EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

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Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Melaleuca alternifolia* (Maiden and Betch) Cheel, *M. linariifolia* Smith, *M. dissitiflora* F. Mueller and/or other species of *Melaleuca*, aetheroleum

Based on Article 16d(1), Article 16f and Article 16h of Directive 2001/83/EC as amended (traditional use)

Final

Herbal substance(s) (binomial scientific name of the plant, including plant part)	*Melaleuca alternifolia* (Maiden and Betch) Cheel, *M. linariifolia* Smith, *M. dissitiflora* F. Mueller and/or other species of *Melaleuca*, leaf and terminal branchlets
Herbal preparation(s)	*Melaleuca alternifolia*, aetheroleum
Pharmaceutical forms	Herbal preparation in liquid and semi-solid dosage forms for cutaneous use or in liquid dosage form for oromucosal use.
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Abbreviations

ASI	Acne Severity Index
CoNS	Coagulase-Negative *Staphylococci*
EMA	European Medicines Agency
ESCOP	European Scientific Cooperative On Phytotherapy
EO/LTTO	Eucalyptus Oil and Lemon Tea Tree Oil pediculicide
IgA	Immunoglobulin A
GI	Gingival Index
MBC	Minimum Bactericidal Concentration
MDCK	Madin–Darby canine kidney (cell line)
MIC	Minimal Inhibitory Concentration
MICs	Minimal Inhibitory Concentrations
MIC_{90}	Minimal Inhibitory Concentration required inhibiting the growth of 90% of organisms
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSB	Mitis Salivarius-Bacitracin agar
MSSA	Methicillin- susceptible *Staphylococcus aureus*
OPC	Oropharyngeal candidiasis
PBI	Papillary Bleeding Index
RHL	Recurrent herpes labialis
SCCP	Scientific Committee on Consumer Products
TTO	Tea Tree Oil
TTO/LO	Tea Tree Oil and Lavender Oil pediculicide
VAS	Visual Analogue Scale
VRE	Vancomycin-resistant enterococci
VSC	Volatile Sulphur Compounds

1. Introduction

1.1. Description of the herbal substance(s), herbal preparation(s) or combinations thereof

- Herbal substance(s)

Melaleuca alternifolia tree is a member of the botanical family Myrtaceae. The name tea tree was established for the plant because the leaves were used to prepare an aromatic tea.

The term *"Tea Tree"* includes species of the genus *Leptospermum* and *Melaleuca* (more than 150 species) of the family Myrtaceae. The best known and economically most important species is the Australian Tea Tree (Saller *et al.* 1998).

- Herbal preparation(s)

The preparation with pharmacological interest is the oil from the leaves (called tea tree oil, TTO), because it has been reported as having immuno-stimulatory property and activity against bacterial, viral and fungal organisms. It is also known that it can attenuate inflammation and may help wound healing (Carson *et al.* 2006).

There are several historical terms for TTO, including ''melaleuca oil'' and ''ti tree oil'', ''ti tree'' being a Maori and Samoan common name for plants in the genus *Cordyline*. The term ''*Melaleuca* oil'' has been selected as the official approved name by the Therapeutic Goods Administration of Australia (Carson & Riley 2001).

About 2% essential oil can be obtained from the leaves of the Australian Tea Tree by extraction with lipophilic organic solvent or by steam distillation. According to the European Pharmacopoeia TTO is obtained by steam distillation from the foliage and terminal branchlets of *Melaleuca alternifolia* (Maiden and Betch) Cheel, *M. linariifolia* Smith, *M. dissitiflora* F. Mueller and/or other species of *Melaleuca*. It is a clear, mobile, colourless or pale yellow liquid with no visible trace of water and has a distinct pleasant odour like turpentine with a high content of terpenes (> 50 to 60%) and a specific weight of 0.89. It is almost insoluble in water, but mixes well with most organic solvents (Saller *et al.* 1998).

TTO is produced mainly from *Melaleuca alternifolia* on large-scale plantations in the states of New South Wales and Queensland in Australia. Prior to commercial cultivation, the natural habitat of *Melaleuca alternifolia* was limited to the area around the Clarence and Richmond Rivers in north-eastern coast of New South Wales. Other *Melaleuca* species, including *Melaleuca dissitiflora* and *Melaleuca linariifolia*, have produced oils which meet the international standard, such as ''cajuput'' oil (also ''cajeput'' or ''cajaput'') from *Melaleuca cajuputi* and ''niaouli'' oil from *Melaleuca quinquenervia* (Carson & Riley 2001).

TTO is composed of terpene hydrocarbons, mainly monoterpenes, sesquiterpenes and their associated alcohols. According to Carson *et al.* (2006), the early reports on the number of components TTO was put at up to 48, however in 1989 a paper was published reporting on the examination of over 800 samples of TTO and concluded that there were approximately 100 components (Brophy 1989). This wide variation and the potential for batch-to-batch variation led in 1996 to an international standard for "Oil of Melaleuca – terpinen-4-ol type (TTO)". Prior to this there was an Australian standard. The Australian standard specified that the 1,8-cineole content of TTO must not exceed 15%, while terpinen-4-ol content must exceed 30% (Carson & Riley 2001).

The chemical composition of TTO consists largely of cyclic monoterpenes of which about 50% are oxygenated and about 50% are hydrocarbons (Cox *et al.* 2000).

The oil contains 42.35% terpinen-4-ol, 20.65% γ-terpinene, 9.76% α-terpinene, 3.71% terpinolene, 3.57% 1,8-cineole, 3.09%, α-terpineol, 2.82% p-cimene, 2.42% α-pinene, 1.75% limonene, 1.05% δ-cadinene, 0.94% α-thujene, 0.94% aromadendrene, 0.87% myrcene, 0.73% β-pinene, 0.40% sabinene, and 0.34% α-phellandrene (Bozzuto *et al.* 2011).

Since the exact composition of TTO is variable, according to the Australian and International Standards Organizations, the substance known as TTO from *Melaleuca alternifolia* has a chromatographic profile within given ranges (Halcón & Milkus 2004).

The European Pharmacopoeia and the International Standard ISO 4730 require TTO to have a minimum content of 30% of terpinen-4-ol and a maximum content of 15% of 1,8-cineole. Terpinen-4-ol is the major TTO component and has shown strong antimicrobial and anti-inflammatory properties (in Mondello *et al.* 2006), while 1,8-cineole is probably an undesirable allergen in TTO products (Carson & Riley 2001).

Table 1: Main constituents of tea tree oil

	From European Pharmacopoeia		**From ISO 4730-2004**	
Constituent	*Minimum (%)*	*Maximum (%)*	*Minimum (%)*	*Maximum (%)*
α-pinene:	*1*	*6*	*1*	*6*
sabinene		*3.5*	*Trace*	*3.5*
α-terpinene	*5*	*13*	*5*	*13*
limonene	*0.5*	*4*	*0.5*	*1.5*
1,8-cineole		*15*	*Trace*	*15*
γ-terpinene	*10*	*28*	*10*	*28*
p-cymene	*0.5*	*12*	*0.5*	*8*
terpinolene	*1.5*	*5*	*1.5*	*5*
terpinen-4-ol	*30*		*30*	*48*
aromadendrene		*7*	*Trace*	*3*
α-terpineol	*1.5*	*8*	*1.5*	*8*
δ-cadinene			*Trace*	*3*
globulol			*Trace*	*1*
viridiflorol			*Trace*	*1*
ledene (syn. viridiflorene)			*Trace*	*3*

TTO is incorporated in topical formulations for the treatment of cutaneous infections (Carson *et al.* 2006; Hammer *et al.* 2006). The concentrations of TTO found in commercially available products range from 2 to 5%. Terpinen-4-ol is the main antimicrobial compound, but other components, such as α-terpineol, also have similar antimicrobial activities (Carson *et al.* 2006).

TTO has to be stored in air-tight containers, protected from light and heat, because proper storage and handling are needed to avoid the formation of oxidation products which have greater potential for skin sensitisation (British Pharmaceutical Codex 1949, WHO 2004). A shelf-life of 12 months after opening is recommended for formulated TTO products by the Australian Government – Rural Industries Research and Development Corporation (2007).

TTO has been used for many years as a component in cosmetic products. It has also been used as an ingredient in medicinal products for its antimicrobial properties especially in treating cutaneous infections. It has been listed in various reference books including the British Pharmaceutical Codex

1949 and books on Essential Oils (Penfold & Morrison 1950) and the World Health Organisation in 2004 has published a monograph on "Aetheroleum Melaleucae Alternifoliae".

- Combinations of herbal substance(s) and/or herbal preparation(s) including a description of vitamin(s) and/or mineral(s) as ingredients of traditional combination herbal medicinal products assessed, where applicable.

Not applicable.

1.2. nformation about products on the market in the Member States

Regulatory status overview

Member State	Regulatory Status				Comments
Austria	☐ MA	☐ TRAD	☐ Other TRAD	☒ Other Specify:	Only in combination with several other essential oils in medicinal products on the market. In cosmetics and food supplements.
Belgium	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Bulgaria	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No medicinal products
Cyprus	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Czech Republic	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No medicinal products
Denmark	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No medicinal products (a cutaneous liquid authorised from 1993 to 2009)
Estonia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No medicinal products
Finland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No medicinal products
France	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No medicinal products
Germany	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No medicinal products
Greece	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Hungary	☐ MA	☒ TRAD	☐ Other TRAD	☐ Other Specify:	
Iceland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Ireland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Italy	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No medicinal products
Latvia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Liechtenstein	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Lithuania	☐ MA	☐ TRAD	☐ Other TRAD	☒ Other Specify:	Food supplements
Luxemburg	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Malta	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
The Netherlands	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Norway	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Poland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	

Member State	Regulatory Status				Comments
Portugal	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No medicinal products
Romania	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No medicinal products
Slovak Republic	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Slovenia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No medicinal products
Spain	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No medicinal products
Sweden	☐ MA	☐ TRAD	☒ Other TRAD	☐ Other Specify:	
United Kingdom	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Medicinal products in combination with non-herbal ingredients authorised since before 1970. There was a monograph in the BPC of 1949

MA: Marketing Authorisation
TRAD: Traditional Use Registration
Other TRAD: Other national Traditional systems of registration
This regulatory overview is not legally binding and does not necessarily reflect the legal status of the products in the MSs concerned.

1.3. Search and assessment methodology

This assessment report reviews the scientific literature data available for *Melaleuca alternifolia* essential oil*,* and from the WHO monograph, European Pharmacopoeia monograph, British Pharmaceutical Codex monograph, ESCOP monograph, PubMed, EMA library and the internet, as well as available information on products marketed in the European Community, including pharmaceutical forms, indications, posology and methods of administration.

The keywords "*Melaleuca alternifolia*", "tea tree oil", in all text fields were used. The information ad references provided by the Australian Tea Tree Industry (ATTIA Ltd.) following the call for submission of scientific data were also taken into consideration. Only clinical studies with tea tree oil were included in the assessment report.

2. Historical data on medicinal use

2.1. Information on period of medicinal use in the Community

Melaleuca alternifolia oil has been used as medicinal by Australian Bundjabung Aborigines for several millennia for bruises, insect bites, and skin infections. European colonists soon recognized the therapeutic properties and began to distil oil from its leaves (Carson & Riley 2001). Members of the crew of James Cook described at the end of the eighteenth century the use of the TTO. It was rediscovered in the 1920s as a topical antiseptic with more effective activity than phenol (Bozzuto *et al.* 2011).

The essential oil was distilled for the first time in 1925 and due to its antiseptic, antibacterial and antifungal effects became a standard antiseptic agent for surgery, especially for dental surgery (Saller *et al.* 1998).

The monograph on TTO of the British Pharmaceutical Codex of 1949 reports that TTO has germicidal properties and has been used as a local application in the treatment of furunculosis, tinea, paronychia,

impetigo, trush and stomatitis, and as inhalant in coryza. In veterinary practice it has been used in the treatment of mange and eczema and in sores and skin diseases of parasitic origin.

TTO has been used for its bactericidal and fungicidal properties as a disinfectant component in several medicinal combination products with non-herbal ingredients authorised in UK since before 1970.

A cutaneous liquid containing TTO has been authorised in Denmark from 1993 to 2009 for disinfection in acne and in fungal infections on the foot.

In Sweden a cutaneous liquid is marketed since 1988 and a oromucosal and cutaneous solution is registered in Hungary since 2004.

In Australia, the Complementary Medicines Evaluation Committee (CMEC) recommended in 1999 to the TGA that registration applications for uncompounded TTO products, intended for topical use and with low level claims of a first aid nature, can be approved by the TGA without requiring prior consideration and recommendation by CMEC (CMEC extracted ratified minutes).

There is a significant number of 100% TTO medicinal products authorised in Australia to date.

Table 2 shows a consistent and long standing use of TTO demonstrated for more than 30 years, since 1930, internationally and for more than 15 years, since 1933, in the European Community. A wide range of traditional indications have been described for local application including the nasal, mouth and throat regions.

TTO has been used as an antiseptic for special and general dental surgery and in denture and mouth washes (MacDonald 1930, Anonymous 1933, Penfold & Morrison 1937, Penfold & Morrison 1950). It has also been indicated for a variety of skin conditions including bacterial and fungal infections of the skin such as acne, furunculosis, dermatophytosis (tinea pedis, tinea cruris, tinea barbae), pityriasis versicolor (tinea versicolor), parionychia, impetigo, empyema, dermatitis, eczema, psoriasis, skin rashes, impetigo contagiosa, pediculosis, ringworm, thrush, infected pustules, intertrigo and nail infections (caused by *Candida albicans*), parasitic skin diseases (Penfold & Morrison 1937, Penfold & Morrison 1950, Humphery 1930, Martindale 1993, British Pharmaceutical Codex 1949, Walker 1972, WHO 2004, Williamson 2003, Lawless 1994, Drury 1991).

Many different foot problems have been treated by TTO including onychomycosis infections of toenails, bromidrosis, malodour, cracks, fissures, peeling, callused heels, inflammation of corns, calluses, bunions, hammertoes, post-operative wound healing (Walker 1972, WHO 2004). It has also been used for the treatment of infected, colonised, dirty wounds, diabetic gangrene and chronic leg ulcers, burns and wounds (Penfold & Morrison 1937, Penfold & Morrison 1950, Humphery 1930, WHO 2004).

Throat, nasal and mouth conditions including acute nasopharyngitis, catarrh, thrush, stomatitis, tonsillitis, mouth ulcers, sore throat, coughs and colds, nasopharyngitis, sinus congestion, tonsillitis, pyorrhoea, gingivitis are traditional indications for use of TTO (Penfold & Morrison 1937, Penfold & Morrison 1950, Humphery 1930, British Pharmaceutical Codex 1949, WHO 2004).

TTO has been used for vaginal infections and gynaecological conditions including vaginitis, cystitis and cervicitis (Penfold & Morrison 1937, Penfold & Morrison 1950, Humphery 1930, WHO 2004), irrigation of bladder and urethra (Anonymous 1933), symptomatic treatment of colitis (WHO 2004) and as an inhalant in coryza (British Pharmaceutical Codex 1949).

