RIRDC Completed Projects in 2006 - 2007 and Research in Progress as at June 2007

TEA TREE OIL

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RIRDC Completed Projects for the Tea Tree Oil Program in 2006-07 and Research in Progress as at June 2007
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Foreword

This *Research in Progress, June 2007*, contains short summaries of continuing projects as well as those that were completed during 2006-2007 for RIRDC's Tea Tree Oil Sub-program. This program aims to support the continued development of an environmentally sustainable and profitable Australian tea tree oil industry that has established international leadership in marketing, value-adding, product reliability and production.

The complete report on all the programs is only available in electronic format on our website at http://www.rirdc.gov.au

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Peter O'Brien
Managing Director
Rural Industries Research and Development Corporation

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COMPLETED PROJECTS − To improve existing products and develop new ones

Project Title:	**Fabrication of electronic materials from the Australian essential oils**
RIRDC Project No.:	UJC-13A
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Objectives	1. Fabricate high quality plasma polymerised thin films from essential oils available in Australia. 2. Procure an Ellipsometer and study the optical properties of the fabricated thin films to find suitable applications. 3. Fabrication of thin film and study the semi-conducting properties.
Background	More than 60 billion pounds of polymer products is used per year only in USA, which includes the polymers like Polyethylene. The total polymer industry around the world is worth billions of dollars and employs several million people. Most of the commercially available polymers are made out of chemicals and are not biodegradable, and are hazardous to the environment. The aim of this research is to fabricate polymers from natural resources and hence adding value to our resources.
Research	A plasma polymerisation unit has been fabricated and installed to develop polymer thin films from essential oils. The deposition parameters were changed to optimise the thin film properties. Different essentials oils were tested to find the feasibility of polymerisation. a-Pinene and d-Limonene and tea tree oil were used as monomers for fabrication of the thin films. The Atomic Force Microscope was used to study the surface properties and found that very smooth films can be obtained from tea tree oil. We have also tried different substrate materials. An ellipsometer was ordered to study the optical properties. We have installed an electrical characterisation set up for the measurement of electrical properties.
Outcomes	Even though we could polymerise the monomers, the quality of the thin film was different. Some of the cheapest essential oils like a-pinene and d-Limonene could produce polymer thin films under ideal conditions but the roughness of the surface was very high. The optical properties of the thin films derived from Tea Tree Oil was studied and found that the refractive index of the thin film is close to that of glass. A setup is made ready for electrical characterisation.
Implications	The properties of the thin polymer films show that they are potential candidates for electrical and biomedical applications. More tests are under progress to study the behaviour of the films under ideal and varying physical and environmental conditions. Initial studies show a biodegradable nature of the fabricated thin film.
Publications	We are waiting for more results before communicating the results in the public domain.

COMPLETED PROJECTS − To enhance the ability of the industry to provide products that meet appropriate safety and efficacy

Project Title	Preparation of SCCP submission for TTO-Stage 1
RIRDC Project No.:	CIN-1A
Researcher: Organisation: Phone: Fax: Email:	Mr John Issa Cintox Pty Ltd PO Box 168 SUMMER HILL NSW 2130. (02) 9705 9909 (02) 9705 9919 johnissa@cintox.com.au
Objectives	To prepare a safety dossier on tea tree oil for submission to the Scientific Committee on Consumer Products (SCCP) in conjunction with the tea tree oil industry.
Background	The European SCCP (Scientific Committee on Consumer Products) recently concluded that insufficient data was available on the safety and stability of tea tree oil. The industry is addressing this situation by commissioning approved tests in order to obtain a more complete safety dossier on tea tree oil.
Research	A comprehensive review of the available scientific literature on the toxicity of tea tree oil and its main constituents has been conducted. This review was conducted independently by Dr Jesper Bo Nielsen, Associate Professor, Institute of Public Health, Environmental Medicine, University of Southern Denmark. Several literature studies, not previously considered by the SCCP, have been identified and presented in this dossier. Additional new studies have been either conducted by ATTIA/RIRDC or have been contributed by individual companies in response to the SCCP request: • Percutaneous absorption • Mouse micronucleus assay • Mouse Local Lymph Node Assays (LLNA) • Serial dilution patch test and Repeated Open Application Test (ROAT) • Predictive testing for irritancy and allergenicity of tea tree oil in normal subjects (Aspres and Freeman 2003) • In-use stability study • Method for the characterisation of tea tree oil and determination of peroxide levels and p-cymene as markers for oil degradation status in neat oil and in formulated products • Stability of formulated products • Peroxide levels of retained samples • Company adverse event data

