?	?	?	?	?	?	***0***
T082	?	?	?	?	?	?	?	?	?	?	***0***
T088	?		?	?	?	?					***0***
T091						?					***0***
T092						?					***0***
T100						3					***1***
T101						?	?	?	?	?	***0***
T127											***0***
T128											***0***
T132											***0***
T134										?	***1***
T176											***0***
T198											***0***
T008			4								***1***
T017											***0***
T045											***0***
T066	2	2		2	2	2	2	2	2	2	***9***
T071								?	?	?	***0***
T074											***0***
T089				?	?		?	?	?	?	***0***
T115						1	1	1		1	***4***
T141					?						***0***
T146				?				?	?	?	***0***
T161											***0***
T183		?	?	1	1	?	1	1	?	?	***4***
T184		1									***1***
T196											***0***
T210										?	***0***
T211	?	?	?	?	?	?		?	?	?	***0***
T216	3	3	3	3	3	3	3	3	3	3	***10***
T218	?				?	?					***0***
T030											***0***
T050											***0***
T051											***0***
T053											***0***
T063											***0***
T123											***0***
T150											***0***
T162											***0***
T169	?	?	?	?	?	?	?	?	?	?	***0***
T175											***0***
T178						?					***0***
T191							3	3			***2***
T192											***0***
T204											***0***
T207											***0***
T209											***0***
T214											***0***
T217											***0***
T219											***0***
# reactors	**2**	**3**	**2**	**3**	**3**	**4**	**5**	**5**	**2**	**3**	
total score	**5**	**6**	**7**	**6**	**6**	**9**	**10**	**10**	**5**	**6**	

Table 8: Individual total MCLI scores (2 readings) to 100% tea tree oil in secondary patch tests

Subject	Tea tree oil (100%)										*Oils reacted*	Response
(gender)	A	B	C	D	E	F	G	H	I	J	***to at 1-4 (n)***	**type**
Dropper bottle oils												
T014 (F)	3	2	2	2	2	2	2	2	2	2	***10***	ICD
T015 (F)	1	1	1	1	1	1	1	1	1	1	***10***	mild ICD
T052 (F)	2	2	1	8	2	2	2	1	2	2	***10***	indist.
T082 (F)	2	2	5	2	2	2	2	8	2	5	***10***	ICD
T088 (F)	2	1							±		***2***	mild ICD
T091 (F)	2	2	5	2	2	5	5	2	2	3	***10***	ICD
T092 (F)	3	4	8	8	4	4		6	3	2	***9***	indist.
T100 (M)	1	5	1	2	5	1	1	5	8	2	***10***	ICD
T101 (M)	8	8	8	4	4	4	8	8	6	4	***10***	ACD
T127 (M)	2	2		2	6	3	2		±	6	***7***	indist.
T128 (F)	2	2		2	2	2	2	2	2	2	***9***	ICD
T132 (F)	±							±			***0***	
T134 (M)	1	±				±	1	1	±	1	***4***	mild ICD
T176 (F)	1	1	1	1	1	1	1	1	1	1	***10***	mild ICD
T198 (M)											***0***	
no. reactors	**13**	**12**	**9**	**11**	**11**	**11**	**11**	**11**	**10**	**12**		
total score	**30**	**32**	**32**	**34**	**31**	**27**	**27**	**37**	**29**	**31**	**310**	
proportion (/15)	**2.0**	**2.1**	**2.1**	**2.3**	**2.1**	**1.8**	**1.8**	**2.5**	**1.9**	**2.1**	**20.7**	
Stored oils												
T008 (F)			±	±				±	±	±	***0***	
T017 (F)	4	4	2	4	4	3	4	6	4	4	***10***	ACD
T045 (F)			1	1	±		1	1	1		***5***	mild ICD
T066 (F)	6	6	6	6	6	6	6	6	6	6	***10***	ACD
T071 (F)											***0***	
T074 (M)								±			***0***	
T089 (F)	4	1	2	2	±	±	±	±	1	±	***5***	mild ICD
T115 (F)	1	3	±	1	1	4	6	2		±	***7***	indist.
T141 (F)			1	±			1	1	±		***3***	mild ICD
T146 (F)	1	±	1	1	±	±	±	1	1	1	***6***	mild ICD
T161 (F)			1	1				±	1		***3***	mild ICD
T183 (M)	4	4	4	2	3	4	4	4	3	3	***10***	ACD
T184 (F)	3	1	1	±	2		±	2	2	±	***6***	mild ACD
T196 (F)											***0***	
T210 (F)											***0***	
T211 (M)	1	±	1	1	±	±	±	1	1	1	***6***	mild ICD
T216 (F)	8	8	8	8	8	8	8	8	8	8	***10***	ACD
T218 (F)	±	±					±	±		±	***0***	
no. reactors	**9**	**7**	**11**	**10**	**6**	**5**	**7**	**10**	**10**	**6**		
total score	**32**	**27**	**28**	**27**	**24**	**25**	**30**	**32**	**28**	**23**	**276**	
proportion (/18)	**1.8**	**1.5**	**1.6**	**1.5**	**1.3**	**1.4**	**1.7**	**1.8**	**1.6**	**1.3**	**15.3**	
TOTALS												
no. reactors	**22**	**19**	**20**	**21**	**17**	**16**	**18**	**21**	**20**	**18**		
total score	**62**	**59**	**60**	**61**	**55**	**52**	**57**	**69**	**57**	**54**	**586**	

ICD = irritant contact dermatitis; ACD = allergic contact dermatitis; indist. = ICD and ACD indistinguishable

Table 9: Individual total ICDRG scores (2 readings) to 100% tea tree oil in secondary patch tests