Table 2: Traditional use of tea tree oil

Reference	Documented Use / Traditional Use	Herbal preparation	Posology	Safety	Comments
Humphery 1930 Australia	a) Cleaning of dirty or infected wounds and pus dissolution b) help wound healing c) peryonichia. d) as a gargle to clear up sore throats in the early stages e) for use in the vagina with no irritation f) help in clearing head cold symptoms. g) for nasopharynx h) for several parasitic skin diseases	a)-f) 35% TTO saponified solution g) TTO diluted with paraffin	a) various water dilutions commencing from 2.5% to 10% b) 2.5% dilution to be applied as impregnated dressing and changed every 24 hours c) 10% water dilution d) 20 drops in a glass of warm water e) Stronger dilutions f) a few drops inhaled from handkerchief g) as a spray h) as an ointment	No apparent damage to the tissues even in quite strong solutions.	Infections that had resisted treatments of various kinds for months were cured in less than a week.
MacDonald 1930 Australia	as an antiseptic for special and general dental surgery	Ti-Trol – 100% TTO Melasol – 40% TTO in water soluble emulsion			
Anonymous 1933 Great Britain	a) Use in dental, medical and surgical practice b) Use in a wide range of septic conditions c) for irrigation of bladder and the urethra	a), b) Ti-Trol (100% TTO) a)–c) Melasol (40% TTO in water soluble emulsion)	c) 100% Melasol solution		powerful non-poisonous and non-irritant disinfectant
Penfold and Morrison 1937 Australia	Extensive application in surgical and dental practice. Chronic leg ulcers and wounds Germicidal even in presence of blood and organic matter. Peryonichia (paronychia), empyema, gynaecological conditions, skin conditions including psoriasis, impetigo contagiosum, pediculosis, ringworm (tinea). Throat and mouth condition including acute nasopharyngitis, catarrh, thrush, aphthous stomatitis, tonsillitis, mouth ulcers, sore throat, pyorrhoea, gingivitis.	Ti-Trol (100% TTO) Melasol (40% TTO in water soluble emulsion)			Ti-Trol quickly healed an unhealing head wound; Ti-Trol cleared tinea in many cases; TiTrol and Melasol healed leg ulcers with pus not responding to other treatments; Melasol healed a chronic case of diabetic gangrene

Reference	Documented Use / Traditional Use	Herbal preparation	Posology	Safety	Comments
British Pharmaceutical Codex 1949 Great Britain	Germicidal properties. Local application for treatment of furunculosis, tinea, paronychia, impetigo, eczema, thrush, stomatitis. Inhalant in coryza.	TTO		Stored in well-closed containers, protected from light and in a cool place	
Penfold and Morrison 1950 Australia	Extensive application in surgical and dental practice. Germicidal even in presence of blood and organic matter. Perionychia (paronychia), empyema, gynaecological conditions, diabetic gangrene. Skin conditions including psoriasis, impetigo contagiosa, pediculosis, ringworm (tinea). Throat and mouth condition including acute nasopharyngitis, catarrh, thrush, aphthous stomatitis, tonsillitis, mouth ulcers, sore throat, pyorrhoea, gingivitis. Skin injuries and abrasions. Antiseptic agent in denture and mouth washes.	100% TTO or a water soluble oil emulsion without relating to a specific indication			Pleasant odour, non-poisonous, non-irritant, non-corrosive. Ability to penetrate pus, acts to deslough, leaving a healthy surface. The germicidal activity is maintained and even increased in presence of organic matter.
Walker 1972 USA	Common foot problems: onychomycotic toenails	Ti-Trol – 100% TTO	To be applied twice daily		helps make nails smoother and firmer but had little effect on organisms
Walker 1972 USA	Common foot problems: a) bromidrosis b) deodorant, healing of cracks and fissures, peeling and callused heels , inflammation of corns, calluses, bunions, hammertoes c) Post-operative wound healing of chemical matricectomies and post-surgical sutured wounds d) Relief of post-treatment dryness following copper sulphate iontophoresis for tinea pedis e) fungal preventative associated with tinea pedis	Melasol (40% TTO in water soluble emulsion) e) 8% TTO in ointment preparation	apply once daily or hydrotherapy daily application to the affected areas post-operative dressing, to be applied twice daily		
Martindale 1982 UK	Added to many disinfectant preparations	TTO		Stored in cool place in air-tight containers, pro-	

Reference	Documented Use / Traditional Use	Herbal preparation	Posology	Safety	Comments
				tected from light	
Drury 1991 England	a) Arthritis b) Boils and abscesses c) Bruises d) Burns and sunburn e) Cuts and abrasions f) Tinea pedis g) Paronychia	100% TTO Melasol	a) Mixing 3 to 5 drops of TTO into a small amount of baby oil and massaging it deeply into the joints. b) Application the oil directly to the boil three times a day or use of Melasol (40% solution of TTO) in castor oil soap c) TTO dabbed directly onto the bruise d) Gently coat with TTO antiseptic cream or in severe cases with pure tea tree oil e) Apply in its pure form or diluted into a soothing antiseptic cream f) Apply pure TTO twice a day g) Soak infected nail in tea tree oil for 5 minutes and massage well twice a day for up to two weeks	a) None reported b) Slight temporary stinging c) None reported	
Martindale 1993 UK	Reported to have bactericidal and fungicidal properties and is used topically for various skin disorders	TTO		Stored in air-tight containers, protected from light	
Lawless 1994 England	a) Tinea pedis (Athlete's foot) b) Boil (furuncle)/ abscess c) Cut / wounds d) Paronychia	100% TTO	a) Apply neat tee tree oil to the affected areas. Soaking the feet for 5-10 minutes a day in a tea tree foot bath (5-10 drops in a bowl of warm water) b) Dab with neat tee tree oil. Repeat 2 or 3		

Reference	Documented Use / Traditional Use	Herbal preparation	Posology	Safety	Comments
			times a day. c) Dab a few drops of pure tea tree oil. d) Soak the infected nails in pure tea tree oil for 2 or 3 minutes, massaging the solution into the nailbed. Repeat 3 times a day until the infection clears		
World Health Organization 2004 International	Uses supported by clinical data: topical application for symptomatic treatment of common skin disorders such as acne, tinea pedis, bromidrosis, furunculosis and mycotic onychia (onychomycosis) and of vaginitis due to Trichomonas vaginalis or Candida albicans, cystitis and cervicitis. Uses described in pharmacopoeias and in traditional medicine: as an antiseptic and disinfectant for the treatment of wounds. Uses described in folk medicine: symptomatic treatment of burns, colitis, coughs and colds, gingivitis, impetigo, nasopharyngitis, psoriasis, sinus congestion, stomatitis, tonsillitis	TTO	external application at concentrations of 5-100%, depending on skin disorder being treated	Contraindicated for cases of known allergy to plants of the Myrtaceae family. Not for internal use. Keep out of reach of children. Store in a well-filled airtight container, protected from heat and light	

2.2. Information on traditional/current indications and specified substances/preparations

The leaves were macerated in water for a long period (hours or even days) and then used as infusion or impregnated dressing especially in treating common cold, sore throat, insect bites, wounds or fungal skin infections as well as in delousing (Saller *et al.* 1998).

The essential oil had been used during the Second World War as a general antimicrobial agent and insect repellent, and provided in the first aid kits of serving Australian soldiers. The essential oil is nowadays used as a strong antimicrobial and antifungal agent in creams, soaps, toothpastes and other preparations and it has been used both externally and internally by both herbalists and aromatherapists (Lis-Balchin *et al.* 2000).

In modern times, TTO is reputed to have several medicinal properties including antibacterial, antifungal, antiviral, anti-inflammatory and analgesic properties. For its antibacterial activity is today popular as a topical antimicrobial agent (Carson *et al.* 1998). It has been recommended in the treatment of many cutaneous conditions, including acne, eczema, furunculosis, onychomycosis and tinea (Carson *et al.* 2006).

TTO enjoys remarkable popularity as a topical antimicrobial agent and, although it is marketed mainly for its well-documented antibacterial, antifungal and antiviral properties, the oil also has anti-inflammatory, analgesic, insecticidal and antipruritic properties (Edmondson *et al.* 2011). Currently it is also incorporated as the principal antimicrobial or as a natural preservative in many pharmaceutical and cosmetic products intended for external use (Cox *et al.* 2000).

TTO has a number of characteristics which suggest potential for its use in wound treatments or protectants against fly strike. It has documented insecticidal effects, which could be of use in the treatment of larvae in strikes, and repellent effects (Callander & James 2011).

In Australia, it has also a long history of clinical use in the treatment of foot problems such as tinea pedis and toenail onychomycosis. Dermatologic studies have been conducted in the treatment of acne, dandruff, head lice, and recurrent herpes labialis, in which effects were found to be either similar or better than traditional treatment, and often with fewer side effects. A few published studies report the successful use of TTO in treating mucous membrane infections, including *Trichomonas vaginalis*, and against oral bacteria and oropharyngeal candidiasis (Halcón & Milkus 2004). 100% TTO is listed by the Australian Therapeutic Goods Administration. A wide range of claims for use are permitted [Quoted at Austteam Tea Tree Oil Conference, 1995].

In Denmark it has been authorised for disinfection in acne and in fungal infections on the foot (1993-2009).

In Sweden TTO is used against itch at mild athlete´s foot, for uncomplicated insect bites and for treatment of mild acne, in Hungary for treatment of skin infection, stomatitis, gingivitis, cut wounds, excoriation and acne.

2.3. Specified strength/posology/route of administration/duration of use for relevant preparations and indications

TTO is usually topically applied at concentrations 1.0%-100% for treating microbial infections (Combest 1999).

Tea tree preparations containing 10% and 100% TTO have been used in clinical trials to treat tinea pedis and onychomycosis, respectively (Buck *et al.* 1994; Tong *et al.* 1992).

For treating athlete's foot, it is advised to dilute the concentrated oil with an equal amount of water or vegetable oil and apply to the affected area three times a day with a cotton ball (Combest 1999). A topically applied 5% solution appears to be effective in treating acne (Bassett *et al.* 1990).

Several published reports have addressed minimum inhibitory and bactericidal concentrations of TTO against clinical isolates of S*taphylococcus aureus*. A study of 105 clinical isolates of *Staphylococcus aureus* using a broth microdilution method found the MIC_{90} (Minimal Inhibitory Concentration required to inhibit the growth of 90% of organisms) of TTO to be 0.5%. A later study of 100 clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) found the MIC_{90} of TTO to be 0.32% (Halcón & Milkus 2004).

In Australia, in 1995 the Medicines Evaluation Committee approved undiluted TTO as a "mild antiseptic for minor cuts, abrasions, bites and stings and minor burns". [Quoted at Austteam Tea Tree Oil Conference, 1995]

According to the posology of medicinal products licensed in Europe for application on the skin TTO should always be diluted before use. In Sweden it is diluted in olive oil or baby oil 1:9 and dabbed on the afflicted areas of the skin 1-3 times daily. The rate of dilution in Denmark was 1:9 as well. The use is not recommended for children under 12 years of age. In acne or athlete's foot the maximum duration of use is 1 month of treatment.

In Hungary the daily dose for cutaneous use is 10-15 drops (corresponding to 0.33–0.5 ml or 0.3147-0.47205 g) to be stirred in 50 ml of lukewarm water and the solution is applied on the skin with a sterile cotton wool or gauze. In case of stomatitis and gingivitis 5-10 drops (corresponding to 0.17–0.33 ml or 0.15735-0.47205 g) to be mixed in 100 ml of water for gargle several times daily (1 ml is 30 drops and 1 g is about ~32 drops). If the symptoms do not improve after 5 days treatment the use of products should be stopped.

A number of papers, documents and letter on the sales of TTO in Europe, provided by Interested Parties, represent a body of data that, as a whole, substantiates the medicinal use of undiluted TTO in Europe for at least 15 years (Drury 1991, Drury 1995, Lawless 1994, Lawless 1996). In these papers and documents the use of undiluted TTO is specified and posology is given. In addition this was supported by wide spread evidence of use by way of magazine articles, sales figures and books.

Table 3: Information on preparations of TTO grouped according to the traditional use

Herbal preparation **Pharmaceutical form**	**Indication**	**Strength** **Posology**	**Period of medicinal use**
solution readily miscible in water containing 35% of TTO (saponified)	a) to dissolve pus, to clean surface of infected wounds b) to wash or syringe out dirty wounds to loosen and remove debris. c) to help with healing d) as an ointment for several parasitic skin diseases	a) 35% TTO saponified solution at various water dilutions commencing from 2.5% b) 10% watery lotion c) Dressings dipped in 2.5% solution to be applied to wound and changed every 24 hours d) TTO diluted with paraffin (no further specification)	1930 Humphery Australia
TTO for local application	Use as an antiseptic for special and general dental surgery	100% TTO or 40% TTO in water soluble emulsion (Melasol)	1930 MacDonald Australia
TTO for local application	Extensive application in surgical and dental practice. Chronic leg ulcers and wounds including an ability to penetrate pus, acts to deslough, leaving a healthy surface. Germicidal properties retained even in presence of blood and organic matter. Skin conditions including psoriasis, impetigo contagiosum, pediculosis, ringworm (tinea).	Refers to 100% oil or a water soluble oil emulsion (Melasol) without relating to a specific indication	1937 Penfold and Morrison Australia
TTO for local application	Impetigo	Not specified	1949 British Pharmaceutical Codex (UK)
TTO for local application	Extensive application in surgical and dental practice. Ability to penetrate pus, acts to deslough, leaving a healthy surface. Germicidal properties retained even in presence of blood and organic matter. Skin conditions including psoriasis, impetigo contagiosa, pediculosis, ringworm (tinea). Skin injuries and abrasions.	Refers to 100% oil or a water soluble oil emulsion (Melasol) without relating to a specific indication	1950 Penfold and Morrison Australia
TTO for local application	Added to many disinfectant preparations	No further specification	1982 Martindale (UK)
Cutaneous liquid	For uncomplicated insect bites	TTO diluted in olive oil or baby oil 1:9 (10%) and dabbed on the afflicted areas of the skin 1-3 times daily. Maximum duration of use 1 month. Not recommended for children under 12 years of age.	Since 1988 (Sweden)
TTO for local application	a) Boils and abscesses b) Burns and sunburn c) Cuts and abrasions d) Insect bites	a) 100% TTO to be applied directly to the boil 3 times daily or use of Melasol (40% solution of TTO in castor oil soap and containing about 13% of isopropyl alcohol) b) TTO to be applied directly to the burn or in form of a	1991 (first edition 1989) (UK) 1995 (FR) Drury

Herbal preparation Pharmaceutical form	Indication	Strength Posology	Period of medicinal use
		non greasy antiseptic cream c) Apply TTO pure or diluted into a soothing antiseptic cream A9 TTO to be dabbed directly onto bites	
TTO for local application	Used topically for various skin disorders for its bactericidal and fungicidal properties	No further specification	1993 Martindale (UK)
TTO for local application	Cut / wounds/burns/insect bites	Apply neat TTO to the affected areas several times a day until the skin has healed.	1994 (UK) 1996 (Germany) Lawless
TTO for local application	Mild antiseptic for minor cuts, abrasions, bites and stings and minor burns		Tea Tree Oil conference, 1995
TTO for local application	For treating microbial infections.	TTO concentrations ranging from 1.0% to 100%	1999 Combest (US)
Cutaneous (and oromucosal) liquid	Treatment of skin infection, cut wounds, excoriation	0.33 – 0.5 ml to be stirred in 50 ml of lukewarm water and the solution is applied on the skin with a sterile cotton wool or gauze.	Since 2004 (Hungary)
TTO for local application	Uses described in pharmacopoeias and in traditional medicine: as an antiseptic and disinfectant for the treatment of wounds. Uses described in folk medicine: symptomatic treatment of burns, psoriasis	external application at concentrations of 5-100%, depending on skin disorder being treated	2004 World Health Organization International
TTO	As a disinfectant	Several published reports have addressed minimum inhibitory and bactericidal concentrations of TTO against clinical isolates of *Staphylococcus aureus*. A study of 105 clinical isolates of using a broth microdilution method found the105 clinical isolates of *Staphylococcus aureus* MIC_{90} _= 0.5%. 100 clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) MIC_{90} = 0.32%.	(Halcón & Milkus 2004).
TTO for local application	Treatment of furunculosis	Not specified	1949 British Pharmaceutical Codex (UK)
Cutaneous liquid	For treatment of mild acne	TTO diluted in olive oil or baby oil 1:9 (10%) and dabbed on the afflicted areas of the skin 1-3 times daily. Maximum duration of use 1 month. Not recommended for children under 12 years of age.	Since 1988 (Sweden)
Cutaneous liquid	Disinfection in acne	Before use dilute 1 part of oil with 9 parts of olive oil or similar oil. To be applied 1-3 times daily.	1993-2009 (Denmark)