Outcomes	A current safety dossier has been prepared jointly by the Australian Tea Tree Oil Industry Association (ATTIA) and the Australian Government's Rural Industries Research and Development Corporation (RIRDC) and been submitted to the SCCP
Implications	Overall, the safety of tea tree oil to human health has been clearly demonstrated by many years of use in a wide range of products. Company adverse event reporting data show that there is no epidemic associated with tea tree oil use. Exposure of tea tree oil to air and light results in oxidation of some of its components. These oxidation products increase the toxicity profile of tea tree oil. While tea tree oil is a weak skin sensitiser, oxidised tea tree oil has a much greater propensity to cause skin sensitisation. Thus, the use of oxidised tea tree oil should be avoided. When used and stored properly, tea tree oil is not expected to pose a risk to human health. The Australian Tea Tree Oil Industry Association has developed a Code of Practice and Guidelines to ensure the quality of tea tree oil supplied to the market. Similarly, advice to consumers on the proper storage and use of tea oil products and use-by dates after opening will also help to ensure that consumers will not be using degraded tea tree oil. The scientific testing undertaken in the preparation of this submission will be of economic benefit to the tea tree oil industry as a more acceptable oil will lead to more sales as a direct result of the safety and stability of the oil being better defined.
Publications	Submission to the Scientific Committee on Consumer Products (SCCP) - Toxicological summary and safety assessment for tea tree oil: A joint submission made by the Australian Tea Tree Industry Association and the Rural Industries Research & Development Corporation. A summary booklet outlining the major findings in the above submission is currently under development.

COMPLETED PROJECTS − To enhance the ability of the industry to provide products that meet appropriate safety and efficacy

Project Title	Skin sensitisation: Local lymph node assay
RIRDC Project No.:	GUI-1A
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Objectives	To undertake a Local Lymph Node Assay in Mice (OECD429) to identify the contact allergenic potential of tea tree oil in order to provide a rational basis for risk assessment to the sensitising potential of tea tree oil to humans.
Background	The European SCCP (Scientific Committee on Consumer Products) recently concluded that insufficient data was available on the safety and stability of tea tree oil. The industry is addressing this situation by commissioning approved tests in order to obtain a more complete safety dossier on tea tree oil.
Research	In order to study a possible contact allergenic potential of tea tree oil, a number of LLNA tests were undertaken in accredited laboratories in Europe and the United States of America. Concentrations of 5%, 25% and 50% of tea tree oil were tested in the LLNA assay. Topical applications at 25% and 50% in PEG 400 resulted in a stimulation index greater than three which classifies tea tree oil as an extremely weak sensitiser at those concentrations.
Outcomes	While tea tree oil has been shown to be an extremely weak skin sensitiser, oxidised tea tree oil has a much greater propensity to cause skin sensitisation. Thus, the use of oxidised tea tree oil should be avoided. When used and stored properly, tea tree oil is not expected to pose a risk to human health.
Implications	The scientific testing undertaken in the preparation of this submission will be of economic benefit to the tea tree oil industry as a more acceptable oil will lead to more sales as a direct result of the safety and stability of the oil being better defined.
Publications	Submission to the Scientific Committee on Consumer Products (ACCP) - Toxicological summary and safety assessment for tea tree oil: A joint submission made by the Australian Tea Tree Industry Association and the Rural Industries Research & Development Corporation. A summary booklet outlining the major findings in the above submission is currently under development.

COMPLETED PROJECTS − To enhance the ability of the industry to provide products that meet appropriate safety and efficacy