Subject	**Tea tree oil (100%)**										***Oils reacted***	**Response**
(gender)	A	B	C	D	E	F	G	H	I	J	***to at 1-4 (n)***	**type**
Dropper bottle oils												
T014 (F)	1	IR	IR	IR	IR	IR	IR	IR	IR	IR	***1***	ICD
T015 (F)	?	?	?	?	?	?	?	?	?	?	***0***	mild ICD
T052 (F)	?	?	?	6	?	?	?	?	?	?	***1***	indist.
T082 (F)	IR	?	3	?	?	?	?	6	?	3	***3***	ICD
T088 (F)	?	?									***0***	mild ICD
T091 (F)	IR	IR	3	IR	IR	3	3	IR	IR	1	***3***	ICD
T092 (F)	1	2	6	6	2	2		4	1	1	***9***	indist.
T100 (M)	?	3	?	?	3	?	?	3	6	?	***5***	ICD
T101 (M)	6	6	6	2	2	2	6	6	4	2	***10***	ACD
T127 (M)	?	?		?	4	1	?			4	***3***	indist.
T128 (F)	IR	IR		IR	IR	IR	IR	IR	IR	IR	***0***	ICD
T132 (F)											***0***	
T134 (M)	?						?	?		?	***0***	mild ICD
T176 (F)	?	?	?	?	?	?	?	?	?	?	***0***	mild ICD
T198 (M)											***0***	
no. reactors	**3**	**3**	**4**	**3**	**4**	**4**	**2**	**4**	**3**	**5**		
total score	**8**	**11**	**18**	**14**	**11**	**8**	**9**	**19**	**11**	**11**	**120**	
proportion (/15)	**0.5**	**0.7**	**1.2**	**0.9**	**0.7**	**0.5**	**0.6**	**1.3**	**0.7**	**0.7**	**8.0**	
Stored oils												
T008 (F)											***0***	
T017 (F)	2	2	1	2	2	1	2	4	2	2	***10***	ACD
T045 (F)			?	?			?	?	?		***0***	mild ICD
T066 (F)	4	4	4	4	4	4	4	4	4	4	***10***	ACD
T071 (F)											***0***	
T074 (M)											***0***	
T089 (F)	2	?	?	?					?		***1***	mild ICD
T115 (F)	?	1		?	?	2	4	?			***3***	indist.
T141 (F)			?				?	?			***0***	mild ICD
T146 (F)	?		?	?				?	?	?	***0***	mild ICD
T161 (F)			?	?					?		***0***	mild ICD
T183 (M)	2	2	2	?	1	2	2	2	1	1	***10***	ACD
T184 (F)	1	?	?		?			?	?		***1***	mild ACD
T196 (F)											***0***	
T210 (F)											***0***	
T211 (M)	?		?	?				?	?	?	***0***	mild ICD
T216 (F)	6	6	6	6	6	6	6	6	6	6	***10***	ACD
T218 (F)											***0***	
no. reactors	**7**	**5**	**4**	**3**	**4**	**5**	**5**	**4**	**4**	**4**		
total score	**17**	**15**	**13**	**12**	**13**	**15**	**18**	**16**	**13**	**13**	**145**	
proportion (/18)	**0.9**	**0.8**	**0.7**	**0.7**	**0.7**	**0.8**	**1.0**	**0.9**	**0.7**	**0.7**	**8.1**	
TOTALS												
no. reactors	**10**	**8**	**8**	**6**	**8**	**9**	**7**	**8**	**7**	**9**		
total score	**25**	**26**	**31**	**26**	**24**	**23**	**27**	**35**	**24**	**24**	**265**	

ICD = irritant contact dermatitis; ACD = allergic contact dermatitis; indist. = ICD and ACD indistinguishable

A paired two-tailed t-test comparing the series of proportional MCLI scores for each of the sections showed that the dropper bottle oils had contributed disproportionately to the total reaction scores ($p < 0.0001$). The difference in scores was not evident if the ICDRG scale was used, as the disproportionate contribution of dropper bottle oils was manifested mainly as erythema which was often clearly irritant, and therefore not given a numerical score. The proportion of subjects who reacted during each month of primary testing did not appear to increase with time as the oils aged, nor did there seem to be any seasonal effect.

Four individuals who had not reacted to tea tree oil on the primary test were subsequently tested with both series of oils. In two individuals there was no clearly distinguishable difference between the two series, while in a third there was limited erythema to both series but it was more marked in response to the dropper bottle oils. In the fourth subject, who did not respond to any substance during the primary (prick and patch) testing, there was no response to the stored oils, but clearly visible erythema and pruritis (itch) at all sites of the dropper bottle oils (Figure 3).

Figure 3: The reactions of a previously unresponsive individual to stored tea tree oil (LHS) in comparison to dropper bottle oils (RHS). While the sites of application of stored oils are barely visible, dropper bottle oils caused erythema and pruritis.

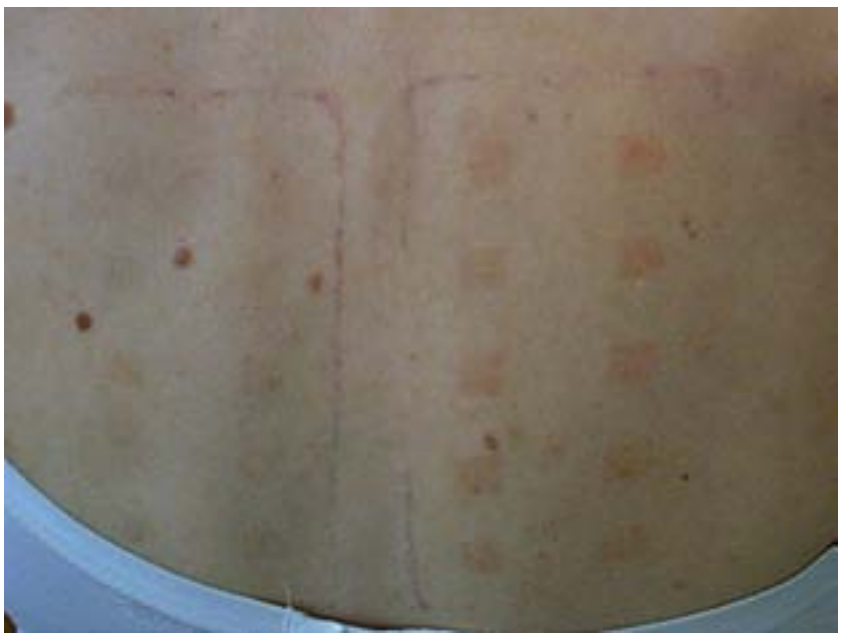


Table 10: GC analysis of the 10 tea tree oil samples stored in two different ways and used for secondary patch testing