Herbal preparation Pharmaceutical form	Indication	Strength Posology	Period of medicinal use
		Maximum duration of use 1 month. Not recommended for children under 12 years of age.	
Water based gel	Treatment of acne	5% water based gel applied daily for 3 months	1990 Bassett *et al.*(clinical trial)
TTO for local application	Boil (furuncle)/ abscess	Dab with neat tee tree oil. Repeat 2 or 3 times a day.	1994 (UK) 1996 (Germany) Lawless
Cutaneous (and oromucosal) liquid	Treatment of acne	0.33 – 0.5 ml to be stirred in 50 ml of lukewarm water and the solution is applied on the skin with a sterile cotton wool or gauze	Since 2004 (Hungary)
TTO for local application	Uses supported by clinical data (reference to Bassett *et al.* 1990): topical application for symptomatic treatment of common skin disorders such as acne and furunculosis	5% water based gel applied daily for 3 months	2004 World Health Organization International
Solution (saponified) readily miscible in water containing 35% of TTO	Peryonichia	a) 10% watery lotion to be applied as impregnated dressing to be changed every 24 hours. Moisten the dress with water if it becomes dry b) pure 35% TTO solution	1930 Humphery Australia
TTO for local application	Peryonichia (paronychia), ringworm (tinea).	Refers to 100% oil or a water soluble oil emulsion (Melasol*) without relating to a specific indication	1937 Penfold and Morrison Australia
TTO for local application	Tinea, paronychia	Not specified	1949 British Pharmaceutical Codex (UK)
TTO for local application	Perionychia (paronychia)	Refers to 100% oil or a water soluble oil emulsion (Melasol) without relating to a specific indication	1950 Penfold and Morrison Australia
1) Undiluted TTO 2) Melasol* – 40% TTO in water soluble emulsion (mixed with 13% isopropyl alcohol) 3) 8% extract of TTO in lanolin as an ointment	Common foot problems: a) Reduce bromidrosis b) to eliminate odour and healing cracks and fissures, peeling and callused heels c) to reduce inflammation of corns, calluses, bunions, hammertoes d) Post-operative wound healing of chemical matricectomies e) post-surgical sutured wounds healing	a) half once of Melasol in 22 gallons of water: apply once daily or as a whirlpool additive for hydrotherapy b) Melasol – 40% TTO in water soluble emulsion (mixed with 13% isopropyl alcohol): daily application c) Melasol – 40% TTO in water soluble emulsion (mixed with 13% isopropyl alcohol): daily application to irritated areas	1972 Walker USA

* a preparation containing 40% of TTO in a soap base called Melasol in Australia and Ti.Trol solution in England (Anonimous 1933)

Herbal preparation Pharmaceutical form	Indication	Strength Posology	Period of medicinal use
	f) Relief of post-treatment dryness following copper sulphate iontophoresis for tinea pedis g) onychomycosis h) prevention of tinea pedis	d) Melasol – 40% TTO in water soluble emulsion (mixed with 13% isopropyl alcohol) post-operative dressing e) Melasol – 40% TTO in water soluble emulsion (mixed with 13% isopropyl alcohol): apply twice daily f) Melasol – 40% TTO in water soluble emulsion (mixed with 13% isopropyl alcohol): daily massages before iontophoresis and application twice a week after iontophoresis g) TTO: apply twice daily (morning and evening, 1 to 6 months) h) 8% extract of TTO in lanolin as an ointment	
TTO for local application	a) Tinea pedis b) Paronychia	a) Apply pure TTO twice a day b) Soak infected nail in TTO for 5 minutes and massage well twice a day for up to two weeks	1991 (first edition 1989) (UK) 1995 (FR) Drury
TTO for local application	a) Tinea pedis (Athlete's foot) b) Paronychia	a) Apply neat TTO to the affected areas or b) Soak the feet for 5-10 minutes a day in a TTO foot bath (5-10 drops in a bowl of warm water) c) Soak the infected nails in pure TTO for 2 or 3 minutes, massaging the solution into the nailbed. Repeat 3 times a day until the infection clears	1994 (UK) 1996 (Germany) Lawless
Cutaneous liquid	Against itch at mild athlete´s foot	TTO diluted in olive oil or baby oil 1:9 (10%) and dabbed on the afflicted areas of the skin 1-3 times daily. Maximum duration of use 1 month. Not recommended for children under 12 years of age.	Since 1988 (Sweden)
Cutaneous liquid	Disinfection in fungal infections on the foot	Before use dilute 1 part of oil with 9 parts of olive oil or similar oil. To be applied 1-3 times daily. Maximum duration of use 1 month. Not recommended for children under 12 years of age.	1993-2009 (Denmark)
TTO for local application	Onychomycosis	100% TTO	Tong *et al.* 1992 (clinical trial)
TTO for local application	Tinea pedis	10% TTO	Buck *et al.* 1994 (clinical trial)
TTO for local application	Athlete's foot	dilute the concentrated oil with an equal amount of water or vegetable oil and apply to the affected area three times a day with a cotton ball	1999 Combest US
TTO for local application	Uses supported by clinical data: topical application for	external application at concentrations of 5-100%,	2004

Herbal preparation Pharmaceutical form	Indication	Strength Posology	Period of medicinal use
	symptomatic treatment of common skin disorders such as tinea pedis, bromidrosis and mycotic onychia (onychomycosis)	depending on skin disorder being treated	World Health Organization International
Solution readily miscible in water containing 35% of TTO (saponified)	To clear up sore throats in the early stages	20 drops in a glass of warm water used as a gargle	1930 Humphery Australia
TTO for local application	Use as an antiseptic for special and general dental surgery.	100% TTO or 40% TTO in water soluble emulsion (Melasol)	1930 MacDonald Australia
TTO for local application	Throat and mouth condition including acute nasopharyngitis, catarrh, thrush, aphthous stomatitis, tonsillitis, mouth ulcers, sore throat, pyorrhoea, gingivitis.	Refers to 100% oil or a water soluble oil emulsion (Melasol) without relating to a specific indication	1937 Penfold and Morrison Australia
TTO for local application	Thrush and stomatitis.	Not specified	1949 British Pharmaceutical Codex
TTO for local application	Extensive application in surgical and dental practice. Throat and mouth condition including acute nasopharyngitis, catarrh, thrush, aphthous stomatitis, tonsillitis, mouth ulcers, sore throat, pyorrhoea, gingivitis. Antiseptic agent in denture and mouth washes.	Refers to 100% oil or a water soluble oil emulsion (Melasol) without relating to a specific indication	1950 Penfold and Morrison Australia
TTO for local application	Treatment of stomatitis, gingivitis.	0.17 – 0.33 ml (0.15735-0.47205 g) to be mixed in 100 ml of water for gargle several times daily.	Since 2004 (Hungary)
TTO for local application	Uses described in folk medicine: symptomatic treatment of gingivitis, stomatitis, tonsillitis	External application at concentrations of 5-100%, depending on skin disorder being treated	2004 World Health Organization
TTO for local application	a) As an aid to clear head cold symptoms. b) as a spray for nasopharynx	a) A few drops inhaled from handkerchief b) TTO diluted with paraffin	1930 Humphery, Australia
TTO for local application	Nasopharyngitis, catarrh	Refers to 100% oil or a water soluble oil emulsion (Melasol) without relating to a specific indication	1937 Penfold and Morrison Australia
TTO for local application	As inhalant in coryza	Not specified	1949 British Pharmaceutical Codex
TTO for local application	Nasopharyngitis, catarrh	Refers to 100% oil or a water soluble oil emulsion (Melasol) without relating to a specific indication	1950 Penfold and Morrison Australia
TTO for local application	Uses described in folk medicine: symptomatic treatment of coughs and colds, nasopharyngitis, sinus congestion		2004 World Health Organization

Long-standing use for at least 30 years, 15 of them within the European community, is therefore demonstrated for the undiluted TTO and for the following preparations and indications:

1) Liquid preparation containing 0.5% to 10% of essential oil to be applied to the affected area 1-3 times daily for treatment of small superficial wounds and insect bites. Traditional use of this preparation is substantiated by the presence in the BPC 1949, by the European market overview (in Sweden since 1988, registered in Hungary since 2004) and by the widespread use in Australia documented since 1930. For the same indication 1-2 drops (0.033-0.066 ml) of the undiluted essential oil are applied to the affected area using a cotton bud 1-3 times daily.

2) Oily liquid or semi-solid preparation, containing 10% of essential oil, to be applied to the affected area 1-3 times daily or 0.7-1 ml of essential oil stirred in 100 ml of lukewarm water to be applied as an impregnated dressing to the affected areas of the skin for treatment of small boils (furuncles and mild acne). Traditional use of this preparation is substantiated by the presence in the BPC 1949 (treatment of furunculosis), by the European market overview (in Sweden since 1988, in Denmark from 1993 to 2009) and by the widespread use in Australia. The undiluted essential oil is to be applied to the boil using a cotton bud 2-3 times daily.

3) Oily liquid or semi-solid preparation, containing 10% of essential oil, to be applied to the affected area 1-3 times daily for the relief of itching and irritation in cases of mild athlete´s foot. Traditional use of this preparation is substantiated by the European market overview (in Sweden since 1988, in Denmark from 1993 to 2009) and by the widespread use in USA, documented since 1972, and in Australia documented since 1930. For the same indication 0.17-0.33 ml of essential oil in is diluted in an appropriate volume (a bowl) of warm water to soak feet for 5-10 minutes a day. The undiluted essential oil is to be applied to the affected area using a cotton bud 2-3 times daily until the condition is cleared up.

4) 0.17–0.33 ml of TTO to be mixed in 100 ml of water for rinse or gargle several times daily for symptomatic treatment of minor inflammation of oral mucosa. Traditional use of this preparation is substantiated by the presence in the BPC 1949 (stomatitis) and by the European market overview (registered in Hungary since 2004) and by the widespread use in Australia documented since 1937.

3. Non-Clinical Data

3.1. Overview of available pharmacological data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

Based on results of laboratory and animal studies, there are several likely mechanisms by which a topical TTO preparation may facilitate healing in chronic *Staphylococcus*-infected wounds. Preliminary studies suggest both reduction in microbial load and changes in immune function related to TTO applications. Terpinen-4-ol, linalool, and α-terpineol are the most studied active antibacterial components of TTO (Halcón & Milkus 2004).

3.1.1. Primary pharmacodynamics

Antibacterial activity

The oil exhibits a broad spectrum of antimicrobial activity *in vitro* although its efficacy *in vivo* remains relatively unsubstantiated. Antibacterial activity against *Staphylococcus aureus,* both methicillin-susceptible (MSSA) and -resistant (MRSA) has been demonstrated (Carson *et al.* 1996).

Minimum inhibitory concentrations (MICs) have been determined for many organisms including coagulase-negative staphylococci (0.06-3% v/v), *Staphylococcus aureus* (including MRSA) (0.12-0.5%), *Streptococcus* spp. (0.03-0.12%), vancomycin-resistant enterococci (VRE) (0.5-1%), *Acinetobacter baumannii* (0.06-1%), *Escherichia coli* (0.12-0.25%), *Klebsiella pneumoniae* (0.12-0.5%), *Candida albicans* (0.12-0.25%), other *Candida* species (0.12-0.5%) and *Malassezia furfur* (0.12-0.25%). The wide range of organisms susceptible to TTO suggests that it may be useful for skin antisepsis. Furthermore, many organisms that colonise skin transiently have been shown to be more susceptible to TTO than commensal organisms (Carson *et al.* 1998).

MICs of TTO range from 0.06 to 0.5% (v/v) for *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus* spp., and 2 to 8% (v/v) for *Pseudomonas aeruginosa* (Longbottom *et al.* 2004).

A study was carried out to evaluate the activities of TTO against lactobacilli and a range of organisms associated with bacterial vaginosis. MIC data indicated that a variety of anaerobic and aerobic bacteria are susceptible to TTO. The data also show that all lactobacilli tested were appreciably more resistant to TTO than organisms known to be associated with bacterial vaginosis, with at least a twofold difference in MIC_{90} results. Therefore, authors suggested that previous clinical success reported by Blackwell may be due, in part, to the susceptibility of bacterial vaginosis-associated organisms to TTO and the relative resistance of commensal *Lactobacillus* spp. The authors suggested that this difference in susceptibility could allow formulation of products that will selectively kill or inhibit certain organisms while having a minimal effect on the commensal lactobacilli (Hammer *et al.* 1999).

In vitro studies established that MIC and MBC (minimum bactericidal concentration) of TTO range from 0.003 to 2% (v/v). Studies indicate that several oral bacteria are susceptible, suggesting that TTO may be used in oral healthcare products and in maintenance of oral hygiene (Hammer *et al.* 2003a).

TTO and α-terpineol and terpinen-4-ol shows to have antibacterial activity against growth of *Staphylococcus aureus* and *Escherichia coli* biofilms at concentration about 0.78%. Terpinen-4-ol seems to have the most potent activity (Budzyńska *et al.* 2011).

The *in vitro* activity of TTO against MRSA has been shown many times with minimum inhibitory concentrations ranging from 0.25% to 2% (Edmondson *et al.* 2011).

The broad-spectrum antimicrobial activity of TTO is mainly attributed to terpinen-4-ol and 1,8-cineole, major components of the oil, and includes antibacterial, antifungal, antiviral, antiprotozoal and antimycoplasmal activities, all promoting TTO as therapeutic agent (Furneri *et al.* 2006, Carson *et al.* 2006).

McMahon *et al.* (2007) has suggested that the treatment of both Gram-positive and Gram-negative bacteria with low levels of TTO results in organisms becoming less susceptible to antibiotics when compared to cells not treated with TTO. One interpretation of these data is that cells undergo an adaptive response that produced cross-tolerance to conventional antimicrobial agents in addition to potentially protecting cells from TTO.

The effect of sub-lethal challenge with TTO on the antibiotic resistance profiles of staphylococci has been studied. Isolates of MRSA and MSSA and coagulase-negative staphylococci (CoNS) were habituated to sub-lethal concentrations of TTO (72 h). Following habituation, the minimum inhibitory concentrations (MIC) of antibiotics and TTO were determined. Habituated MRSA/MSSA cultures had higher ($P < 0.05$) MIC values than control cultures for the examined antibiotics. Habituated MRSA/MSSA cultures also displayed decreased susceptibility to TTO. Conclusions of the authors were that TTO habituation 'stress-hardens' MRSA and MSSA was evidenced by transient decreased antibiotic susceptibility and stable decreased TTO susceptibility. Although TTO habituation did not decrease susceptibility of CoNS to TTO, such cultures showed transient decreased antibiotic susceptibility.

Results suggested that application of TTO at sub-lethal concentrations may reduce the efficacy of topical antibiotics used with TTO in combination therapies (McMahon *et al.* 2008).

Carson (2009), Thomsen *et al*. (2009) and Hammer & Riley (2009) attempted to reproduce the results of McMahon *et al.* (2007), but were unsuccessful. The authors have suggested that exposure to sub-inhibitory concentrations of TTO does not appear to affect the susceptibility or resistance to conventional antibiotics.

Carson *et al.* (2002) investigated the mechanisms of action of TTO and three of its components, 1,8-cineole, terpinen-4-ol, and α-terpineol, against *Staphylococcus aureus* ATCC 9144. They reported that treatment with the test compounds at the MIC and two times the MIC, reduced the viability of *Staphylococcus aureus*, particularly the treatment with terpinen-4-ol and α –terpineol. None of the compounds caused lysis, as determined by measurement of the optical density at 620 nm, although cells became disproportionately sensitive to subsequent autolysis. *Staphylococcus aureus* organisms treated with TTO or its components at the MIC or two times the MIC showed a significant loss of tolerance to NaCl.

When the compounds were tested at one-half the MIC, only 1,8-cineole significantly reduced the tolerance of *Staphylococcus aureus* to NaCl. Electron microscopy of terpinen-4-ol-treated cells showed the formation of mesosomes and the loss of cytoplasmic contents. The authors concluded that the predisposition to lysis, the loss of 260-nm-absorbing material, the loss of tolerance to NaCl, and the altered morphology seen by electron microscopy all suggest that TTO and its components compromise the cytoplasmic membrane.

Antiviral activity

In their review paper Carson *et al.* (1996) stated that the antiviral activity of TTO was first shown using tobacco mosaic virus and tobacco plants. In field trials TTO (spray concentration 0, 100, 250 or 500 ppm) was sprayed on plants that were then experimentally infected with tobacco mosaic virus. After 10 days, there were significantly fewer lesions per square centimetre of leaf in plants treated with TTO than in controls.

Another study has been conducted in 2001 by Schnitzler *et al.* with herpes simplex viruses that were incubated with various concentrations of TTO; these treated viruses were then used to infect cell mono-layers. After 4 days, the numbers of plaques formed by TTO-treated virus and untreated control virus were determined and compared. The concentration of TTO inhibiting 50% of plaque formation was 0.0009% for herpes simplex virus type 1 and 0.0008% for herpes simplex virus type 2, relative to controls. These studies also showed that at the higher concentration of 0.003%, TTO reduced herpes simplex virus-1 titres by 98.2% and HSV-2 titres by 93.0%. In addition, by applying TTO at different stages in the virus replicative cycle, TTO was shown to have the greatest effect on free virus (prior to infection of cells). Another study evaluated the activities of 12 essential oils, including TTO, for activity against herpes simplex virus -1 in Vero cells. Again, TTO was found to exert most of its antiviral activity on free virus, with 1% oil inhibiting plaque formation completely and 0.1% TTO reducing plaque formation by approximately 10%. Pre-treatment of the Vero cells prior to virus addition or post-treatment with 0.1% TTO after viral absorption did not significantly alter plaque formation (Carson *et al*. 2006).