Project Title:	**Stability testing of tea tree oil**
RIRDC Project No.:	USC-9A
Researcher:	Associate Professor David Leach
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Objectives	The objective of this study is to determine the changes in tea tree oil composition and peroxide value over 12 months under simulated in-use conditions. The proposed study protocol is based on principles outlined by the EMEA (European Agency for the Evaluation of Medicinal Products) in 'Guidance On In-Use Stability of Human Medicinal Products'. Analysis methods are based on authoritative European reference texts and should be acceptable to the SCCP and related relevant European authorities.
Background	Tea tree products are successfully marketed abroad and have become popular in Europe and North America. With this popularity has come increased scrutiny. Some tea tree oil products have been shown anecdotally and scientifically to cause dermal irritation and allergic sensitisation. Previous studies have indicated that intermediate peroxides formed by the oxidation of specific monoterpenes resulting in dimers and ultimately monoterpene polymers which crystallise, making the degraded oil yellow and viscous. The degraded tea tree oils are shown to have very high peroxide values. Tea tree oil, like all essential oils, is a blend of different, but closely related molecules called monoterpenes and sesquiterpenes. However, approximately 75% of the oil is comprised of only 4 molecules: a-terpinene (10%); γ-terpinene (20%); terpinen-4-ol (40%); p-cymene (1 – 3%); and 1,8-cineole (3%). In the presence of oxygen monoterpenes gradually oxidise forming peroxyl radicals.
Research	Oil of *Melaleuca,* terpinen-4-ol type (tea tree oil) from two separate production batches (60175 and 60176) supplied to CPP by TP Health Ltd., Ballina, Australia. The oil samples conform to the definition and identification/quality profile of the European Pharmacopoeia (EP) monograph 04/2002:1837 'Tea Tree Oil – Melaleuca aetheroleum' and ISO 4730 'Oil of *Melaleuca*, terpinen-4-ol type (Tea Tree oil). Tea tree oil was stored in 100 mL capped (airtight), amber glass bottles completely filled (no initial air space), and stored at room temperature (25°C) in a suitable space away from heat sources and light. At the start of the study the bottles were removed from the storage cabinet and 0.15 mL and 6.17 mL of tea tree oil for GC analysis and peroxide value determination were withdrawn from each bottle which was then recapped after a total exposure to air for 1 minute and returned to the cabinet. Every seven days for three weeks each bottle was removed from the storage cabinet to an area within the laboratory illuminated by normal room lighting and opened for the duration of 1 minute while 0.5 mL oil was removed to simulate ongoing regular product usage before the bottle was tightly recapped and returned

	to the cabinet. On the 28th day only the 0.15 mL and 6.17mL aliquots of oil were removed from each bottle for testing and each bottle remained uncapped for 1 minute before being sealed again and returned to the dark cabinet. This procedure was repeated for the next 11 months. The analysis test results over the specified 12 x 28 day periods provide a compositional profile and information with respect to changes in peroxide value of tea tree oil under a defined simulated in-use regime.
Outcomes	The results from the in-use stability testing of the two tea tree oil batches indicated that both samples remained relatively stable over the 12 month test period. There was a gradual increase in peroxide value over the 12 month period from 2 to 9 meqO_2/kg. The major components of tea tree oil remained stable for 6 months after which time downward trends in α-terpinene and γ-terpinene and an upward trend in p-cymene were observed. There was no significant change in the minor tea tree oil components over the 12 month period. At the end of the 12 month storage period both batches of oil still conformed to monograph specifications for tea tree oil.
Implications	This in-use stability study confirmed that neat tea tree oil is stable for 12 months and therefore presents no additional health hazards.
Publications	RIRDC Final report.

COMPLETED PROJECTS - To establish production systems that are both ecologically sustainable and profitable

Project Title:	**Breeding and cloning tea tree for greater profitability**
RIRDC Project No.:	DAN-199A
Researcher:	Dr Trevor Olesen
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Objectives	To improve the profitability of the tea tree industry by production and distribution of highly improved seed and selected clones.
Background	The project builds on two earlier RIRDC/ATTIA tea tree breeding projects (1993-1996; 1996-2000) that substantially improved the quality of seed available to industry.
Research	The main breeding strategy is based on establishing large trials which, after culling inferior trees, are converted to orchards to supply genetically improved seed. Complementing this, interim measures (best bush seed and a clonal seed orchard) have also delivered genetically improved seed to industry.
Outcomes	Oil yield from project seed has been progressively increased from 148 kg/ha at the beginning of the breeding program to c. 250 kg/ha, equivalent to a gain of 70% in 12 years. Desirable changes in oil quality have also been achieved in parallel with the increase in yield. Even greater gains are expected from elite clonal selections, which are currently being field assessed, and through improved seed from the project's seed orchard program.
Implications	Adoption of project seed would result in improved profitability to the industry equivalent to c. $4M at current prices and costs, increasing the long-term viability of the Australian tea tree industry.
Publications	Doran JC, Baker GR, Williams ER, Southwell IS (2006) Genetic gains in oil yields after nine years of breeding *Melaleuca alternifolia* (Myrtaceae). Aust. J. Exp. Ag. **46**, 1521-1527.