Component	ISO 4730 range %	Dropper bottle oil (av. %)	Stored oil (av. %)	Mean change (%)	t-test (p value)
α-pinene	1-6	1.8	2.7	-33.4	<0.0001
sabinene	tr-3.5	0.5	0.6	-19.8	0.0013
α-terpinene *	5-13	5.3	9.1	-41.3	<0.0001
limonene	0.5-4	1.1	1.2	-12.4	<0.0001
p-cymene #	0.5-12	7.4	4.3	84.0	<0.0001
1,8-cineole	0-15	3.5	3.6	-4.3	0.0051
γ-terpinene *	10-28	16.3	21.1	-23.1	<0.0001
terpinolene	1.5-5	2.9	3.5	-16.4	<0.0001
terpinen-4-ol	30->	41.5	37.0	12.2	<0.0001
α-terpineol	1.5-8	3.2	2.8	14.6	0.0014
aromadendrene	tr-7	1.8	1.6	15.9	<0.0001
ledene	0.5-6.5	1.3	1.2	11.6	0.0002
δ-cadinene	tr-8	1.3	1.1	18.2	<0.0001
globulol	tr-3	0.4	0.3	27.0	0.0031
viridiflorol	tr-1.5	0.4	0.3	35.3	0.0039
1,2,4-trihydroxymenthane		<0.2	<0.2	0.0	
total sesquiterpenoids		12.6	9.8	32.0	<0.0001

* decreases with oxidation
# increases with oxidation

The two series of oils were submitted for GC analysis in order to determine whether the repeated opening of the bottles and aspiration of oil into the dropper for administration had caused significant oxidation of the oil. The results of the GC analysis are presented in Table 10. GC analyses of the dropper bottle and stored oils show that significant oxidation of the oils had occurred by the commencement of the second phase of the trial.

Together with retesting 100% tea tree oil, the secondary patch tests also involved testing 10% tea tree oil and a number of the major components of tea tree oil at concentrations approximating those found in oil meeting the ISO 4730 standard. . The individual results for 10% tea tree oil on secondary tests are presented in Tables 11 and 12 (MCLI and ICDRG scales respectively). The results for tea tree oil components are presented in Tables 13 and 14 (MCLI and ICDRG scales respectively).

Table 11: Individual total MCLI scores (2 readings) to 10% tea tree oil in secondary patch tests

Subject	Tea tree oil (10%)										*Oils reacted*	Response
(gender)	A	B	C	D	E	F	G	H	I	J	*to at 1-4 (n)*	type
Dropper bottle oils												
T014 (F)											***0***	ICD
T015 (F)											***0***	mild ICD
T052 (F)			±								***0***	indist.
T082 (F)											***0***	ICD
T088 (F)											***0***	mild ICD
T091 (F)											***0***	ICD
T092 (F)											***0***	indist.
T100 (M)											***0***	ICD
T101 (M)	1	±	1		±	±	±	1		1	***4***	ACD
T127 (M)											***0***	indist.
T128 (F)											***0***	ICD
T132 (F)											***0***	
T134 (M)											***0***	mild ICD
T176 (F)											***0***	mild ICD
T198 (M)											***0***	
no. reactors	**1**	**0**	**1**	**0**	**0**	**0**	**0**	**1**	**0**	**1**		
total score	**1**	**0**	**1**	**0**	**0**	**0**	**0**	**1**	**0**	**1**	**4**	
proportion (/15)	**0.1**	**0.0**	**0.1**	**0.0**	**0.0**	**0.0**	**0.0**	**0.1**	**0.0**	**0.1**	**0.3**	
Stored oils												
T008 (F)									±		***0***	
T017 (F)	3		3			4	1	4	1	2	***7***	ACD
T045 (F)				±	±	±					***0***	mild ICD
T066 (F)											***0***	ACD
T071 (F)											***0***	
T074 (M)		±	±	±	±	±	±	±	±		***0***	
T089 (F)											***0***	mild ICD
T115 (F)											***0***	indist.
T141 (F)											***0***	mild ICD
T146 (F)											***0***	mild ICD
T161 (F)											***0***	mild ICD
T183 (M)	4	1	±	±		2	±	4	1	1	***6***	ACD
T184 (F)											***0***	mild ACD
T196 (F)											***0***	
T210 (F)											***0***	
T211 (M)											***0***	mild ICD
T216 (F)	6	6	6	6	6	6	6	6	6	6	***10***	ACD
T218 (F)											***0***	
no. reactors	**3**	**2**	**2**	**1**	**1**	**3**	**2**	**3**	**3**	**3**		
total score	**13**	**7**	**9**	**6**	**6**	**12**	**7**	**14**	**8**	**9**	**91**	
proportion (/18)	**0.7**	**0.4**	**0.5**	**0.3**	**0.3**	**0.7**	**0.4**	**0.8**	**0.4**	**0.5**	**5.1**	
TOTALS												
no. reactors	**4**	**2**	**3**	**1**	**1**	**3**	**2**	**4**	**3**	**4**		
total score	**14**	**7**	**10**	**6**	**6**	**12**	**7**	**15**	**8**	**10**	**95**	

Response type as determined for 100% oil (Tables 8 and 9). ICD = irritant contact dermatitis; ACD = allergic contact dermatitis; indist. = ICD and ACD indistinguishable

Table 12: Individual total ICDRG scores (2 readings) to 10% tea tree oil in secondary patch tests

Subject	**Tea tree oil (10%)**										***Oils reacted***	**Response**
(gender)	A	B	C	D	E	F	G	H	I	J	***to at 1-4 (n)***	**type**
Dropper bottle oils												
T014 (F)											***0***	ICD
T015 (F)											***0***	mild ICD
T052 (F)											***0***	indist.
T082 (F)											***0***	ICD
T088 (F)											***0***	mild ICD
T091 (F)											***0***	ICD
T092 (F)											***0***	indist.
T100 (M)											***0***	ICD
T101 (M)	?		?					?		?	***0***	ACD
T127 (M)											***0***	indist.
T128 (F)											***0***	ICD
T132 (F)											***0***	
T134 (M)											***0***	mild ICD
T176 (F)											***0***	mild ICD
T198 (M)											***0***	
no. reactors	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**		
total score	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	
proportion (/15)	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	
Stored oils												
T008 (F)											***0***	
T017 (F)	1		1			2	?	2	?	?	***4***	ACD
T045 (F)											***0***	mild ICD
T066 (F)											***0***	ACD
T071 (F)											***0***	
T074 (M)											***0***	
T089 (F)											***0***	mild ICD
T115 (F)											***0***	indist.
T141 (F)											***0***	mild ICD
T146 (F)											***0***	mild ICD
T161 (F)											***0***	mild ICD
T183 (M)	2	?				?		2	?	?	***2***	ACD
T184 (F)											***0***	mild ACD
T196 (F)											***0***	
T210 (F)											***0***	
T211 (M)											***0***	mild ICD
T216 (F)	4	4	4	4	4	4	4	4	4	4	***10***	ACD
T218 (F)											***0***	
no. reactors	**3**	**1**	**2**	**1**	**1**	**2**	**1**	**3**	**1**	**1**		
total score	**7**	**4**	**5**	**4**	**4**	**6**	**4**	**8**	**4**	**4**	**50**	
proportion (/18)	**0.4**	**0.2**	**0.3**	**0.2**	**0.2**	**0.3**	**0.2**	**0.4**	**0.2**	**0.2**	**2.8**	
TOTALS												
no. reactors	**3**	**1**	**2**	**1**	**1**	**2**	**1**	**3**	**1**	**1**		
total score	**7**	**4**	**5**	**4**	**4**	**6**	**4**	**8**	**4**	**4**	**50**	