TTO has an interesting antiviral activity against influenza A/PR/8 virus subtype H1N1 in Madin–Darby canine kidney (MDCK) cells. It has been found that TTO had an inhibitory effect on influenza virus replication at doses below the cytotoxic dose; terpinen-4-ol, terpinolene, and α-terpineol were the main active components (Garozzo *et al.* 2009).

The mechanism of action of TTO and its active components against Influenza A/PR/8 virus subtype H1N1 was investigated in MDCK cells. The effect of TTO and its active components on different steps of the replicative cycle of influenza virus was studied by adding the test compounds at various times after infection. These experiments revealed that viral replication was significantly inhibited if TTO was added within 2 h of infection, indicating an interference with an early step of the viral replicative cycle of influenza virus and suggesting that TTO could inhibit viral uncoating by an interference with acidification of intra-lysosomal compartment (Garozzo *et al.* 2011).

Antifungal activity

The antifungal activity of TTO was known anecdotally especially amongst the aboriginal people of Australia.

In 1998 Hammer *et al*. studied the *in vitro* TTO activity against *Candida albicans* and non-*albicans Candida* species. The minimum killing TTO concentration for killing isolates was 0.25% and 0.5% for *Candida albicans* and non-*albicans Candida* species, respectively.

Mondello *et al.* (2003) investigated the *in vitro* antifungal activity of TTO (ISO 4730-2004) against clinical isolates of pathogenic yeasts including strains of *Candida albicans* resistant to fluconazole and/or itraconazole, as well as the *in vivo* activity in an experimental vaginal infection using fluconazole–itraconazole-susceptible or -resistant strains of *Candida albicans.* The susceptibility testing of *Candida* spp., and *Cryptococcus neoformans* to TTO, fluconazole and itraconazole was conducted using a microbroth method according to the National Committee for Clinical Laboratory Standards (NCCLS 1997) for both dilution antifungal susceptibility testing of yeasts (Liu *et al.* 2009).

TTO was active against all tested strains, with MICs ranging from 0.03% (for *Cryptococcus neoformans*) to 0.25% (for some strains of *Candida albicans* and other *Candida* spp.). Fluconazole- and/or itraconazole-resistant *Candida albicans* isolates had TTO MIC_{50}s and MIC_{90}s of 0.25% and 0.5%, respectively. The MIC_{90} for *Candida albicans* strains was found to be the same (0.25%) reported by Hammer *et al*. (1998) against the same fungus using a TTO mixture with relatively similar proportions of terpinen-4-ol and 1,8-cineole. Moreover neither fungistatic nor fungicidal activities were strongly influenced by lowering the pH of the incubation medium to pH 5, thus supporting the use of TTO for skin and mucosal infections.

The results of the *in vivo* investigations on the animal model (oophorectomized – ovary removal surgery female rats of the Wistar strain) of vaginal candidiasis demonstrated that TTO administered intravaginally using a dose volume of 0.1 ml at concentrations of 1%, 2.5% and 5% is effective in resolving experimental *Candida albicans* infection, with both fluconazole-susceptible and –resistant isolates. In the case of the fluconazole-susceptible organism, treatment with TTO was comparable to a standard treatment with fluconazole, used as positive control, whereas no effect was observed in rats treated with TTO diluted with polisorbate 80 used as negative control. The results showed that TTO exerted a marked acceleration of clearance of the yeast, as demonstrated by a statistically significant decrease in CFU counts in the first 2 weeks after the vaginal treatment, with a substantial TTO dose dependence of fungal clearance, although the difference was not statistically significant. With all dose regimens, the infection was cleared in 3 weeks, whereas the untreated control rats remained infected. TTO (5%) also caused a rapid clearance of the fluconazole-resistant strain from the vagina of experimentally infected rats. There was a statistically highly significant difference at all time-points considered between control (or fluconazole-treated rats) and those treated with TTO. Again the infection was resolved in 3 weeks by TTO, whereas all other animals, either untreated or fluconazole-treated, were still infected at the end of the 3 week period.

In a follow up study, Mondello *et al.* (2006) confirmed the previous result with the animal experimental model as reported on the *in vivo* activity of terpinen-4-ol, considered the main bioactive component of

TTO. Using the same methodology as detailed in their previous paper they concluded that terpinen-4-ol was a likely mediator of the *in vitro* and *in vivo* activity of TTO and claimed that their results were the first to demonstrate that terpinen-4-ol could control *Candida albicans* vaginal infections. They concluded that the purified compound held promise for the treatment of vaginal candidiasis, particularly the azole-resistant forms.

Antimycotic properties of TTO and its principal components were compared with the activity of 5-fluorocytosine and amphotericin B. The majority of the organisms were sensitive to the essential oil, with TTO and terpinen-4-ol being the most active oils showing antifungal activity at minimum inhibitory concentration values lower than other drugs (Oliva *et al.* 2003).

The *in vitro* activities of TTO against *Malassezia* yeast species were shown. Ketoconazole was the most active of the imidazoles in the agar dilution assay, followed by miconazole and econazole, which were similar in activity. *Malassezia furfur* was the least susceptible species. *Malassezia sympodialis*, *Malassezia slooffiae*, *Malassezia globosa*, and *Malassezia obtusa* showed similar susceptibilities. Tea tree oil was active against all *Malassezia* species, for which the MICs were similar. Ketoconazole was also the most active of the imidazoles in the broth dilution assay. Miconazole and econazole showed similar activities against each species, but demonstrated differences in activity between species. The MICs of tea tree oil were similar for *M. furfur* and *M. sympodialis*, but the minimum fungicidal concentrations (MFCs) were several dilutions lower for *M. furfur*. The authors concluded that individual *Malassezia* species vary in their susceptibility to several antifungal agents, with *M. furfur* being the least susceptible of the species tested, whereby TTO may be a suitable alternative topical agent (Hammer *et al.* 2000).

In another study investigating *in vitro* antifungal activity of TTO components, the highest activity, with minimum inhibitory concentrations and minimum fungicidal concentrations of <0-25%, was shown by terpinen-4-ol, α-terpineol, linalool, α-pinene and β-pinene, followed by 1,8-cineole. All TTO components, except β-myrcene, had antifungal activity. This study identified that most components of TTO have activity against a range of fungi (Hammer *et al.* 2003b).

Carson *et al.* (2006) summarised the antifungal activity of TTO against a range of fungal species published by a number of researchers obtained from over 15 papers: MICs were in the range between 0.03 and 0.5% and fungicidal concentrations from 0.12 to 2%. The exception to these ranges was *Aspergillus niger* with MFC values up to 8%. However the authors noted that these assays were conducted with fungal conidia that are known to be relatively impervious to chemical agents. Subsequent assays show that germinated conidia are significantly more susceptible to TTO than non-germinated conidia. They also noted that TTO vapours have also been demonstrated to inhibit fungal growth and affect sporulation.

Hammer *et al.* (2004) investigated the mechanism of action of TTO and its components against *Candida albicans, C. glabrata* and *Saccharomyces cerevisiae.* Yeast cells were treated with TTO or components, at one or more concentrations, for up to 6 hours. During that time, alterations in permeability were assessed by measuring the leakage of 260 nm absorbing materials and by the uptake of methylene blue dye. Membrane fluidity was measured by 1,6-diphenyl-1,3,5-hexatriene fluorescence. The effects of TTO on glucose-induced medium acidification were quantified by measuring the pH of cell suspensions in the presence of both TTO and glucose. The results showed that treatment of *Candida albicans* with TTO and its components at concentrations of between 0.25 and 1.0% altered both permeability and membrane fluidity. Membrane fluidity was also increased when *Candida albicans* was cultured for 24 hours with 0.016%-0.06% TTO, as compared with control cells. For all three organisms, glucose-induced acidification of the external medium was inhibited in a dose-dependent manner in the presence of TTO at concentrations of 0.2%, 0.3% and 0.4%. It was

concluded that the data from the study supported the hypothesis that TTO and components exert their antifungal actions by altering membrane properties and compromising membrane-associated functions.

Antiseptic and disinfectant activity

Effective skin antisepsis and disinfection are key factors in preventing many healthcare-acquired infections associated with skin microorganisms, particularly *Staphylococcus epidermidis*. The antimicrobial efficacy of chlorhexidine digluconate, a widely used antiseptic in clinical practice, alone and in combination with TTO was studied. Chlorhexidine digluconate exhibited antimicrobial activity against *Staphylococcus epidermidis* in both suspension and biofilm (MIC 2–8 mg/l) as well as TTO (2–16 g/l), but no synergistic effect was found for combination of chlorhexidine digluconate with TTO (Karpanen *et al.* 2008).

A study was conducted to determine the frequencies at which single-step mutants resistant to TTO and rifampicin occurred amongst the Gram-positive organisms *Staphylococcus aureus*, *Staphylococcus. epidermidis* and *Enterococcus faecalis*. For TTO, resistance frequencies were very low at $<10^{-9}$. Single-step mutants resistant to TTO were undetectable at two times the MIC for *Staphylococcus aureus* RN4220 and derivative mutator strains or at 3× MIC for the remaining *Staphylococcus aureus* strains, including a clinical MRSA isolate. Similarly, no mutants were recovered at 2× MIC for *Staphylococcus. epidermidis* or at 1× MIC for *E. faecalis*. Resistance frequencies determined *in vitro* for rifampicin (8× MIC) ranged from 10^{-7} to 10^{-8} for all isolates, with the exception of the *Staphylococcus aureus* mutator strains, which had slightly higher frequencies. Data suggest that Gram-positive organisms such as *Staphylococcus* spp. and *Enterococcus* spp. have very low frequencies of resistance to TTO (Hammer *et al.* 2008).

An investigation was carried out to determine the effect of Burnaid, a commercial TTO preparation, against *Enterococcus faecalis* ATCC29212, *Staphylococcus aureus* ATCC29213, *Escherichia coli* ATCC25922 and *Pseudomonas aeruginosa* ATCC27853.The organisms were suspended in sterile saline (density of 0.5 McFarland Standard) and inoculated onto horse blood agar (*E. faecalis* and *Staphylococcus aureus*) or Mueller-Hinton agar (*Escherichia coli* and *P. aeruginosa*). 100 µl of Burnaid unsterilized, Burnaid sterilized and the base product (Tinasolve™) were placed in duplicate in wells cut into the agar plates. Sterility and inactivation cultures were also performed on the samples. None of the samples were found to be contaminated with bacteria prior to testing. Only *Staphylococcus aureus* and *Escherichia coli* showed zones of growth inhibition around the Burnaid and Tinasolve. Zones of growth inhibition (22 mm) were similar for the active product (Burnaid) and the base (Tinasolve™). There was no bactericidal activity against *E. faecalis* or *P. aeruginosa*. In view of these findings and literature indicating the cytotoxicity of TTO against human fibroblasts and epithelial cells, it is recommended that this product should not be used on burn wounds (Faoagali *et al.* 1997).

Assessor's comment: This study suggests not using TTO preparations for the care of burn wounds.

Antiprotozoal activity

Carson *et al.* (2006) reported that results have been published showing that TTO has antiprotozoal activity. TTO caused a 50% reduction in growth (compared to controls) of the protozoa *Leishmania major* and *Trypanosoma brucei* at concentrations of 403 mg/ml and 0.5 mg/ml, respectively. TTO at high concentration corresponding to 300 mg/ml killed all cells of *Trichomonas vaginalis* and there is also anecdotal *in vivo* evidence that TTO may be effective in treating *T. vaginalis* infections.

3.1.2. Secondary pharmacodynamics

Antitumor activity

The potential anti-tumoral activity of TTO, distilled from *Melaleuca alternifolia*, was analysed against human melanoma M14 WT cells and their drug-resistant counterparts, M14 adriamicin-resistant cells. Both sensitive and resistant cells were grown in the presence of TTO at concentrations ranging from 0.005 to 0.03%. Both TTO and its main active component terpinen-4-ol were able to induce caspase-dependent apoptosis of melanoma cells and this effect was more evident in the resistant variant cell population. Freeze-fracturing and scanning electron microscopy analyses suggested that the effect of the crude oil and of the terpinen-4-ol was mediated by their interaction with plasma membrane and subsequent reorganization of membrane lipids. In conclusion, TTO and terpinen-4-ol were able to impair the growth of human M14 melanoma cells and appear to be more effective on their resistant variants, which express high levels of P-glycoprotein in the plasma membrane, overcoming resistance to caspase-dependent apoptosis exerted by P-glycoprotein-positive tumour cells (Calcabrini *et al.* 2004).

Human melanoma cells (M14 WT) grown in the presence of the antitumor drug adriamycin (M14 ADR) express the multidrug transporter P-gp. TTO and terpinen-4-ol proved to be capable of inhibiting the growth of melanoma cells and of overcoming multidrug resistance. The major inhibitory effect was found after treatment with 0.01% terpinen-4-ol. The effect of TTO on melanoma cells appears to be mediated by its interaction with the lipid bilayer of the plasma membrane. The experiments indicate that TTO and its main active component, terpinen-4-ol, can also interfere with the migration and invasion processes of drug-sensitive and drug-resistant melanoma cells (Bozzuto *et al.* 2011).

Liu *et al.* (2009) reported that TTO showed strong *in vitro* cytotoxicity towards human lung cancer cell line (A549), human breast cancer cell line (MCF-7) and human prostate cancer cell line (PC-3) with IC50 values (24 hr incubation) of 0.012%, 0.031% and 0.037%, respectively.

Antioxidant activity

The antioxidant activity of Australian TTO was determined using two different assays. In the 2,2-diphenyl-1-picrylhydrazyl assay, 10 µl/ml crude TTO in methanol had approximately 80% free radical scavenging activity, and in the hexanal/hexanoic acid assay, 200 µl/l crude TTO exhibited 60% inhibitory activity against the oxidation of hexanal to hexanoic acid over 30 days. The results indicate that TTO has an antioxidant activity. Inherent antioxidants, i.e., R-terpinene, R-terpinolene, and γ-terpinene were separated from crude TTO and identified chromatographically using silica gel open chromatography, C18-high-pressure liquid chromatography, and gas chromatography-mass spectrometry. Their antioxidant activities decreased in the following order in both assays: α-terpinene > α-terpinolene > γ-terpinene (Kim *et al.* 2004).

Estrogenic activity

Following 3 case reports of gynecomastia in prepubertal boys (4, 7, and 10 years old) after repeated topical use of products containing lavender, one of them in combination with TTO, *in vitro* studies on estrogenic and anti-androgenic activity of both essential oils separately were performed. It was shown that they exert *in vitro* apparently dose-related oestrogen-like activity by inducing growth in MCF-7 cells and anti-androgenic effects by increasing luciferase activity in breast-cancer (MDA-kb2) cells in presence of the androgen-receptor agonist dihydrotestosterone (DHT) at 0.1 nM. Other components in the products used by the boys may also possess endocrine-disrupting activity that contributed to the gynecomastia, but those components were not tested because lavender oil was the only one present in all the products and TTO was considered chemically similar (Henley *et al.* 2007).

The estrogenic potential of TTO was confirmed with a similar *in vitro* experimental model. However, the only three constituents of TTO which demonstrated through an *in vitro* dermal penetration study to be able to penetrate human skin to any measurable degree (terpinen-4-ol, α-terpineol and eucalyptol) did not show any estrogenic activity when analysed separately and as mixture in a ratio penetrating the

skin. It was concluded that the components of TTO which responsible of the estrogenic potential *in vitro* may not be bioavailable (Nielsen 2008).

Also the SCCP in its opinion concluded that the estrogenic potential of TTO shown *in vitro* is not supported by *in vivo* studies to elucidate the relevance of this finding for the *in vivo* situation. Moreover, since the hormonal active ingredients of TTO were shown not to penetrate the skin, the hypothesized correlation of the finding of 3 cases of gynecomastia to the topical use of TTO is considered implausible (SCCP 2008).