RIRDC RESEARCH IN PROGRESS − To improve existing products and develop new ones

Project Title	Tea tree oil to prevent staphylococcal infections in dialysis patients
RIRDC Project No.:	UWA-100A
Start Date:	06/11/06
Finish Date:	10/11/08
Researcher:	Professor Thomas V. Riley
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Objectives	1. To demonstrate that tea tree oil products are efficacious in the prevention of staphylococcal infections associated with dialysis catheters. 2. To demonstrate that tea tree oil products are a suitable alternative to existing products used to prevent staphylococcal infections associated with dialysis catheters. 3. To provide clinical data on the efficacy and safety of tea tree oil products.
Current Progress	The protocol, information sheet and consent form were considered by the Clinical Drugs Trials Committee (CDTC) of Sir Charles Gairdner Hospital on 19th April 2007. They had some minor revisions and these were accepted by the Chair of the committee, Associate Prof. D A Joyce on 22 June, 2007. The application to the Human Research Ethics Committee (HREC) was considered at their meeting on the 8th May 2007. The Committee raised the question of gynecomastia and tea tree oil and the chief investigator Dr Brian Hutchison was asked to comment. A reply was drafted and sent to the HREC on 6th June. Dr Hutchison is awaiting their reply and approval for the trial to commence. The Research Governance Unit (RGU) of Sir Charles Gairdner Hospital has recently been instituted and now oversees the standards of research at the hospital. A meeting was held with their representative on 2nd May 2007. They raised the issues of indemnity and insurance as the hospital now requires 10 million dollars in clinical trial insurance and indemnity before any trial can proceed. Novsel Australia who are supplying the tea tree ointment to be used in the trial have kindly agreed to supply both. We are waiting for this to be finalised. Once this has been done the RGU will give their approval for the trial. Once approval has been obtained from the HREC and RGU the CTN form can be lodged with the TGA. Once permission from the TGA has been received by Dr Hutchison, the trial can begin.

RESEARCH IN PROGRESS – To enhance the ability of the industry to provide products that meet appropriate safety and efficacy

Project Title	Allergy to tea tree oil: Qualitative aspects and risk assessment
RIRDC Project No.:	SCF-1A
Start Date:	1/6/06
Finish Date:	30/6/07
Researcher:	Dr Lisa O'Brien
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Objectives	To evaluate the safety profile of tea tree oil by elicitation of dose-response patch testing and use testing.
Current Progress	A study was carried out to evaluate the safety profile of tea tree oil by elicitation dose-response patch testing and use testing in subjects who had been previously fount to be allergic to tea tree oil. A total o14 study subjects and 7 control subjects were tested. An additional two study subjects (currently being sought) are required to produce the necessary statistical power to provide conclusive results.

RESEARCH IN PROGRESS − To enhance the ability of the industry to provide products that meet appropriate safety and efficacy

Project Title	Effects of tea tree oil on biofilm formation
RIRDC Project No.:	UWA 90A
Start Date:	04/07/05
Finish Date:	06/07/07
Researcher:	Dr Katherine Hammer
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Objectives	1. Demonstrate that tea tree oil can inhibit the formation of microbial biofilm. 2. Investigate the effects of tea tree oil on existing biofilm. 3. Investigate the mechanism(s) by which biofilm formation is inhibited. 4. Explore potential medical and industrial applications of biofilm inhibition.
Current Progress	Objective 1 has been achieved, with tea tree oil shown to inhibit biofilm formation by the Gram negative bacteria *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia* and *Vibrio harveyi*, the Gram positive bacterium *Staphylococcus epidermidis* and the yeast *Candida albicans*. However, there is some data from experiments with *V. harveyi* indicating that the inhibition of biofilm formation may simply be a direct result of reduced microbial growth, rather than an effect specifically against biofilm. This possibility requires further investigation and can be addressed to some extent under Objective 3. Objective 2 has largely been achieved, with work showing that the treatment of existing biofilm with tea tree oil for 24 hours results in the loss of viability within the biofilm, but not necessarily loss of the biofilm structure itself, also referred to as the biofilm biomass. This means that although the organisms may no longer be alive within the biofilm, its structure remains intact. Objectives 3 and 4 have yet to be achieved. Protocols for investigating whether tea tree oil interferes with the cell-to-cell communication that is critical for biofilm initiation and development (also known as quorum sensing) have been developed and these experiments will commence in the final stages of the project.