Response type as determined for 100% oil (Tables 8 and 9). ICD = irritant contact dermatitis; ACD = allergic contact dermatitis; indist. = ICD and ACD indistinguishable

Table 13: Individual total MCLI scores (2 readings) to tea tree oil components in secondary patch tests

Subject	Component (key below)											*Reactions*	Response
(gender)	1	2	3	4	5	6	7	8	9	10	11	*at 1-4 (n)*	type
Dropper bottle oils													
T014 (F)								±				***0***	ICD
T015 (F)												***0***	mild ICD
T052 (F)												***0***	indist.
T082 (F)												***0***	ICD
T088 (F)												***0***	mild ICD
T091 (F)												***0***	ICD
T092 (F)			±	±	±						1	***1***	indist.
T100 (M)			±									***0***	ICD
T101 (M)												***0***	ACD
T127 (M)												***0***	indist.
T128 (F)												***0***	ICD
T132 (F)												***0***	
T134 (M)												***0***	mild ICD
T176 (F)												***0***	mild ICD
T198 (M)												***0***	
no. reactors	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**		
total score	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**1**	
proportion (/15)	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.1**	**0.1**	
Stored oils													
T008 (F)								±				***0***	
T017 (F)								1	1		±	***2***	ACD
T045 (F)				±								***0***	mild ICD
T066 (F)									6			***1***	ACD
T071 (F)												***0***	
T074 (M)			±	±			±	±		±	±	***0***	
T089 (F)									±			***0***	mild ICD
T115 (F)									3	±		***1***	indist.
T141 (F)												***0***	mild ICD
T146 (F)												***0***	mild ICD
T161 (F)												***0***	mild ICD
T183 (M)												***0***	ACD
T184 (F)												***0***	mild ACD
T196 (F)												***0***	
T210 (F)												***0***	
T211 (M)												***0***	mild ICD
T216 (F)					±				6			***1***	ACD
T218 (F)												***0***	
no. reactors	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**4**	**0**	**0**		
total score	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**16**	**0**	**0**	**17**	
proportion (/18)	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.1**	**0.9**	**0.0**	**0.0**	**0.9**	
TOTALS													
no. reactors	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**4**	**0**	**1**		
total score	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**16**	**0**	**1**	**18**	

Component key: 1) 1% α-phellandrene; 2) 5% α-pinene; 3) 25% γ-terpinene; 4) 10% α-terpinene; 5) 5% α-terpineol; 6) 5% 1.8-cineole; 7) 5% aromadendrene; 8) 1% limonene; 9) 5% *p*-cymene; 10) 40% terpinen-4-ol; 11) 1% viridiflorene (ledene)

Response type as determined for 100% oil (Tables 8 and 9). ICD = irritant contact dermatitis; ACD = allergic contact dermatitis; indist. = ICD and ACD indistinguishable

Table 14: Individual total ICDRG scores (2 readings) to tea tree oil components in secondary patch tests

Subject	Component (key below)											*Reactions*	Response
(gender)	1	2	3	4	5	6	7	8	9	10	11	*at 1-4 (n)*	type
Dropper bottle oils													
T014 (F)												***0***	ICD
T015 (F)												***0***	mild ICD
T052 (F)												***0***	indist.
T082 (F)												***0***	ICD
T088 (F)												***0***	mild ICD
T091 (F)												***0***	ICD
T092 (F)											?	***0***	indist.
T100 (M)												***0***	ICD
T101 (M)												***0***	ACD
T127 (M)												***0***	indist.
T128 (F)												***0***	ICD
T132 (F)												***0***	
T134 (M)												***0***	mild ICD
T176 (F)												***0***	mild ICD
T198 (M)												***0***	
no. reactors	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**		
total score	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	
proportion (/15)	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	
Stored oils													
T008 (F)												***0***	
T017 (F)								?	?			***0***	ACD
T045 (F)												***0***	mild ICD
T066 (F)									4			***1***	ACD
T071 (F)												***0***	
T074 (M)												***0***	
T089 (F)												***0***	mild ICD
T115 (F)									1			***1***	indist.
T141 (F)												***0***	mild ICD
T146 (F)												***0***	mild ICD
T161 (F)												***0***	mild ICD
T183 (M)												***0***	ACD
T184 (F)												***0***	mild ACD
T196 (F)												***0***	
T210 (F)												***0***	
T211 (M)												***0***	mild ICD
T216 (F)									4			***1***	ACD
T218 (F)												***0***	
no. reactors	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**3**	**0**	**0**		
total score	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**9**	**0**	**0**	**9**	
proportion (/18)	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.5**	**0.0**	**0.0**	**0.5**	
TOTALS													
no. reactors	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**3**	**0**	**0**		
total score	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**9**	**0**	**0**	**9**	

Component key: 1) 1% α-phellandrene; 2) 5% α-pinene; 3) 25% γ-terpinene; 4) 10% α-terpinene; 5) 5% α-terpineol; 6) 5% 1.8-cineole; 7) 5% aromadendrene; 8) 1% limonene; 9) 5% *p*-cymene; 10) 40% terpinen-4-ol; 11) 1% viridiflorene (ledene)

Response type as determined for 100% oil (Tables 8 and 9). ICD = irritant contact dermatitis; ACD = allergic contact dermatitis; indist. = ICD and ACD indistinguishable

The reason for using 10% tea tree oil was to assist with distinguishing between allergic and irritant reactions, as the former is far less concentration dependent than the latter. Therefore

it was expected that reactions would be elicited in most allergic individuals even with 10% oil, whereas irritant reactions would be unlikely to persist at such a low concentration. Allergic contact dermatitis (ACD) is also characterised by the reaction spreading beyond the area of substance contact, whereas irritant reactions are often sharply demarcated. All individuals classified as ACD reacted to all 10 samples of 100% oil and also reacted more mildly to at least some of the 10% tea tree oil samples when assessed on the MCLI scale. The only exception was one subject who did not react to all oils at 100% nor to any of the 10% oils samples, and was therefore classified as only mild ACD. Component testing resulted in very few reactions, even in individuals classified as allergic. The component that most frequently elicited a response was 5% *p*-cymene.