3.2. Overview of available pharmacokinetic data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

TTO contains terpenes, sesquiterpenes, hydrocarbons, and related alcohols. Because of the high lipophilicity of its components it has been postulated that TTO is likely to be rapidly and completely absorbed from the skin and mucous membranes (ESCOP 2009). On the other hand, *in vitro* experiments indicated that, after application of TTO to human epidermal membranes mounted in diffusion cells in the pure form and as a 20% solution in ethanol, only a small proportion of the applied amount (2-4% and 1.1-1.9% respectively) penetrated into or through human epidermis (Cross *et al.* 2008).

The major compound of TTO, terpinen-4-ol, is able to permeate human epidermis. The permeation depends on the applied preparation whereas a semisolid O/W emulsion or an ointment is superior to a cream (Reichling *et al.* 2006). The skin absorption rate of TTO was investigated *in vitro* using diffusion cell permeation experiments with heat separated human epidermis to evaluate the capability of terpinene-4-ol, the main component of the oil, to permeate human skin. Flux values (the absorption rate per unit area, µl/cm^2 h) of three different semisolid preparations containing 5% TTO were 0.067 for an oil/water emulsion, 0.051 for white petrolatum and 0.022 for a cream. Apparent permeability constants (Papp cm/s) can be calculated from flux values, taking the applied drug concentration into account. Papp values for the cream (2.74) and pure oil (1.62) were quite comparable, whereas white petrolatum (6.36) and the semi-solid oil/water emulsion (8.41) gave higher values indicating penetration enhancement (Reichling *et al.* 2006).

Considerable research has been done on the metabolism of monoterpenes. After dermal and/or oral absorption, liver P450 mono-oxygenases are involved in biotransformation. Subsequently, 60-80% of absorbed monoterpenes are excreted as glucuronides (Villar *et al.* 1994).

Cal and Krzyaniak (2006), Cal *et al.* (2006) and Cal (2008) studied the penetration behaviour of TTO and pure constituents using a flow-through diffusion cells, human skin preparations and *in vivo* human studies which represented infinitive dose and occlusive application conditions. Application times of 1, 4 or 8 hours. Neat TTO, neat terpene-4-ol and 5% terpene-4-ol (grape seed oil/carbomer hydrogel and o/w emulsion) were tested. After the exposure period, the receptor fluid and skin layers were analysed in the *in vitro* studies and the skin layers in the *in vivo* studies. TTO or pure terpene-4-ol caused a significant increase in the skin accumulation of terpene-4-ol in the hydrophilic skin layers (dermis and epidermis). In contrast to the results of Cross *et al.* (2008) and Reichling *et al.* (2006) which used only epidermis, terpene-4-ol was not detected in the receptor fluid at any stage of the study of Cal *et al.* (2006) which utilised epidermal and dermal layers. TTO or pure terpene-4-ol caused a significant increase in the skin accumulation of terpene-4-ol in the hydrophilic skin layers (dermis and epidermis). These sets of data, accumulation in the skin layers and diffusion into the acceptor fluid, suggest that *in vivo* terpene-4-ol may penetrate into the blood circulation.

Assessor's comment: In conclusion, the process of terpene penetration into the skin and through the skin can be considered to be strongly dependent on the experimental model used (choice of

membrane, hydration level and dose) and on the carrier for the penetrating terpene, while in vivo the effect of evaporation – shown to be 98% needs to be considered.

3.3. Overview of available toxicological data regarding the herbal substance(s)/herbal preparation(s) and constituents thereof

3.3.1. Single dose toxicity

The acute oral LD50 in rats has been reported as 1.9-2.6 ml/kg (1.4-2.7 g/ kg of b.w.) (Hammer *et al.* 2006, Carson *et al.* 1998, Halcón & Milkus 2004). Rats receiving 1.5 g/kg or more appeared lethargic and ataxic 72 hours post dose. By day 4 all but one animal at this dose had regained locomotor function (Hammer *et al.* 2006).

No acute inhalation toxicity was evident in response to exposure with TTO/ethanol/CO_2 in rats, but methodological weaknesses with the study were noted (SCCP, 2008).

Postulated lethal dose for a 3-year-old child was calculated to be 26 ml (Halcón & Milkus 2004).

The dermal LD50 in rabbits is > 5 g/kg (Council of Europe Committee of Experts on Cosmetic Products 2001).

3.3.2. Repeated dose toxicity

No deaths or toxic effects were reported in a 30 days-skin irritation study in rabbits using a 25% TTO in liquid paraffin other than slight initial irritation (Council of Europe Committee of Experts on Cosmetic Products 2001).

Renal toxicity has been observed in separate studies following oral administration of terperne-4-ol, cineole and cumene (similar to p-cymene). Taking into consideration the typical levels of these components in TTO, a NOEL of 117 mg/kg/day has been theoretically estimated for TTO (Nielsen 2005).

This conclusion could be substantiated by available information on repeated dose systemic toxicity of TTO constituents. Based on repeated-dose toxicity data in literature, the SCCP in 2008 has established NOAEL values in animals of six main components in Tea Tree Oil. These NOAEL's have been summarized in the table below (Norwegian Food Safety Authority (Matthylsyne) 2012):

Compound	Max content in TTO*)	Established or Estimated NOAEL (mg/kg/day)
Terpinen-4-ol	≤30%	400
1,8 Cineole	<15%	300
α-Terpinene	5-13%	60
p-Cymene	0.5-12%	75
α-Terpineol	1.5-8%	500
α-Pinene	1-6%	250

*)According to the European Pharmacopoeia

Terpinen-4-ol did not induce changes in the morphology or function of the kidneys of male Sprague-Dawley rats following 28 days of repeated oral exposure to 400 mg/kg b.w. and was considered to be non-toxic (Schilcher & Leuschner 1997). Thus the NOAEL after oral exposure may be estimated to be 400 mg/kg.

Cineole given to B6C3F1 mice by gavage for 28 days at doses up to 1200 mg/kg/day did not result in any changes. When given encapsulated at doses corresponding to 600 – 5607 mg/kg/day, some hypertrophy of hepatocytes was seen, but was not considered significant (National Toxicology

Program, cited in De Vincenzi *et al.* 2002). Cineole (8 or 32 mg/kg b.w. was given by gavage to male SPF CFLP mice 6 days per week for 80 weeks. No changes were evident in mice given cineole when compared to control mice (Roe *et al.* 1979). Based on the studies on hepatic and renal toxicity evaluated by BIBRA (British Industrial Biological Research Association), a NOAEL for cineole might be estimated as 300 mg/kg b.w., which is in agreement with the evaluation from the Norwegian Food Control Authorities in 1999 (EFSA 2012).

Based on the available information on repeated dose systemic toxicity of TTO constituents, the SCCP opinion estimated a derived NOAEL for TTO of 117 mg /kg b.w. /day for renal effects (SCCP 2008, Norwegian Food Safety Authority (Matthylsyne) 2012).

Several reports of oral toxicity can be found in the literature. Data indicate that due to its systemic toxicity, TTO should only be used as a topical agent.

General toxicology profile of TTO indicates that severe reactions would be extremely rare if TTO is not ingested (Halcón & Milkus 2004).

3.3.3. Genotoxicity

TTO produced a negative result in the *in vitro* Ames test (Saller *et al.* 1998). In December 2004 the Scientific Committee on Consumer Products (SCCP) noted that TTO is not mutagenic in the Ames test although they stated that there were insufficient details of the study and the study was deemed inadequate. They further noted that, as TTO has antimicrobial properties, an Ames test would be of limited value (SCCP 2004).

In 2005 Evandri *et al.* evaluated the mutagenic and antimutagenic activity of essential oils TTO and *Lavandula angustifolia* (lavender oil) the bacterial reverse mutation assay in *Salmonella typhimurium* TA98 and TA100 strains and in *Escherichia coli* WP2 uvrA strain, with and without an extrinsic metabolic activation system. The results showed that neither essential oil had mutagenic activity on the two tested Salmonella strains or on *Escherichia coli*, with or without the metabolic activation system, providing further evidence of the lack of mutagenic potential of TTO.

These results were also supported by a paper published by Fletcher *et al.* (2005) using Salmonella strains TA102, TA100 and TA98 in the Histidine Reversion Assay Ames test: neither TTO nor terpinen-4-ol, one of the major constituents of TTO, induced reverse mutations in any of the tester strains examined with or without metabolic activation, confirming that they are not mutagens.

Two papers were found evaluating the mutagenic potential of TTO components:

Gomes-Carneiro *et al.* (2005) investigated the genotoxicity of β-myrcene, α-terpinene and (+) and (-)-α-pinene by the Salmonella/microsome assay (TA100, TA98, TA97a and TA1535 tester strains), using a plate incorporation procedure without and with addition of an extrinsic metabolic activation system (rat liver S9 fraction induced by Aroclor 1254) and concluded that these common constituents of essential oil are not mutagenic in the Ames test.

Hammer *et al.* (2006) in a review noted that the following components were non-mutagenic in the Salmonella/microsome (Ames) test or the *Bacillus subtilis* rec- assay: terpinen-4-ol, α-terpinene, 1,8-cineole, cymene, limonene, α -pinene, β-pinene, linalool and β -myrcene. In contrast, terpineol caused a slight but dose related increase in the number of revertants with the TA102 tester strain both with and without S9 mixture. However, no significant effect was seen in the other three bacterial strains, indicating that terpineol induced a base-pair substitution affecting an A–T base pair.

In tests with mammalian cells (comet assay), γ-terpinene did not increase DNA strand breakage in human lymphocytes at 0.1 mM but did at concentrations starting from 0.2 mM. Cineole, D-(+)-

limonene, linalool, l-phellandrene and β -pinene at concentrations ranging from 10 to 1000 µM did not increase the frequency of spontaneous sister-chromatid exchanges in Chinese hamster ovary cells. Another study showed linalool to be non-mutagenic using a Chinese hamster fibroblast cell line. β-myrcene did not have mutagenic activity when tested with human lymphocytes and was not genotoxic in bone marrow cells of rats administered β -myrcene orally.

They concluded that, overall, the available data on the mutagenicity of TTO and its individual constituents indicate low mutagenic potential, using both bacterial and mammalian test systems (Hammer *et al.* 2006).

In contrast γ-terpinene was shown to protect lymphocytes against IQ- and MMC-induced DNA damages at concentrations lower than 0.2 mM (Aydin *et al.* 2005)

An *in vivo* Mouse Micronucleus Assay (ICP Firefly Pty Ltd. 2005) was conducted according to OECD Test Guideline No. 474, which was conducted under GL. TTO was administered by gavage at 1000, 1350 and 1750 mg/kg b.w. TTO. There were no increases in the frequency of micronucleated cells in any of the dose groups. There was a statistically significant depression of PCE viability and PCE+NCE ratio ($P<0.001$) in the high dose group in both sexes when compared with the vehicle control groups at 48 hours. This finding is an indication that there was sufficient exposure of the bone marrow to the test substance to elicit a response. Clinical signs in the high dose group included depressed weight gain, wobbly gait, laboured breathing and rough coat.

TTO in concentrations ranging from 95 µg/ml to 365 µg/ml increased neither the frequencies of micronuclei nor the frequencies of chromosomal aberrations in human lymphocytes. Higher concentrations could not be tested, since at higher concentrations cell viability was significantly reduced. Within that limitation, these results suggest that TTO does not induce chromosome aberration (Pereira *et al*. 2014).

3.3.4. Carcinogenicity

No available data.

Methyleugenol

The Scientific Committee on Consumer Products in its updated "Opinion on Tea Tree Oil" in 2008 stated that since methyleugenol was reported as a minor constituent of TTO *"the content should be indicated. According to the opinion SCCNFP/0373/00 on methyleugenol in fragrances the content in finished leave-on products should not exceed 0.0002% (2 ppm) and in rinse-off products 0.001% (10 ppm)."*

This statement follows the EMEA "Public statement on the use of herbal medicinal products containing methyleugenol" (2005) reporting a content of 0.28 to 0.9% of the natural potential carcinogen methyleugenol in TTO. However HMPC has concluded that "the present exposure to methyleugenol resulting from consumption of herbal medicinal products (short time use in adults at recommended posology) does not pose a significant cancer risk."

The Australian TTO industry reports that these levels of methyleugenol refer to *Melaleuca bracteata*, whereas commercial TTO is derived solely from *Melaleuca alternifolia*; analytical surveys conducted by Australian TTO industry show that *Melaleuca alternifolia* contains only trace levels of methyleugenol.

Southwell *et al.* (2011) quantified the traces of methyleugenol previously reported in TTO ranging from less than 0.01% to 0.06% (mean 0.02%).

3.3.5. Reproductive and developmental toxicity

No data available on TTO.

However, exposure to α-terpinene (125 or 250 mg/kg b.w.), present at approximately 9% in TTO, for nine consecutive days caused decreased body weight gain in pregnant Wistar rats. The offspring of dams given 60 mg/kg b.w. from day 6 to day 15 of pregnancy had delayed ossification and skeletal malformations. At 30 mg/kg b.w. no effects were seen on either dams or offspring. Effects at doses higher than 60 mg/kg b.w. were accompanied by maternal toxicity. The authors suggested a NOAEL for embryofoetotoxicity of 30 mg/kg b.w. for oral exposure of rats to α-terpinene (Araujo *et al.* 1996). These limited data suggest that TTO is potentially embryofoetotoxic, although only if ingested at relatively high levels (Araujo *et al.* 1996).

Hammer *et al.* (2006), noted that the embryofoetotoxicity of α-terpinene (normally present in TTO at 9%) has been evaluated and found that at oral doses of greater than 60 mg/kg b.w. there was delayed ossification and skeletal malformations in the foetuses and this was accompanied by maternal toxicity. The test material was administered to rats from day 6 to day 15 of gestation. The authors concluded that TTO is potentially embryofoetotoxic although only if orally ingested at relatively high doses.

3.3.6. Local tolerance

Skin irritation

Two studies were conducted on groups of 3 female rabbits of the New Zealand strain according to the methodology detailed in OECD guideline 404 and were GLP compliant. In the first study TTO (100%) was applied undiluted on 4x4 cm patches. In the second study, dilutions of 75-12.5% TTO were applied for 4 hours with a semi-occlusive patch application followed by a 14 days observation period. The results showed that, in the first study, TTO (100%) was found to be a mild irritant at 60 minutes post exposure, a severe irritant at 24 and 48 hours, a moderate irritant at 72 hours and a mild irritant 7 and 14 days following a 4 hour semi-occlusive patch application on intact skin. At 21 days the skin had returned to normal. In the second study, TTO (75%) was found to be a mild to moderate irritant, TTO (50%) was found to be a minimal irritant. TTO (at 25% and 12.5%) was found to be a non-irritant (SCCP December 2004).

Draize skin irritancy index was found to be 5.0, based on application of 100% TTO to intact and abraded skin of albino rabbits, thus signifying that TTO could cause dermatitis in some users (Halcón & Milkus 2004).

The acute dermal LD_{50} in rabbits was recorded as in excess of 5.0 g/kg since this dose resulted in 2/10 deaths in rabbits. Furthermore, it was observed at necropsy that neat TTO produced irritant effects and skin abnormalities in rabbits patch tested at this dose for 24 h with occlusion. Pure (100%) TTO applied to the skin of albino rabbits and maintained at 2 g/kg for 24 hours resulted in no signs of toxicity (Halcón & Milkus 2004).

A 30-day dermal irritation test in rabbits using 25% TTO in paraffin on shaved skin did not result in visible signs of irritation. Therefore, TTO should not be used for conditions where skin irritability is already present (e.g. dermatitis) (Halcón & Milkus 2004).

Eye irritation

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

discharge lasting for up to six hours following instillation of 1% TTO and conjunctival redness and discharge for up to 24 hours following instillation of 5% TTO. In both groups, the maximal response was observed after one hour. Based on these observations, the author concludes, that both TTO solutions can be classified as "minimally irritating" (SCCP 2008).

Ototoxicity

TTO was found to produce ototoxicity when applied in the ears of guinea pigs at 100% concentration, but no ototoxicity was found for 2% solutions (Halcón & Milkus 2004).

Skin sensitisation potential

In order to test the potential of TTO to cause skin sensitisation guinea pigs were pre-treated 2 times via intradermal injections and an epidermal induction application of the oil. Two weeks after the induction application, the animals were tested on one flank with the maximum sub-irritant concentration of the oil. No irritant response was observed (Halcón & Milkus 2004).