RESEARCH IN PROGRESS − To enhance the ability of the industry to provide products that meet appropriate safety and efficacy

Project Title	Pilot study of tea tree oil in the decolonisation of MRSA positive wounds
RIRDC Project No.:	UWA-93A
Start Date:	01/11/05
Finish Date:	31/10/07
Researcher:	Dr Christine Carson
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Objectives	1. To determine if tea tree oil (TTO) can eliminate methicillin-resistant *Staphylococcus aureus* (MRSA) from colonised wounds. 2. To determine if TTO is a beneficial treatment for chronic wounds.
Current Progress	Final approvals to conduct the pilot study were received from both the UWA and Silver Chain Human Research Ethics Committees and recruitment began in April. To date, four participants have been recruited and all commenced treatment with the tea tree oil product as planned. Two of the participants were subsequently shown to not be carrying MRSA and were withdrawn from the study in keeping with protocol. A third participant was MRSA positive but developed a wound infection and commenced antibiotics which necessitated withdrawing from the study. Results from the primary swab on the fourth participant are pending. If they are MRSA positive, their TTO treatment will continue. Additional strategies to enhance recruitment are being developed. These include getting Silver Chain staff from other nursing bases to recruit participants.

RESEARCH IN PROGRESS - To establish production systems that are both ecologically sustainable and profitable

Project Title	Diagnostic tools for quality enhancement in Australian essential oil industries
RIRDC Project No.:	ANU-74A
Start Date:	01/06/06
Finish Date:	30/05/07
Researcher:	Dr William Foley
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Objectives	The project aims to exploit the recent discovery of genes that control the production of terpenes in *Melaleuca*. The discovery of these genes provides a means of identifying the genetic differences responsible for the chemotypic variation among species and genotypes of *Melaleuca* plantation cultivars. It is expensive and slow. This project will correlate variations in the terpene profiles of *Melaleuca* chemotypes with variations in the sequences of terpene synthase genes. With this information it will be possible to identify diagnostic genetic variation that can ultimately be converted into diagnostic assays for use by breeders.
Current Progress	We have completed the collection of *Melaleuca alternifolia* leaf samples, covering its natural distribution. The leaf oils have been analysed and the presence of individuals of all known chemotypes has been confirmed. RNA has been extracted from individuals representing all chemotypes, and the construction of cDNA libraries has commenced. DNA has been extracted from all samples collected, and has been tested for purity and quantity. We currently have consistent results in isolating four distinct terpene synthases from genomic DNA, covering up to 60% of the open reading frame with sufficient specificity to allow for direct sequencing. Furthermore, we have been able to apply recently obtained sequence information from *Eucalyptus* as well as publicly available *Melaleuca* sequence to isolating the mevalonate kinase and isopentenyl diphosphate isomerase genes responsible for upstream steps in the terpene biosynthesis pathway. Presently, population wide sequencing of close to 100 individuals is underway to ascertain variation in the sequences available, while further effort is being put into completing the sequence information of all genes to obtain 100% coverage. We are thus close to being able to correlate variations in gene sequences with variations in oil profiles to provide a diagnostic test of high value oils in *Melaleuca*.

RESEARCH IN PROGRESS - To establish production systems that are both ecologically sustainable and profitable

Project Title	Improved tea tree varieties for a competitive market
RIRDC Project No.:	DAN-254A
Start Date:	01/07/06
Finish Date:	01/07/09
Researcher:	Dr. Trevor Olesen
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Objectives	To release improved seed and clones to maximise profit and market access for Australian the tea tree oil producers.
Current Progress	The tea tree breeding project has continually released seed to the industry since 1997. Seed sales total over 7.8kg (enough to plant over 1000 ha). Seed made available is from the best provenances, together with improved seed from both seedling and clonal orchards. Over 970g ($80/g) of seed from the clonal orchard have been sold since 2004 when yield gains of over 70% (averaged over 4 harvests) were confirmed for this seedlot. A yield trial established in 2002 for project released seed showed a 45% increase in yield on the fourth harvest compared with an industry standard. This increase comprised a 7% increase in growth and a 29% increase in oil concentration. The best performing release for the first, second, third and fourth harvests was the clonal seed orchard seed which achieved 91, 75, 43 and 79% (mean 72%) increases in oil yield for the four harvests, respectively. Accompanying these yield improvements were desirable changes in oil quality with project releases giving lower 1,8 cineole and higher terpinen-4-ol levels. Improved seed from second-generation seedling seed orchards is now available. The progeny is to be tested in a yield trial to be established at Bungawalbin later this year. A second clonal orchard for commercial seed production, using 24 elite families, was established at Wollongbar in February 2007. The first available seed from this orchard is expected in 2011. Twenty elite clones are being assessed in a series of plant density/progeny trials at Bungawalbin. Early results suggest that reducing plant density from 33K to 16K plants per ha will have the potential to reduce yields. At first and second harvests, clones (as physiologically mature plants) demonstrated 50% and 9% higher oil concentrations than seedlings respectively. The implication for growers is that clones may return higher early yields than seedlings but this advantage appears to diminish by the second harvest.