Milder reactions recorded on the MCLI scale were in some cases classified as irritant, or not even recorded, on the less stringent ICDRG scale. However, the final assessment of reaction type was consistent between both scales. A summary of the secondary test results is presented in Table 15. Mild ICD has been listed separately from ICD due to the responses being of much lesser clinical significance. The overall reaction rate (% total) has been calculated from a total of 208 subjects as it was deemed appropriate to exclude from the total those individuals who had exhibited some form of reaction to tea tree oil during primary testing but had not attended secondary testing. Relative reaction rates have been calculated for males and females using the group totals adjusted for the excluded individuals. A reaction rate for ACD and indistinguishable responses has also been calculated based on the number of subjects who reported positive pre-exposure to tea tree oil (% pre) as previous exposure is a necessary pre-requisite for ACD to develop, but is not for ICD. Whilst these rates have been calculated from the secondary test results, which means that all subjects had prior exposure due to the primary test, only one subject classified as ACD did not report definite previous exposure before primary testing, and appears to have been sensitised to tea tree oil.

Table 15: Summary of secondary patch test results – Frequency and type of contact dermatitis reactions resulting from exposure to tea tree oil

Classification		Female	Male	Total
ACD	n	4	2	6
	% of total	1.9	1.0	2.9
	% of pre-exposed	3.1	1.5	4.6

	% of gender total	3.1	2.5	
	% of gender pre-exposed	4.1	5.9	
indistinguishable	n	3	1	4
	% of total	1.4	0.5	1.9
	% of pre-exposed	2.3	0.8	3.1
	% of gender total	2.4	1.2	
	% of gender pre-exposed	3.1	2.9	
ICD	n	4	1	5
	% of total	1.9	0.5	2.1
	% of gender total	3.1	1.2	
Mild ICD	n	9	1	10
	% of total	4.3	0.5	4.8
	% of gender total	7.1	1.2	
Not CD	n	6	2	8
	% of total	2.9	1.0	3.8
	% of gender total	4.7	2.5	

ICD = irritant contact dermatitis; ACD = allergic contact dermatitis; indist. = ICD and ACD indistinguishable; not CD = not contact dermatits (i.e. no reaction)

Within this sample population of volunteers drawn from the general community, the rate of allergic contact dermatitis to tea tree oil is 2.9% ACD plus a possible 1.9% which were indistinguishable, to give a range of 2.9-4.8%. The rate of marked ICD is 2.4-4.3%, again with a 1.9% cross-over between these ACD and ICD that was unable to be classified. The total prevalence of both mild and marked ICD was 7.2-10.1%. When the need for prior exposure to tea tree oil in order to elicit ACD is taken into account for definite or possible allergic reactions, the prevalence becomes 4.6-7.6% in this subsection of the sample population.

The contribution of females to the response rates was slightly higher than males, with 3.1-5.5% of women compared to 2.5-3.7% of men experiencing ACD, and similarly marked ICD occurring in 3.1-5.5% compared to 1.2-2.5%, respectively. Yet when calculations took into account pre-exposure, the prevalence of ACD in females was lower at 4.1-7.2% compared to a rate in males of 5.9-8.8%. Total (marked and mild) ICD rates were far higher in females at 10.2-12.6% where the male rate was 2.5-3.7%.

5 Discussion

5.1 Subjects

The sample population tested for this project constituted healthy volunteers drawn from the local community. It is common with such volunteer-based studies to have a greater proportion of women, and thus it was neither unexpected, nor considered a hindrance, that our male:female ratio was 2:3 (Table 1). While 77% of the women were certain of having previously come into contact with tea tree oil in some form, only 42% of the male volunteers gave a positive response to this question. This was also expected, as a recent survey of attendees at a Sydney hospital emergency department showed that 60.9% of females but only 43.8% of males used alternative medicines [32]. Tea tree oil was the most commonly used preparation by this population (13.3% of preparations), but even then was not used by the majority of those surveyed. Therefore it is not unreasonable to suggest that our study population had a higher usage of tea tree oil products (63%) than would be expected in the wider Australian population. Because recruitment was on a voluntary basis it is not surprising that those who were interested in participating were also likely to have used tea tree oil products. Tea tree oil is now found in a vast array of products and it is possible that many individuals are unaware of their exposure to it (a number of our volunteers indicated that they were unsure). For example, exposure may occur outside of the home or work environment, such as use of a tea tree oil soap or handwash provided in a restaurant restroom. Thus it is difficult to accurately gauge the level of exposure to tea tree oil in the population at large.

5.2 Sensitivity tests

5.2.1 Type I Reactions: The Prick Test

The prick test results for the standard series of common allergens (Table 3) indicated that the subject population was quite atopic, with responses to dust mite and rye grass nearing 50%. The prevalence of positive allergen prick tests from two other regions in this state were reported as 22-30% and 27-28%, respectively, for these allergens, and 11-12% for *Alternaria* (36% here) and 10-19% for cat hair (26% here) [33]. There is geographical variation in the prevalence of airborne allergens, and therefore in the rate of allergen reactivity, however, this is not considered sufficient to have caused the much higher rate of

reactivity in our study population. It is possible that these outcomes stem from the "self-selection" of our volunteers, at least some of whom are known to have attended due to a desire to know more about their suspected allergies.

In light of our sample population being more responsive to airborne allergens than would be expected from the wider population, it is perhaps even more significant that so few reactions occurred in response to challenge with 100% tea tree oil. Only four individuals responded to tea tree oil, and these responses were elicited by only one or two oils (A and B) rather than by all 10 oils being tested. No factor unique to these two oils alone was apparent from the analysis of the oil samples. It is therefore unclear why only oils A and B caused reactions. However, it is clear that tea tree oil is very unlikely to cause a type I allergic reaction.