A guinea pig maximization assay using the Magnusson and Kligman method (Pharmaceutical Consulting Service 1989) and albino guinea pigs (20 per group) has been conducted with TTO. During the induction phase, two 0.1 ml intradermal injections were given to the animals. One week later, 5% TTO was applied to the skin at the injection site under occlusion for 48 hours. After a two week period, a 30% TTO challenge dose was applied to the skin under occlusion for 48 hours. There was no evidence of sensitisation in this assay. In a published report, TTO of unknown quality was tested in 10 guinea pigs using an adjuvant maximization protocol. The induction concentration was not given. At an elicitation concentration of 30%, 3/10 guinea pigs gave positive reactions at the 48-hour reading. At 10%, no reactions were observed. The main component of TTO, terpinen-4-ol, gave no response when cross-challenged in the reacting animals. These results may indicate that TTO may be a weak skin sensitizer. The disagreement between the two studies cannot be explained, other that it could have been the result of different quality and oxidation state of the TTO tested.

Three samples of TTO were tested in the Mouse Local Lymph Node Assay (LLNA) (RCC Ltd. Study A69041, Study A78682, Study A78816 2006). Two of the samples were non-oxidised, undegraded oil, while the third was a severely oxidised and degraded. The EC3 (calculated concentration of the test substance which elicited a three-fold increase in the Stimulation Index) values of 24.3% and 25.5% were obtained with the two undegraded oil samples, while the EC3 of the degraded oil was 4.4%. There was a clear dose-response in each case. Another sample of undegraded TTO was sent to a different laboratory (MB Research Laboratories 2007) which could perform immunophenotyping of the lymphocytes. An EC3 value of 8.3% was calculated in this LLNA. Similarly the %B cells, %T cells, and B:T ratio indicated a sensitising response. Overall, these results show that undegraded TTO has a weak potential for sensitisation in this assay system. Degraded TTO had 5-times higher potency, but would still be regarded as a moderate sensitizer.

The peroxide value and p-cymene content are particular useful indicators of the age of the oil and the extent of degradation (Southwell 2006). The peroxide value is a measure of available oxygen, i.e. how much one or more components of the oil have absorbed oxygen in the form of peroxide. Therefore, the Peroxide Value is an indicator of the presence of peroxides. Generally, good quality fresh oils will have a peroxide value below 10 µeq O_2. Peroxides degrade over time and the degradation products, such as 1,2,4-trihydroxymenthane, may have a high irritation and sensitisation potential. The peroxide value will fall as the peroxides decompose. A very old (>10 years old), decomposed oil could have a low peroxide value. Such an oil will have elevated levels of the decomposition products and potentially elevated p-cymene (16% plus). p-Cymene, occurs naturally in TTO (typically 0.5 – 8%).

Southwell (2006) examined 26 TTO samples and demonstrated that the presence of 1,2,4-trihydroxymenthane in TTO is a rare event and in the cases where this breakdown product was found, the oils were extremely old and severely degraded to the extent where the oils would not be compliant to the ISO Standard. Even in extremely degraded oils, 1,2,4-trihydroxymenthane concentrations were less than 5%. Consequently, although 1,2,4-trihydroxymenthane can be detected by GC and GC/MS in aged TTOs, when concentrations are low (<1%), the triol peak can easily be hidden by other 37 oil peaks in the same region and therefore the presence of 1,2,4-trihydroxymenthane is not suitable to check possible degradation of TTO. The use of other degradation products as degradation markers is even more difficult as it has not been possible to consistently and positively identify ascaridole, ascaridole glycol, the keto-epoxide and the di-epoxide that have been tentatively identified in degraded TTOs as well as these products being present in even smaller concentrations than the triol. Furthermore, Southwell also demonstrated a relationship between the levels of p-cymene and 1,2,4-trihydroxymenthane. Thus, Southwell has proposed monitoring the degradation status of TTO by using p-cymene as a reliable marker.

For many years, 1,8-cineole was regarded as an undesirable constituent in TTO due to its reputation as a skin and mucous membrane irritant. However, other studies suggested that this component is not responsible for a large proportion of sensitivity reactions (Carson *et al*. 1998).

Oxidation products are the likely allergens. Since oxidized TTO appears to be a more potent allergen than fresh TTO, human adverse reactions may be minimized by reducing exposure to aged, oxidized oil (Carson & Riley 2001).

Phototoxicity

Although some irritation was observed, undiluted TTO did not produce phototoxic effects on the skin of hairless mice (Carson *et al.* 1998).

3.3.7. Other special studies

Neurotoxicity

A case report documented TTO poisoning after a single dermal application of 120 ml of undiluted TTO to 3 adult intact female purebred Angora cats, one of which died. The cats were severely infested with fleas, so they were shaved and the oil was applied directly to the cats' skin. The shaving produced no nicks on the skin; however, numerous flea bites were visible. The product used to eliminate fleas was labelled for use as a spot treatment for skin lesions, but a catalogue advertised that it would repel fleas when diluted and used as a dip. All animals exhibited hypothermia, incoordination, dehydration and trembling. The surviving 2 cats recovered after 1-2 days (Bischoff & Guale 1998). Neurotoxicity and death have been observed in cats exposed to very high doses of TTO by the dermal route. However, the possibility that these animals were also exposed by the oral route by licking of the skin and fur of the application area cannot be ruled out.

Villar *et al*. (1994) reported that cases of TTO toxicosis have been reported by American veterinarians to the National Animal Poison Control Centre when the oil was applied on derma of dogs and cats. They noted that, in most cases, the oil was used to treat dermatologic conditions at inappropriate high doses. The typical signs observed were depression, weakness, incoordination and muscle tremors. Treatment of clinical signs and supportive care was sufficient to achieve recovery without sequelae within 2-3 days.

Cytotoxicity studies

TTO and components of TTO was tested on several human cell lines *in vitro*. Cytotoxicity with 100% TTO ranged from 0.02 to 2.8 g/l, with epithelial-like cells being the most robust, and liver-derived cells being the most susceptible. Cytotoxicity for the components of TTO was as follows: 1,8-cineole, from 0.14 to 4.2 g/l; terpinen-4-ol, from 0.06 to 2.7 g/l; α-terpineol, 0.02 to 1.1 g/l. These data suggest that topical use of TTO is suitable, as epithelial cell seem to be the most resistant cells to its potential cytotoxicity (Halcón & Milkus 2004).

3.4. Overall conclusions on non-clinical data

Studies on TTO demonstrate that adequate doses have broad spectrum antimicrobial activity with little evidence for inducing tolerance and resistance. There is also some evidence of TTO possessing anti-inflammatory activity.

The cytotoxic activity towards a range of cancer cell types shown by means of *in vitro* studies is not considered relevant for the purpose of this assessment.

The published pharmacokinetic data on TTO are minimal. *In vitro* skin permeation studies using human skin preparations demonstrate that the extent of penetrating of TTO components is very low, with the more polar terpenen-4-ol and α-terpineol being the only components which penetrate to any appreciable levels. The total penetration of TTO is 2-4% and 7% of applied dose under non-occluded and partly occluded conditions. Under infinite dose, occluded conditions terpenen-4-ol can cumulate within the skin which may act as a reservoir for gradual elimination into the circulation. However, these conditions are not representative of the typical use pattern of TTO. As TTO oil is a semi-volatile substance, the majority of the applied dose rapidly evaporates from the surface of the skin before it has the chance to absorb into the skin.

TTO has been reported to cause mild to moderate skin irritation in rabbit studies. Local lymph node assay (LLNA) studies indicate that TTO has mild skin sensitisation potential. Highly degraded TTO has a greater potential for skin sensitisation due to the presences of oxidation by-products. Proper storage and handling of TTO and its formulated products are needed to avoid the development of these by-products and reduce the risk of skin irritation and sensitisation in sensitive individuals (Nielsen 2005).

There are no oral repeated dose toxicity studies available for TTO. However, there are no known indications which require oral administration of TTO. The main route of administration is by dermal application. Repeated dose data are available on some of the main components of TTO. Renal toxicity has been observed in separate studies following oral administration of terperne-4-ol, cineole and cumene (similar to p-cymene). Taking into consideration the typical levels of these components in TTO, a NOEL of 117 mg/kg/day has been theoretically estimated for TTO (Nielsen 2005).

TTO was negative in the Ames assay using *Salmonella typhimurium* TA102, TA100 and TA98 examined with or without metabolic activation and not genotoxic in *in vitro* mammalian cells in concentrations ranging from 95 µg/ml to 365 µg/ml (Pereira *et al*. 2014). It did not induce clastogenicity in the *in vivo* mouse micronucleus assay (Fletcher *et al*. 2005).

Available data on the genotoxicity indicate low genotoxic potential of its major constituents, α-terpineol and γ-terpinene, in bacterial or mammalian test systems. α-Terpineol is slightly mutagenic but only in *Salmonella typhimurium* strain TA102. γ -Terpinene is slightly clastogenic. When tested by the comet assay, it induced DNA damage in human lymphocytes at concentrations starting from 0.2 mM (Aydin *et al*. 2005).

The slight genotoxic potential of α-terpineol and γ -terpinene do not seem to lead to a carcinogenic effect, since both compounds are not listed as carcinogens in IARC and NTP databases.

There are no experimental data on the mutagenic and clastogenic potential of terpinolene (= δ-terpinene, cas nr 586-62-9). This seems not to be a concern, since no genotoxic effects have been reported for TTO, which may contain up to 5% terpinolene. In addition, terpinolene is not listed as a carcinogen in IARC and NTP databases.

While TTO contains trace levels of methyleugenol, the typical use pattern in adults, being short-term dermal use is not expected to pose a significant cancer risk (Nielsen 2005).

4. Clinical Data

4.1. Clinical Pharmacology

4.1.1. Overview of pharmacodynamic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents

The mechanisms of antimicrobial action elucidated so far reflect the terpenic hydrocarbon composition and indicate that cytoplasmic membrane integrity is compromised by treatment with TTO or some of its major components. Alterations in eukaryotic cell membranes have also been observed with TTO and terpinen-4-ol treatment (Longbottom *et al.* 2004, Calcabrini *et al.* 2004).

Pharmacological studies in humans

Pharmacological studies conducted in humans have been discussed in the ESCOP Monograph Supplement 2009. Messager *et al.* 2005 reported on the antimicrobial activity of TTO for hand cleansing. Koh *et al.* 2002 and Pearce *et al.* 2005 reported on the anti-allergenic and anti-inflammatory effects of TTO on histamine and nickel-induced skin reactions.

Khalil *et al.* 2004 have also investigated the regulation of wheal and flare by undiluted TTO on histamine-induced skin responses in human skin. 18 subjects had 25 µl of 100% TTO applied topically to the histamine-induced reaction site at 10 minutes and 20 minutes after histamine injection intradermally to the inner forearm skin. One arm of each subject was the study arm and the other arm (randomly allocated) was the control arm with no control oil applied to the reaction site. The TTO significantly reduced both the flare and wheal response at 30 minutes and 50 minutes respectively after histamine injection. No adverse effects were reported.

Canyon & Speare 2007 conducted head lice (*Pediculus humanus* var. *capitis*) avoidance experiments on the arm of the researcher. Circles of skin (2.5 cm in diameter) were marked out and test materials were applied to a test area. These test materials consisted of 100% TTO, a variety of other oils, neem insect repellent, N,N-Diethyl-3-methylbenzamide (DEET) 69.75 g/l (positive control) and KY-Jelly, inert lubricant gel (negative control). After 2 minutes, 15 lice were placed onto each treated area. TTO repelled 55% of head ice from treated area, followed by peppermint oil (34%) and DEET (26%). TTO was most effective at preventing lice from feeding (60%) followed by lavender oil (40%), peppermint (28%) and DEET (23%).

A summary of these studies is presented in **Table 4**.

Table 4: Pharmacological studies in humans

Indication	Reference	Method	Participants	Posology	Interventions	Outcomes	Comments
Prevention of head lice	Canyon & Speare 2007	Controlled experiments on researcher's forearms	Researcher	Test materials applied with well-soaked cotton bud	100% TTO compared to various other oils and neem insect repellent Positive control: N,N-Diethyl-3-methylbenzamide (DEET) 69.75 g/l Negative control: KY-Jelly, inert lubricant gel Lice applied to treated area after 2 mins	TTO repelled 55% of head ice from treated area, followed by peppermint (34%) and DEET (26%). TTO was most effective at preventing lice from feeding (60%) followed by lavender (40%), peppermint (28%) and DEET (23%). Most repellents were not effective at causing lice to leave the treated site or prevent blood feeding	
Reduction of nickel-induced contact hypersensitivity reaction	Pearce *et al.* 2005	4 arm controlled trial	18 subjects with nickel hypersensitivity (17F/1M; 19-57 years); 18 subjects used 100% TTO. 7subjects used Macadamia oil. 10 subjects used 5% TTO and placebo lotion	25 µl topical application 3 and 5 days after nickel exposure	Treatment 1: 100% TTO (complying with ISO4730); Control 1: 100% macadamia oil Treatment 2: 5% TTO lotion Control 2: placebo lotion (no TTO)	100% TTO significantly reduced the flare area and erythema index when compared to the nickel-only sites. The other substances had no significant effect.	No adverse skin reactions to TTO reported
Effectiveness of hand-cleansing	Messager *et al.* 2005	Study 1: 3 arm controlled trial: Hygienic skin wash (HSW) and 5% TTO in Tween 80 vs. Soft Soap (SS) control; Study 2: 2 arm controlled trial: Alcoholic hygienic skin	Study 1: 13 subjects (8F/5M; 22-52 yrs): Study 2: 14 subjects (8F/6M; 19-53 yrs)	Followed 'EN1499 European Hand washing Method' against *Escherichia coli*: 5 ml of SS (control) or treatment antiseptic product poured into cupped hands pre-moistened with tap water, and 6 steps of hand washing procedure performed. Enough water to create lather and hand wash continued for 60s.	Treatments: HSW(5% TTO); AHSW (5% TTO, 10% alcohol); 5% TTO; 0.001%(v/v) Tween 80 in sterile distilled water; Control: SS recommended by EN1499 (linseed oil; potassium hydroxide; ethanol, sterile distilled water) TTO meets ISO4730;	5% TTO in Tween 80 and AHSW were significantly more active than SS (control); HSW appeared slightly more active than SS (control) but difference not significant. 5% TTO in Tween 80 was significantly more active than HSW.	

Indication	Reference	Method	Participants	Posology	Interventions	Outcomes	Comments
		wash (AHSW) vs. Soft Soap (SS) control		Hands rinsed under tap water for 15s from distal to proximal with fingertips upright.			
Reduction of wheal and flare associated with histamine induced responses	Khalil *et al.* 2004	Controlled trial, participants act as own control. Allocation of arms randomly assigned to control or treatment (alternating fashion)	18 participants (testing 100% TTO)	25 µl of TTO applied topically with a pipette to histamine-induced reaction area after 10 and 20mins	Treatment: 100% TTO Control: no treatment	TTO significantly reduced the wheal and flare response. Significant difference in flare observed 30mins from histamine injection and in wheal observed 50 mins from histamine injection.	No adverse effects
Reduction of histamine induced skin inflammation	Koh *et al.* 2002	Controlled trial, participants act as own control. Allocation of arms randomly assigned to control or treatment (alternating fashion)	21 participants testing 100% TTO (16F/5M; 23-56 yrs); 6 participants testing liquid paraffin (5F/1M; 23-54 yrs)	25µl of TTO applied topically with a pipette to histamine-induced reaction area after 20mins	Treatment 1: 100% TTO Treatment 2: liquid paraffin Control: no treatment	Mean weal volume significantly decreased after TTO application (30mins and 60mins) compared to control. Liquid paraffin had no significant effect on weal or flare. No difference in mean flare area between control and TTO.	No adverse effects

TTO – Tea Tree Oil

F – Female

M – Male

4.1.2. Overview of pharmacokinetic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents

Considerable research has been done on the metabolism of monoterpenes. After rapid dermal and/or oral absorption, liver P450 mono-oxygenases are involved in biotransformation. Subsequently, 60-80% of absorbed monoterpenes are excreted as glucuronides (Villar *et al.* 1994).

Cal and Krzyaniak (2006), Cal *et al.* (2006) and Cal (2008) studied the penetration behaviour of TTO and pure constituents using a flow-through diffusion cells, human skin preparations and *in vivo* human studies which represented infinitive dose and occlusive application conditions. TTO or pure terpene-4-ol caused a significant increase in the skin accumulation of terpene-4-ol in the hydrophilic skin layers (dermis and epidermis).

The process of terpene penetration into the skin and through the skin can be considered to be strongly dependent on the experimental model used (choice of membrane, hydration level and dose) and on the carrier for the penetrating terpene, while *in vivo* the effect of evaporation – shown to be 98% needs to be considered.

Human pharmacokinetic data are not available for tea tree oil. *In vitro* dermal penetration studies using human skin preparations indicate that dermal absorption of TTO components is relatively low, up 2-4% of applied dose and the main components observed to penetrate were terpene-4-ol and α-terpineol. As the components of TTO are semi-volatile, the majority of the applied dose evaporates from the surface of the skin (Cross *et al.* 2008).

4.2. Clinical Efficacy

Clinical trials have been performed to test the efficacy of topical TTO products for a range of conditions including acne, wound healing, mycosis (oral candidiasis, denture stomatitis, onychomycosis, tinea and tinea pedis), protozoan infections, herpes labialis, dandruff, tinea.

4.2.1. Dose response studies

Not applicable.

4.2.2. Clinical studies (case studies and clinical trials)

4.2.2.1. Overview

Clinical studies on effects of TTO were conducted for the following indications (presented in 4.2.2.2):

- Acne vulgaris
- Wound healing
- Protozoan infections
- Mycosis
    - Onychomycosis
    - Oropharyngeal candidiasis
    - Denture stomatitis
    - Tinea pedis
    - Various dermatological mycosis
    - *Candida albicans* vaginal infection
- Recurrent herpes labialis

- Halitosis
- Supragingival plaque
- Minor skin lesions
- Dandruff

Clinical studies conducted with combinations containing TTO (presented in 4.4.4.3):

- Mycosis
  - Onychomycosis
- Pediculosis

4.2.2.2. Clinical studies conducted with TTO

Acne vulgaris

Bassett *et al.* 1990 and Enshaieh *et al.* 2007 conducted randomised controlled trials and reported on the use of TTO for the treatment of mild to moderate acne.

A comparative study of tea-tree oil versus benzoylperoxide in the treatment of acne (Bassett *et al.* 1990).

A single-blind, randomised clinical trial on 124 patients to evaluate the efficacy and skin tolerance of 5% TTO gel in the treatment of mild to moderate acne when compared with 5% benzoyl peroxide lotion was performed. The results of this study showed that both 5% tea-tree oil and 5% benzoyl peroxide had a significant effect in ameliorating the patients' acne by reducing the number of inflamed and non-inflamed lesions (open and closed comedones), although the onset of action in the case of tea-tree oil was slower. Fewer side effects were experienced by patients treated with tea-tree oil (Bassett *et al.* 1990).

The efficacy of 5% topical TTO gel in mild to moderate acne vulgaris: A randomised, double-blind placebo-controlled study (Enshaieh *et al.* 2007).

One study has been conducted on the possible efficacy of TTO in treatment of the acne vulgaris. It was a randomised double-blind clinical trial performed in 60 patients with mild to moderate acne vulgaris. They were randomly divided into two groups and were treated with TTO gel 5% (n=30) or placebo (n=30). They were followed every 15 days for a period of 45 days. Response to treatment was evaluated by the total acne lesions counting and acne severity index (ASI). The data was analysed statistically using t-test and by SPSS program. There was a significant difference between TTO gel and placebo in the improvement of the total acne lesions counting and also regarding improvement of the ASI. In terms of total acne lesions counting and ASI, TTO gel was 3.55 times and 5.75 times more effective than placebo respectively. Side-effects with both groups were relatively similar and tolerable. The authors concluded that topical 5% TTO is an effective treatment for mild to moderate acne vulgaris (Enshaieh *et al.* 2007).

Assessor's Comment: 5% TTO gel showed to ameliorate acne lesions in two studies.

Feinblatt 1960 reported on the use of TTO for the treatment of furunculosis (boils). Thirty five patients (26 males and 9 females) with furuncles located in various sites (18 in the neck, 8 on the back, 6 in the axilla areas, 1 on the scalp, 4 on the face and forehead, 4 on the forearm, 1 on the calf and 1 on the external ear) many of them at multiple sites were enrolled in the study. Ten patients were given expectant treatment and 25 were treated with TTO painting the surface over the furuncle freely with the oil two or three times daily, after thoroughly cleaning the site. Results showed that, of the 10 untreated controls, five of the boils were finally incised and in five cases the infected site of the furuncle was still apparent after eight days. In the 25 cases treated with TTO, only one boil required

incision and in 15 cases the infected site of the furuncle was removed completely in eight days. In six cases the infected site of the furuncle, while still present after eight days, was reduced more than one-half and in three cases the infected site was reduced less than half in eight days. As to local reactions, three patients complained of slight temporary stinging.

In the same paper Feinblatt (1960) described a typical case report of a male patient aged 40 under treatment for diabetes mellitus, who complained of recurrent boils. TTO was applied directly over a large boil (3 x 3 cm), swollen, reddened and painful boil on his neck two or three times daily after thorough cleansing. There was definite improvement within two days, most of the inflammation had disappeared after four days and the skin healed after eight days with no untoward effects or local reactions. The patient repeated the use of TTO whenever a new boil developed and every time the further boils development aborted. The Author concluded that, due to its high germicidal activity against Staphylococcus aureus and on the basis of rapid healing without scarring achieved in the study, TTO may be used as an alternative option before surgical intervention in furunculosis.

Wound healing

Uncontrolled, open-label, pilot study of TTO solution in the decolonisation of MRSA positive wounds and its influence on wound healing (Carson *et al.* 2010, Edmondson *et al.* 2011).

The primary aim of an uncontrolled case series study was to assess whether a TTO solution used in a wound cleansing procedure could decolonise MRSA from acute and chronic wounds of mixed aetiology. The secondary aim was to determine if the TTO solution influenced wound healing outcomes. The product used was a water-miscible 10% v/v TTO solution. Nineteen participants with wounds suspected of being colonised with MRSA were enrolled in a pilot study. Seven were subsequently shown not to have MRSA and were withdrawn from the study. As many as 11 of the remaining 12 participants were treated with a wash solution of 3.3% TTO manually shaken in water; the solution was applied as part of the wound cleansing regimen at each dressing change. Dressing changes were three times per week or daily as deemed necessary by the study nurse following assessment. One participant withdrew from the study before treatment. No participants were MRSA negative after treatment. After treatment had been implemented, 8 out of the 11 treated wounds had begun to heal and reduced in size as measured by computer planimetry. TTO did not appear to inhibit healing and the majority of wounds reduced in size after treatment.

Two adverse events of pain were reported by participants who experienced pain during the cleansing procedure that may or may not have been because of the irrigation with the TTO solution (Edmondson *et al.* 2011).

Assessor's comment: this study shows that treatment with TTO can influence positively wound healing through its antimicrobial activity; limit of the study is the small number of participants.

The Effect of Tea Tree Oil (*Melaleuca alternifolia*) on Wound Healing Using a Dressing Model (Chin & Cordell 2013)

A quasi-experimental study with 10 volunteers, who had wounds assumed to be infected with *Staphylococcus aureus,* were carried out to replicate in humans a 2004 *in vitro* study that used the same dressing model over Petri dishes to determine the antimicrobial effects of the fumes of TTO. Four of the 10 patients were used as matched participants to compare wound healing times between conventional treatment alone and conventional treatment plus fumes of TTO. The results demonstrated decreased healing time in all but one of the participants treated with TTO. However the study has several limitations: the small number of patients, whose characteristics were not compared with patients who did not volunteered and whose abscessed lesions were not all cultured to identify the offending micro-organism; moreover the type of antibiotics used concomitantly by the participants was

not controlled as well as other variables such as the age and the immune system function (Chin & Cordell 2013).

An Integrated Approach to Methicillin-resistant *Staphylococcus aureus* Control in a Rural, Regional-Referral Health care setting (Bowler *et al.* 2010).

A study was performed in a regional-referral hospital, 5 affiliated nursing homes and an outpatient MRSA clinic, in order to identify methods that could be applied in other resource-limited healthcare setting to decrease the prevalence and nosocomial transmission of MRSA.

Residents of the 5 nursing homes were screened for MRSA at baseline and 1 year later to detect the microbial quality improvement. Active surveillance cultures were performed on subsequently admitted nursing home residents, "high-risk" patients admitted to the hospital, and household contacts of clinic patients. The initial phase of decolonization consisted of systemic therapy with minocycline and rifampin and topical therapy with nasal mupirocin ointment and a bath or shower with 5% TTO body wash once per day for 7 days. During the following 5 months only 2% mupirocin nasal ointment two times daily and at least one TTO body wash once per day was applied for the first 5 days of each month. Three separate samples for cultures to document clearance of MRSA colonization were obtained at 1-week intervals 1 month after the completion of decolonization therapy. Samples for follow-up cultures were obtained at month 6 and month 12 after the completion of decolonization therapy. After intervention and follow-up for 12 months or more, the prevalence of MRSA carriage at the nursing homes decreased by 67% (P <0.001), and 120 (82%) of 147 nursing home residents and 111 (89%) of 125 clinic patients remained culture-negative for MRSA. Twenty-three (24%) of 95 new clinic patients had at least 1 MRSA-positive contact. Mupirocin resistance did not develop. In the hospital, the incidence rate of nosocomial MRSA infection decreased from 0.64 infections per 1,000 patient-days before the interventions to 0.40 infections per 1,000 patient-days 1 year after the interventions and to 0.32 infections per 1,000 patient-days 2 years after the intervention (P <0.01).

The author concluded that the use of active surveillance cultures and decolonization therapy was effective in decreasing the prevalence of asymptomatic carriage, the incidence of nosocomial infection, and the overall prevalence of MRSA. However it has to be noted that the number of patients lost to the follow-up was high (105 out of 272 patients who underwent decolonization in the nursing homes and in the MRSA clinic) (Bowler *et al.* 2010).

Chronic Wound Treatment with Topical Tea Tree Oil (Culliton & Halcon 2011)

A case study has been reported on the healing of a chronic lower-extremity wound in an 85-years old and more than 70 years smoker man using on a daily basis an impregnated dressing of 10% TTO in pumpkin seed oil after rinsing the wound. Even though the likelihood of the wound healing was minimal, it is not known whether the wound would have healed without the treatment and the role of pumpkin seed oil in the healing process. The author suggests that this approach could be explored in further studies as a wound treatment of diabetic patients with non-healing foot ulcers (Culliton & Halcon 2011).

Protozoan infections

A clinical investigation to determine the efficacy and safety of TTO use for vaginal douche and topical application in the treatment of trichomonal vaginitis, *Candida albicans* vaginitis and other vaginal infections was performed. The medication studied was a special emulsified 40% solution of Australian TTO with isopropropyl alcohol 13%. Hundred thirty cases of vaginal infections were investigated: trichomonal vaginitis (n=96), *Candida albicans* vaginitis (n=4), nulliparous cervicitis from *Trichomonas vaginalis* (n=20), chronic endocervicitis (n=10). Australian TTO was found to be highly effective in

treatment of tricomonal vaginitis, *Candida albicans* vaginitis, cervicitis and chronic endocervicitis (Peña 1962).

Mycosis

Onychomycosis is a superficial fungal infection that destroys the entire nail unit. It is the most frequent cause of nail disease, ranging from 2% to 13%. Standard treatments include debridement, topical medications, and systemic therapies.

Comparison of two topical preparations for the treatment of onychomycosis: TTO and clotrimazole (Buck *et al.* 1994).

A double-blind, multicenter, randomised controlled trial was performed at two primary care health and residency training centres and one private podiatrist's office to assess efficacy and tolerability of topical application of 1% clotrimazole solution compared with that of 100% TTO for the treatment of toenail onychomycosis.

The participants included 117 patients with distal subungual onychomycosis proven by culture. Patients received twice-daily application of either 1% clotrimazole solution (n=53) or 100% TTO oil (n=64) for 6 months. Debridement and clinical assessment were performed at 0, 1, 3, and 6 months. Cultures were obtained at 0 and 6 months. Each patient's subjective assessment was also obtained 3 months after the conclusion of therapy. Adverse reactions were erythema, irritation and oedema (7.8% in TTO and 5.7% in clotrimazole group), which cause the dropping out of four (3%) of the initial participants.

The baseline characteristics of the treatment groups did not differ significantly. After 6 months of therapy, the two treatment groups were comparable based on culture cure (clotrimazole = 11%, TTO = 18%) and clinical assessment documenting partial or full resolution (clotrimazole = 61%, TTO = 60%). Three months later, about one half of each group reported continued improvement or resolution (clotrimazole = 55%; TTO = 56%).

Topical therapy, including the two preparations presented in this paper, provide improvement in nail appearance and symptomatology. The study shows that use of a topical preparation in conjunction with debridement is an appropriate initial treatment strategy (Buck *et al.* 1994).

Assessor's comment: the study shows efficacy of 100% TTO solution comparable to clotrimazole in the treatment of onychomycosis.

Syed *et al.* 1999 conducted a double blind randomised controlled trial investigating the treatment of onychomycosis. 40 patients were randomly allocated to the Treatment group of 2% butenafine hydrochloride and 5% TTO and 20 patients were randomly allocated to the control group consisting of a TTO cream of unspecified concentration. After 16 weeks of topical application three times daily and covering with an occlusive plastic dressing, 80% in the treatment group were cured and no patients in the control group were cured. TTO in the control cream did not show the expected response and TTO was mixed with butenafine hydrochloride in the treatment group, it is difficult to determine whether the TTO produced any effect in this group. Treatment in the control group was discontinued after 8 weeks so it is possible that the control treatment did not have sufficient time to render its full potency.

Oropharyngeal candidiasis. Oropharyngeal candidiasis is the most common opportunistic infection observed in the patients with HIV/AIDS.

Efficacy of *melaleuca* oral solution for the treatment of fluconazole refractory oral candidiasis in AIDS patients (Jandourek *et al.* 1998, Vazquez & Zawawi 2002).

Efficacy of *Melaleuca* oral solution, an USA branded non-prescription commercial mouthwash, in AIDS patients with fluconazole-resistant oropharyngeal *Candida* infections was investigated in two studies.

A prospective, single centre, open-labelled study was performed on thirteen patients with AIDS and oral candidiasis documented to be clinically refractory to fluconazole, as defined by failure to respond to a minimum of 14 days of > or = 400 mg fluconazole per day. Additionally, patients had in *in vitro* resistance to fluconazole, defined by minimal inhibitory concentrations of > or = 20 µg/ml.

Patients were given 15 ml *Melaleuca* oral solution four times daily to swish and expel for 2-4 weeks.

Resolution of clinical lesions of oral pseudomembranous candidiasis lesions evaluations were performed weekly for 4 weeks and at the end of therapy for clinical signs of oral candidiasis. Quantitative yeast cultures were performed at each evaluation.

A total of 13 patients were entered into the study, 12 were evaluable. At the 2-week evaluation, 7 out of 12 patients had improved, none were cured, and 6 were unchanged. At the 4-week evaluation, 8 out of 12 patients showed a response (2 cured, 6 improved), 4 were non-responders, and 1 had deteriorated. A mycological response was seen in 7 out of 12 patients. A follow-up evaluation 2-4 weeks after therapy was discontinued revealed that there were no clinical relapses in the 2 patients who were cured.

The authors concluded that *melaleuca* oral solution appeared to be effective as an alternative regimen for AIDS patients with oropharyngeal candidiasis refractory to fluconazole (Jandourek *et al.* 1998).

The efficacy of alcohol-based and alcohol-free USA branded non-prescription commercial mouthwashes containing TTO in patients with AIDS and fluconazole-refractory oropharyngeal candidiasis was investigated.

The prospective, single-centre, open-label study was performed in a university-based inner city HIV/AIDS clinic. The study included 27 patients with AIDS and oral candidiasis clinically refractory to fluconazole. Patients were randomised 1:1 to receive either alcohol-based or alcohol-free TTO mouthwash four times daily for 2–4 weeks. Thirteen patients were enrolled into cohort called 1, and treated with 15 ml of an alcohol-based TTO mouthwash 4 times daily for 2 weeks; 14 patients were enrolled into cohort called 2 and treated with 5 ml of an alcohol-free TTO mouthwash 4 times daily for 2 weeks. The different amount of mouthwash used in the two groups was due to need to use an equivalent quantity of TTO because the alcohol-based mouthwash was less concentrated than the non-alcohol-based mouthwash. Additional 2 weeks of therapy were provided for patients who showed clinical improvement but who had not demonstrated a complete clinical response at the end of the initial 2 weeks. The main outcome measure was resolution of clinical lesions of oral candidiasis. Evaluations were performed at 2 and 4 weeks for clinical signs and symptoms of oral candidiasis and quantitative yeast cultures.