5.2.2 Type IV Reactions: The Patch Test

It has been reported that contact sensitisation (indicated by the patch test) appears to be independent of enhanced IgE responsiveness (indicated by the prick test) when assessed in an unselected adult population [34]. Therefore, the fact that our study population exhibited a higher prevalence of airborne allergen reactivity than expected does not allow any prediction of likely sensitivity to contact allergens. Hence it was necessary to determine the contact sensitivity of our population using the European standard series of 23 common contact allergens.

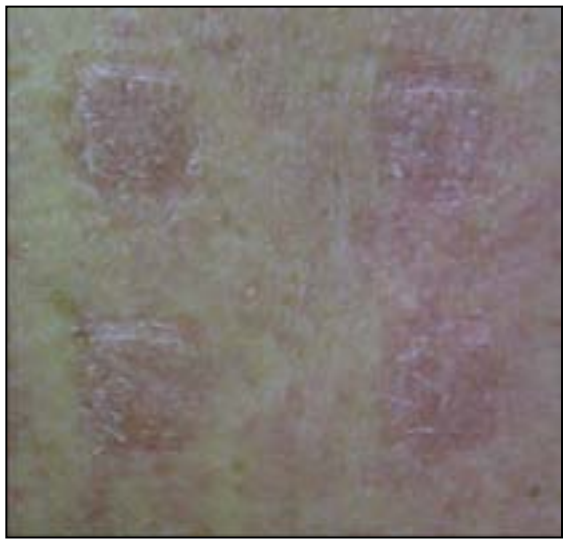
The frequency of sensitivity to a number of the more common contact allergens reported in other studies is listed below as a guide to the relative reactivity of our study population. It should be noted that all of these studies involved patients attending dermatology clinics, except for the first which tested a large unselected population.

Study reference	**This study**	[34]	[35]	[36]	[37]	[38]	[39]
Allergen	**Frequency of sensitivity (%)**						
Nickel sulfate	**20.5**	6.7	9.7	14.8	39.5	12.9	14.3
Fragrance mix	**4.1**	1.1		7.7	14.5	10.5	14
Balsam of Peru	**2.3**	1.1	3.3	5.6	9.2	7.3	10.4
Potassium dichromate	**9.1**	0.5	5.2	5.1	32.9	4.5	2

Our study population appears to be more reactive to contact allergens than might be expected, based on these other reports and the fact that they were selected as healthy individuals. This might stem from the "self-selection" aspect of a volunteer-based study, but may also involve other geographical factors such as extent of exposure to particular allergens, and weather differences compared to other study sites (note that most of this study was performed during the Perth winter). One study indicated that the frequency of strongly positive reactions to only two allergens (nickel and thimerosal) decreased during the central European summer [40], suggesting that seasonal variability may be present but have minimal effect in most cases.

The primary patch testing with tea tree oil (Table 5) indicated that there is a subsection of the population who will experience contact dermatitis to some extent following prolonged occluded exposure to 100% oil. It is important to note that amongst the subjects who were not classified as "reactive", alterations to the surface of the skin were observed in the majority of cases (Figure 4). The appearance matched that described as glistening of the stratum corneum by Wahlberg [41] and classified as an irritant reaction. As there was no erythema it was not classified on the primary tests using the patch test MCLI scoring scale, but was noted in many cases. The penetrative property of pure tea tree oil may have the ability to alter dermal integrity, and should be further investigated.

Figure 4: Alteration to the surface of the skin caused by 100% tea tree oil: a) glistening of the stratum corneum occurred on many subjects; b) more marked drying of the skin surface was visible on some skin types.

(4a)  (4b) 

While there were a number of subjects who did not respond consistently to all oils during both primary (Tables 6 and 7) and secondary (Tables 8 and 9) testing, assessing the MCLI scores of the reactive population for each of the 10 oils provided no indication of a difference between oils. However, there was a difference between the entire group of 10 oils after different levels of usage and methods of storage. The first indication of this was the unexpectedly high number of marked irritant reactions in the first group of subjects to undergo secondary testing using the "dropper bottle oils" which had been used throughout the study. As the remaining subjects undergoing secondary testing were tested using the original "stored oils", the two groups were able to be compared and the suspected disproportionate contribution of the dropper bottle oils to irritant reactions was demonstrated. A further indication of an alteration to the oils was shown by the markedly different responses to the two series of oils after patch testing one previously non-responsive individual (Figure 3), although retesting of another three non-responders did not elicit such a clear difference. Comparative GC analysis of the two series of oils (Table 10) confirmed that significant oxidation of the oil had occurred. While this had not been expected as the oils were stored in sealed brown bottles at 4°C, with hindsight it is perhaps not surprising as the bottles were opened hundreds of times and the oil aspirated into the dropper for administration. At the end of the glass dropper was a rubber teat to facilitate aspiration. The rubber was not visibly degraded, however, it remains possible that some degradation may have occurred.

Secondary testing with 10% preparations of the 10 samples of tea tree oil (Tables 11 and 12) resulted in only four assessable responses on the MCLI scale (and three on the ICDRG scale), all of which were ACD. This translates to a reaction rate in the sample population (excluding those potential reactors who did not attend secondary testing) of 1.9%, which is lower than for 100% tea tree oil (2.9%) where ICD reactions were also evident. This is important information because many tea tree oil products contain 10% oil or less, and are safer to use. Other studies have utilised lower concentrations of tea tree oil in patch testing. Tea tree oil at 1 or 5% has not elicited responses in volunteers [22, 42, 43], yet has caused reactions in diagnostic patch tests on patients suspicious of tea tree oil contributing to their dermatitis [15, 22, 44]. Many of these patients reported dermatitis following use of 100%

oil [13, 15]. In a sensitisation trial with 25% tea tree oil, three of 28 subjects were excluded due to allergic reactions [19, 29].

Testing with 10% oil was useful in distinguishing between irritant and allergic reactions, with all subjects (bar one) classified as ACD exhibiting some response to 10% oil. Confirmation of the nature of a reaction was also often possible by assessing the demarcation of the response, as allergic reactions will often spread beyond the area of direct substance contact. While allergic reactions are far less concentration dependent than irritant reactions [45], it was interesting to find that one subject who was clearly allergic regularly used a shaving cream that contained tea tree oil at 2%. This product had been used prior to participation in the trial, and use had continued during both primary and secondary tests without any adverse effect. There are a variety of possible explanations for this, including the subject having a response concentration threshold between 2 and 10%. Further work is necessary to fully understand the implications of this.

Secondary patch testing to tea tree oil components at concentrations approximating those found in pure oil elicited fewer responses than might have been expected, particularly in individuals classified as allergic (Tables 13 and 14). There were a number of questionable reactions to components, however, as the responses were not sufficiently clear to grade, it would not be appropriate to give any weight to "possible" reactions.

Viridiflorene (ledene) at 1% (ISO range 0.5-6.5, mean in our 10 samples on initial testing 1.2) and limonene at 1% (ISO range 0.5-4.0, our mean 1.2) both caused erythema (MCLI level 1 response) in two different individuals. One of these individuals also exhibited erythema to *p*-cymene at 5% (ISO range 0.5-12, our mean 3.3). Three other subjects also responded to *p*-cymene at higher levels on the grading scales. All of these individuals were classified as exhibiting either allergic or indistinguishable reactions to tea tree oil.

Various components have been implicated in adverse reactions to tea tree oil, including 1,8-cineole [13], d-limonene, α-terpinene, aromadendrene, terpinen-4-ol, *p*-cymene, α-phellandrene, α-pinene, terpinolene [15] and α-terpinene [19, 29]. The latter authors also elicited responses to sesquiterpenoid fractions of tea tree oil. While some of these

components are the same as those that elicited reactions in this trial, there are a number of other components listed that were not responded to by our subjects. This may purely be due to the uniqueness of each individual, but may also be affected by differences in methodology such as component concentration and the diluent used. The study implicating a large number of components [15] tested them at 1% in anhydrous ethanol which might evaporate, rendering the component concentration higher than intended. We and others [13, 29] preferred to use a white paraffin preparation to avoid this possibility. For some years 1,8-cineole was regarded as a cause of irritation, however, this suggestion has been shown to be unfounded in more recent times [13, 15, 46].

The component showing the largest percentage change in the dropper bottle oils compared to the stored oils was *p*-cymene (84% increase), which was also the component that elicited the majority of reactions. The changes in viridiflorene (12.4% decrease) and limonene (11.6% increase) were significant but not remarkably large. It is interesting to note, however, that more of the ACD reactions occurred in the group tested with the stored oils, and more of the ICD reactions occurred in the group tested with dropper bottle oils. This would imply that the oxidation of the oils that was shown in Table 10 did not affect the ability of the oils to elicit allergy, but markedly enhanced their capacity to cause irritant reactions. Yet only one of the responders to *p*-cymene was not classified as ACD, and even then was considered indistinguishable rather than ICD. The components tested in this study constitute ~85% of tea tree oil. Since few reactions were elicited, it is possible that one of the minor components that was not tested plays some role, or there may be an additive or synergistic effect when two or more components are combined. Further work is necessary to understand the contribution of various components to both ICD and ACD.

Marked ICD occurred in 2.4-4.3% of our sample population, noting that there is a 1.9% cross-over between ACD and ICD that was unable to be classified. The total prevalence of both mild and marked ICD was 7.2-10.1%. As the occurrence of ICD does not require prior exposure to a substance, these rates can be considered independent of exposure rates and therefore applicable to the general population. However, the oxidation of the oils during the project (Table 10) is likely to have contributed to an elevated prevalence of ICD, and also has implications for the use of aged oil by consumers. Mild ICD resulting from 48 hour

occluded exposure to 100% tea tree oil is unlikely to occur with normal usage, and hence is not considered clinically important. Marked reactions under these test conditions indicate the possibility of adverse reaction with normal usage, and therefore must be noted.

The prevalence of ACD to tea tree oil in this sample population of volunteers drawn from the general community was 2.9-4.8% (Table 15). A 63% pre-exposure rate amongst our sample population is considered higher than might be expected from a random population sample, and therefore the prevalence of ACD in the community is likely to be lower than found here. When considering only those previously exposed to tea tree oil, this rate increases to 4.3-7.2%. Assuming that the proportion of the community using tea tree oil containing products will increase as markets are expanded, this figure provides important information regarding the likely maximal rates of ACD.

Gender differences in reaction rates to a variety of contact allergens are regularly reported. While the specific allergen dictates which sex exhibits the greater response rate, females seem to be more likely overall to suffer ACD [37, 38, 47]. It is perhaps not surprising therefore that a greater percentage of the female subgroup (3.1-5.5%) than the male subgroup (2.5-3.7%) experienced ACD to tea tree oil. Yet adjusting within each gender for prior exposure to tea tree oil resulted in the prevalence of ACD in pre-exposed females (4.1-7.2%) being lower than in pre-exposed males (5.9-8.8%). This difference is not large, but suggests that males might be more susceptible to sensitisation. The sample size is too small to determine whether there is any foundation to such an idea.

Females also appeared to be more sensitive to the irritating potential of 100% tea tree oil, with 3.1-5.5% experiencing marked ICD compared to 1.2-2.5% of the male subgroup. When considering all (both marked and mild) irritant reactions, rates in females were far higher (10.2-12.6%) than in males (2.5-3.7%). It would seem that the skin of women is more likely than that of men to react adversely to 100% oil, whether it be a mild or marked reaction that occurs.

As tea tree oil is a topical antimicrobial, the prevalence of allergy to it should be compared to other topical antimicrobials. Neomycin sulfate is a topical antibiotic included in the

European standard series because it is a known potential contact allergen. It elicited a reaction in 2.8% of our sample, and has been reported elsewhere to cause reactions in up to 11.6% of subjects (both unselected and dermatology patients). Reactions to bacitracin ointment have been reported in 0.9-9.1% of those tested, and thimerosal elicited responses in 3.4-6.2% of subjects [34, 35, 37-39, 48, 49]. Based on a calculated maximal rate of ACD of approximately 7.2%, and a probable rate far lower, the prevalence of reactions to tea tree oil compares quite reasonably with other topical antimicrobials in common use.

6 Implications and Recommendations

6.1 Implications

The potential impact of this work on the tea tree oil industry in Australia is that of a surer standing in terms of the safety of the product being marketed. Previously there was anecdotal evidence that tea tree oil was safe stemming from the long history of use, together with limited toxicity and other safety study results which were generally known only within the confines of the industry. This project has produced extensive information showing the frequency of adverse reactions in the general population, and provided an indication of the type and extent of reactions.

This knowledge strengthens the case for registration of tea tree oil as a safe topical antimicrobial agent with regulatory bodies such as the FDA in the USA. The information will also allow marketing of the product with appropriate recommendations for use that will be suitable to most individuals. Information on products indicating that a small proportion of individuals may experience adverse effects would be appropriate to ensure that consumers are provided with adequate warning of such possibilities.

6.2 Recommendations

This work shows that while allergic contact reactions to tea tree oil are relatively infrequent, they do still occur in some individuals, and therefore it would be beneficial to consumers for the possibility of such a reaction to be indicated on product labelling.

In addition to allergic responses following contact with tea tree oil, there is a proportion of individuals who may experience irritated skin following prolonged exposure to tea tree oil. It is possible that this may be avoided by decreasing the level of exposure with regard to both time and concentration of the oil. Whilst a significant problem occurred in a limited number of subjects, the majority of people displayed some alteration to the surface of the skin after exposure to 100% tea tree oil for 48 hours. In light of other work that indicates that tea tree oil is active against microorganisms at concentrations far below 100%, it seems reasonable to suggest that use of 100% oil is unnecessary for therapeutic purposes. Use of a tea tree oil product that contains a clinically useful concentration of the oil, rather than pure

oil, would vastly reduce the possibility of eliciting an irritant response in most cases. The value to the tea tree oil industry of enhanced production and marketing of tea tree oil products, rather than sales of pure oil only, has enormous potential. In addition, the benefits to consumers of effective and safer products may well lead to expansion of the market.

These results showing safety levels comparative to other topical antimicrobials, together with scientific evidence of antimicrobial efficacy, indicate that clinical trials are now essential to strengthen the position of tea tree oil in international markets.

7 Communications Strategy

One of the aims of this project was to disseminate the results to the industry, as well as the biomedical community and the wider population.

The Australian tea tree industry has been made aware of our progress and initial results via RIRDC publications in both hard copy and on the internet, and will have access to the published final report. ATTIA has also been very supportive of the work, and will disseminate our findings to its members. Preliminary results have been presented to various international industry groups by T V Riley and C F Carson.

Community awareness of tea tree oil was initially raised during the recruitment phase of the project as a result of articles in local print media, including the Claremont Nedlands POST, the Community Newspapers News Chronicle, the UWA Leader, and the SCGH Newsletter. As hundreds of volunteers were involved in the study, this alone probably resulted in increased awareness of tea tree oil in numerous acquaintances. In addition, J E Greig was interviewed on TAFE Magazine by Kathy Pickup, which was broadcast live on Golden West Network television. Feedback from this program was good.

In addition to the above communications strategies that were part of this RIRDC project, an article on the current safety and toxicity data for tea tree oil was published by our group. The details of this Leading Article are as follows:
Carson CF, Riley TV, Cookson BD. (1998) Efficacy and safety of tea tree oil as a topical antimicrobial agent. *Journal of Hospital Infection* **40:** 175-178.

Finally, the results of this project are being prepared for publication in international peer-reviewed medical journals. The journals selected will be those that are listed on widely used literature databases, thus providing extensive exposure of the information. In addition, opportunities will be sought to present the information at international scientific meetings in the near future.

8 Appendix

Pesticide screening

The analysis of tea tree oil by the NSW Agriculture Chemical Residues Laboratory screened for the following pesticides:

Organochlorines (OCs)

chlordane	o,p-DDD	endosulfan sulphate
dieldrin	p,p-DDE	endrin
p,p-DDT	o,p-DDE	heptachlor
o,p-DDT	α-endosulfan	heptachlor epoxide
p,p-DDD	β-endosulfan	lindane

Organophosphates (OPs)

carbophenothion	fenitrothion	monocrotophos
chlorfenvinphos	fensulphothion	omethoate
chlorpyrifos	fenthion	parathion
chlorpyrifos methyl	guthion	phosmet
diazinon	iodofenphos	pirimiphos methyl
dichlorvos	malathion	profenofos
dimethoate	methacrifos	prothiofos
fenamiphos	methidathion	sulprofos
fenchlorphos		

Synthetic Pyrethroids (SPs)

cyfluthrin	cypermethrin	fenvalerate
cyhalothrin	deltamethrin	

The minimum reporting level for OCs and SPs is 0.3mg/ml, and for OPs is 0.1mg/ml. No oil sample contained detectable levels of any of these pesticides.

Metal analysis of tea tree oil samples (values only shown if above detection limit)

Element	full name	detection limit	Tea tree oil									
			A	B	C	D	E	F	G	H	I	J
Sb (µg/ml)	antimony	0.05										
As (µg/ml)	arsenic	0.50										
Ba (µg/ml)	barium	20.0										
Br (µg/ml)	bromine	0.50		1.50								
Cd (mg/kg)	cadium	0.05			0.08		0.05					0.06
Ca (µg/ml)	calcium	500.00										
Ce µg/ml)	cerium	0.50										
Cs (µg/ml)	caesium	0.10										
Cr (µg/ml)	chromium	0.5										
Co (µg/ml)	cobalt	0.10										
Cu (mg/kg)	copper	0.5	0.7	0.6	1.3	2.1	0.8	0.7	0.9	0.7	1	0.6
Eu (µg/ml)	europium	0.05										
Au (ng/ml)	gold	1.0					2.5					
Hf (µg/ml)	hafnium	0.05										
Ir (ng/ml)	iridium	2.0										
Fe (µg/ml)	iron	50.00										
La (µg/ml)	lanthanum	0.05										
Pb (mg/kg)	lead	0.5			0.7	0.7		0.7			0.8	
Lu (µg/ml)	lutetium	0.01										
Hg (µg/ml)	mercury	0.2										
Mo (µg/ml)	molybdenum	1.0										
Ni (mg/kg)	nickel	0.5	0.5		0.6							
K (%)	potassium	0.20										
Rb (µg/ml)	rubidium	2.0										
Sm (µg/ml)	samarium	0.02										
Sc (µg/ml)	scandium	0.01										
Se (µg/ml)	selenium	1.0										
Ag (µg/ml)	silver	0.5										
Na (µg/ml)	sodium	50.000										
Ta (µg/ml)	tantalum	0.10										
Te (µg/ml)	tellurium	0.5										
Th (µg/ml)	thorium	0.50										
Sn (µg/ml)	tin	50.0										
W (µg/ml)	tungsten	0.50										
U (µg/ml)	uranium	0.50										
Yb (µg/ml)	ytterbium	0.02										
Zn (mg/kg)	zinc	0.5	1.7	2.8	4.1	7.3	<0.5	1.1	<0.5	0.9	<0.5	1.3
Zr (µg/ml)	zirconium	20.0										

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