All *Candida albicans* isolates showed some degree of in *in vitro* resistance to fluconazole. Overall, using a modified intent-to-treat analysis, 60% of patients demonstrated a clinical response to the TTO mouthwash (7 patients cured and 8 patients clinically improved) at the 4-week evaluation.

The authors concluded that both formulations of the TTO mouthwash appeared to be effective alternative regimens for patients with AIDS suffering from oropharyngeal candidiasis refractory to fluconazole (Vazquez & Zawawi 2002).

Assessor's comment: These studies show a positive effect of TTO commercial preparations in patients with AIDS affected by oropharyngeal candidiasis. No information on the concentration of TTO in the preparations used in the studies is available. Moreover the studies were conducted on a small number of patients.

Denture stomatitis

In vitro and *in vivo* activity of *Melaleuca alternifolia* mixed with tissue conditioner on *Candida albicans* (Catalán *et al.* 2008).

Denture stomatitis is an inflammatory reaction of the palatal and alveolar mucosa underlying removable dental prostheses. Denture stomatitis is more commonly seen in the maxillary mucosa than in the mandibular mucosa.

A study was performed to identify *in vitro* and *in vivo* activity of TTO mixed with different tissue conditioners on the *Candida albicans* strain. Microbiological tests were used to isolate *Candida albicans* from patients with denture stomatitis. The *in vitro* antifungal activity of TTO against *Candida albicans* was determined when it was applied directly and when it was mixed with tissue conditioners (Fitt, Lynal, Coe-Comfort). For the *in vivo* activity the responses of 27 denture stomatitis patients divided in three arms (each of them with 9 patients) were evaluated over a period of 12 days: the control group received Coe-Comfort tissue conditioner, treatment group 1 received 1 ml TTO mixed with 4 ml Coe-Comfort and treatment 2 group received 2 ml Nystatin mixed with 3 ml Coe-Comfort.

In the *in vitro* study, Coe-Comfort or Fitt conditioners mixed with 1 ml, 20% (v/v) of TTO exhibited a total inhibition of *Candida albicans*. Patients treated with TTO mixed with Coe-Comfort showed a significant decrease in palatal inflammation compared with those treated with Coe-Comfort (P = 0.001). In addition, a significant inhibition of *Candida albicans* growth was observed with TTO mixed with Coe-Comfort compared with only Coe-Comfort (P = 0.000004). There was no difference between the treatment arms at day 12. The data did however suggest the decrease in *Candida albicans* was faster with Treatment 1 (TTO) than with Treatment 2 (Nystatin). Conclusions of authors were that TTO mixed with Coe-Comfort tissue conditioner is effective in treating denture stomatitis (Catálan *et al.* 2008).

Assessor's comment: This study has been conducted on a small number of patients, but suggests that TTO can be useful as an adjuvant in the care of denture stomatitis.

Treatment of tinea pedis

Satchell *et al.* 2002a and Tong *et al.* 1992 conducted randomised controlled trials and reported on the use of TTO for the treatment of tinea pedis.

Treatment of interdigital tinea pedis with 25% and 50% TTO solution: A randomised, placebo-controlled, blinded study (Satchell *et al.* 2002a).

A randomised, controlled, double-blinded study to determine the efficacy and safety of 25% and 50% TTO in the treatment of interdigital tinea pedis was conducted. One hundred and fifty-eight patients with tinea pedis clinically and microscopy suggestive of a dermatophyte infection were randomised to receive either placebo, 25% or 50% TTO mixed in ethanol and polyethylene glycol solution. Patients applied the solution twice daily to affected areas for 4 weeks and were reviewed after 2 and 4 weeks of treatment. There was a marked clinical response seen in 68% of the 50% TTO group and 72% of the 25% TTO group, compared to 39% in the placebo group. Mycological cure was assessed by culture of skin scrapings taken at baseline and after 4 weeks of treatment. The mycological cure rate was 64% in the 50% TTO group and 55% in the 25% TTO group, compared to 31% in the placebo group. Four (3.8%) patients applying TTO (one in the 25% group and three in the 50%) developed moderate to severe dermatitis that improved quickly on stopping the study medication (Satchell *et al.* 2002a).

Assessor's comment: This randomised, controlled, double-blinded study showing efficacy of 50% and 25% TTO versus placebo in the treatment of interdigital tinea pedis. The study indicates also the potential development of dermatitis during TTO treatment.

TTO in the treatment of tinea pedis (Tong *et al.* 1992).

One hundred and four patients completed a randomised, double-blind trial to evaluate the efficacy of 10% w/w TTO cream compared with 1% tolnaftate and placebo creams in the treatment of tinea pedis. Significantly more tolnaftate-treated patients (85%) than TTO (30%) and placebo-treated patients (21%) showed conversion to negative culture at the end of therapy ($p < 0.001$); there was no statistically significant difference between TTO and placebo groups. All three groups demonstrated improvement in clinical condition based on the four clinical parameters of scaling, inflammation, itching and burning. The TTO group (24/37) and the tolnaftate group (19/33) showed significant improvement in clinical condition when compared to the placebo group (14/34; $p = 0.022$ and $p = 0.018$ respectively). TTO cream (10% w/w) appears to reduce the symptomatology of tinea pedis as effectively as tolnaftate 1% but is no more effective than placebo in achieving a mycological cure (Tong *et al.* 1992).

Assessor's comment: This RCT shows efficacy of cream containing 10% TTO in improving symptoms of tinea pedis but without significant effects against the basic cause of pathology.

Treatment of vaginal infections of *Candida albicans* with TTO (Belaiche 1985a).

A clinical study with TTO on 28 patients (average age 34), in full oestro-progestinic activity affected by vaginitis caused by *Candida albicans* was carried out. One vaginal capsule weighting 2 g and containing 0.2 grams of TTO was administered every night before sleeping for 90 days. Only one woman had felt vaginal burning at the end of the first week and she stopped the treatment. 23 out of 27 patients showed a complete cure with disappearance of burning and white discharge (leucorrhea). 4 of them had to continue the treatment due to the persistence of leucorrhea. Biological examinations showed the disappearance of *Candida albicans* in 21 patients (Belaiche 1985a).

Treatment of skin infections with TTO (Belaiche 1985b).

A clinical study with TTO was conducted in 27 patients affected by different dermatological disorders with the following results:

- 3 cases of intertrigo infected with *Candida albicans:* application of pure TTO for 6 weeks - 2 months showed positive effects.
- 4 cases of angular stomatitis infected with *Candida albicans* and streptococci: twice a day application of TTO was successful in 3 out of 4 patients.
- 2 cases of staphylococcal and streptococcal impetigo in children: twice a day application of TTO caused improvement in 10-15 hours.
- 6 cases of staphylococcal acne: local treatment determined amelioration of the lesions, without a complete healing, acting on the infection and not on the sebaceous glands activity.
- 11 cases of nail infections by *Candida albicans:* treatment with pure TTO twice a day for 3 months, was successful in 8 patients with the first positive result in the first week; no significant improvement in 3 patients.
- 1 case of pytiriasis versicolor [tinea versicolour caused by *Malassezia* and/or *Trichophytum*]: twice a day application of TTO controlled the event after 20 hours (Belaiche 1985b).

Australian TTO: a natural antiseptic fungicidal agent (Shemesh & Mayo 1991)

A clinical trial with Australian TTO was undertaken for the treatment of various dermatological disorders for six months in 50 patients. Several forms of TTO preparations were used: pure oil (100%), lozenges with 1% TTO plus 2.5 mg ground leaf; and a 5% cream. 50 patients were supplied TTO for a

period of 1 to 4 weeks, depending on the severity of the condition being treated. All patients who completed treatment were either cured, all showed remarkable improvement in their presenting condition. One patient stopped the treatment after one day because of mild erythematous skin sensitivity to the 100% TTO (Shemesh & Mayo 1991).

Recurrent herpes labialis

Use of deception to achieve double-blinding in a clinical trial of TTO for the treatment of recurrent herpes labialis (Carson *et al.* 2008).

In a randomised, placebo-controlled trial of TTO for the treatment of recurrent herpes labialis (RHL), or cold sores, deception was used to prevent volunteers from identifying their treatment allocation. Volunteers received placebo (n=102) or TTO (n=112) ointment in preparation for their next episode of RHL and were told, falsely, that the aroma of the ointments had been changed to prevent identification of the treatment group. At the trial's end, of the volunteers who had used their ointment and presented for treatment assessment (n=100), approximately 50% correctly guessed their treatment allocation (P=0.774). Amongst volunteers that had not presented for treatment assessment (n=114), 12 volunteers did not provide blinding data and 46 did not open their tube. For the 56 volunteers who opened their tube, less than half of those receiving TTO (44.4%) and only a small proportion of those on placebo (17.2%) were able to correctly identify their treatment allocation. Among the volunteers that were not treated, the P-value was 0.083. This study showed that the ethical use of deception may provide effective blinding in challenging circumstances (Carson *et al.* 2008).

Halitosis

Antimicrobial activity of garlic, TTO, and chlorhexidine against oral microorganisms (Groppo *et al.* 2002).

Antimicrobial activities of TTO, garlic, and chlorhexidine solutions against oral microorganisms were compared in a five week study consisting of thirty subjects. The first week was considered baseline. All subjects used a control solution (second week), and were randomly divided into the three groups (third week): G1- 0.12% chlorhexidine in a vehicle solution; G2 - 2.5% solution of a garlic (*Allium sativum* L.) aqueous extract 1:1; and G3 - 0.2% TTO in vehicle solution and 0.5% Tween 80. Dishes containing blood agar and Mitis Salivarius Bacitracin agar (MSB) were inoculated with the subjects' saliva (collected twice a week). Total microorganisms and mutans *streptococci* were counted in blood agar and MSB, respectively.

Chlorhexidine and garlic groups showed antimicrobial activity against mutans streptococci, but not against other oral microorganisms. The TTO group showed antimicrobial activity against mutans *streptococci* and other oral microorganisms. Maintenance of reduced levels of microorganisms was observed only for garlic and TTO during the two consecutive weeks (fourth and fifth). Unpleasant taste (chlorhexidine 40%, TTO 30%, garlic 100%), burning sensation (chlorhexidine 40%, TTO 60%, garlic 100%), bad breath (chlorhexidine 40%, TTO 20%, garlic 90%), and nausea (chlorhexidine 0%, TTO 10%, garlic 30%) were reported. The authors concluded that garlic and TTO might be an alternative to chlorhexidine (Groppo *et al.* 2002).

Supragingival plaque

Clinical and antibacterial effect of tea tree oil – a pilot study (Arweiler *et al.* 2000)

Arweiler *et al.* 2000 reported the results from a pilot, non-randomised study on the effect of TTO on supragingival plaque formation and vitality. The study was performed with eight patients, which after professional tooth cleaning were asked to refrain any mechanical cleaning and to rinse the mouth with placebo (water) for 1 week, with chlorhexidine 0.1% (positive control) in a second and 0.34% TTO

water solution with milk as emulsifier in a third test week. Every test week was followed by a 10-day washout in which normal tooth brushing with standard toothpaste was performed. The TTO reduced neither the plaque index nor the plaque area relative to the placebo although there was a reduction in the amount of vital bacteria compared to placebo. Chlorhexidine significantly reduced plaque area and vital bacteria compared to placebo and reduced plaque index.

The effects of a tea tree oil-containing gel on plaque and chronic gingivitis (Soukoulis & Hirsch 2004)

The use of TTO for oral conditions such severe gingivitis was studied in a double-blind, longitudinal, non-crossover trial with 49 medically fit non-smokers (24 males and 25 females) aged 18-60 years. Subjects were randomly assigned to three groups and given either 2.5% TTO-gel, 0.2% chlorhexidine gel, or a placebo gel to be applied with a toothbrush twice daily. Treatment effects were assessed using the Gingival Index (GI), Papillary Bleeding Index (PBI) and plaque staining score at four and eight weeks. The TTO group had significant reduction in PBI and GI scores. However, TTO did not reduce plaque scores, which tended to increase over the latter weeks of the study period. The Authors concluded that topical application of TTO gel to inflamed gingival tissue may be useful as an adjuvant of chemotherapeutic periodontal therapy.

Minor skin lesions

A randomised, controlled trial of TTO topical preparations versus a standard topical regimen for the clearance of MRSA colonisation (Dryden *et al.* 2004)

Two topical MRSA eradication regimes were compared in hospital patients: a standard treatment included mupirocin 2% nasal ointment, chlorhexidine gluconate 4% soap, silver sulfadiazine 1% cream versus a TTO regimen. The TTO regimen comprised TTO 10% cream applied to the anterior nostrils three times a day for five days; TTO 5% body wash all over the body at least once a day for five days; TTO 10% cream to skin lesions, wounds and ulcers, and also to axillae or groins as an alternative to the body wash. One hundred and fourteen patients received standard treatment and 56 (49%) were cleared of MRSA carriage. One hundred and ten received TTO regimen and 46 (41%) were cleared. There was no significant difference between treatment regimens (Fisher's exact test; P ¼ 0:0286). Mupirocin was significantly more effective at clearing nasal carriage (78%) than TTO cream (47%; P ¼ 0:0001), but TTO treatment was more effective than chlorhexidine or silver sulfadiazine at clearing superficial skin sites and skin lesions. The TTO preparations were effective, safe and well tolerated and could be considered in regimens for eradication of MRSA carriage (Dryden *et al.* 2004).

Assessor's comment: this study shows the efficacy of a cream containing TTO 10% to clean skin lesions, wounds and ulcers.

TTO as an alternative topical decolonisation agent for methicillin-resistant *Staphylococcus aureus* (Caelli *et al.* 2000)

Clearance of MRSA was also investigated by Caelli *et al.* 2000 who conducted a pilot randomised controlled trial on 30 hospital inpatients aged between 32 and 82 years. Fifteen patients were randomised to the TTO treatment group consisting of 4% TTO nasal ointment and 5% TTO body wash. Fifteen patients were randomised to the standard treatment group consisting of 2% mupirocin nasal ointment and triclosan body wash. The TTO treatment combination appeared to perform better than the standard treatment of mupirocin and triclosan although the difference was not statistically significant.

Assessor's comment: this is a pilot study with a too small number of patients.

Dandruff

Treatment of dandruff with 5% TTO shampoo (Satchell *et al.* 2002b).

The efficacy and tolerability of 5% TTO on mild to moderate dandruff vs. placebo was investigated in a randomised, single-blind, parallel-group study. One hundred twenty-six male and female patients, aged 14 years and older, were randomly assigned to receive either 5% TTO shampoo or placebo, which was used daily for 4 weeks. The dandruff was scored on a quadrant-area-severity scale and by patient self-assessment scores of scaliness, itchiness, and greasiness. The 5% TTO shampoo group showed a 41% improvement in the quadrant-area-severity score compared with 11% in the placebo group ($P < 0.001$). Statistically significant improvements were also observed in the total area of involvement score, the total severity score, and the itchiness and greasiness components of the patients' self-assessments. The scaliness component of patient self-assessment improved but was not statistically significant. There were no adverse effects. 5% TTO appears effective and well tolerated in the treatment of dandruff (Satchell *et al.* 2002b).

Assessor's comment: this study shows efficacy and good tolerability of a 5% TTO shampoo in the treatment of dandruff.

Finally a case study describing a 5-day successful use of vaginal pessaries containing 200 mg of TTO in vegetable basis for the treatment of vaginal discharge typical of anaerobic vaginosis was reported by Blackwell 1991.

Clinical Treatment of Ocular Demodecosis by Lid Scrub With Tea Tree Oil (Gao *et al*. 2007)

Gao *et al.* 2007, following an *in vitro* observation that *Demodex* is resistant to a wide range of antiseptic solutions but susceptible to TTO in a dose-dependent manner, reported on the results of a retrospective review of an *in vivo* treatment with TTO of eleven patients with ocular *Demodex.* They found that *Demodex* count dropped to zero for two consecutive visits in less than four weeks in eight patients. Ten out of eleven patients showed different degrees of symptomatic relief and notable reduction of inflammatory signs. A significant visual improvement was noted in six out of twenty-two eyes which was associated with the development of a stable lipid tear film. The TTO lid scrub effectively eradicated ocular *Demodex* and resulted in subjective and objective improvements, which was interpreted a result in understanding , but caused notable irritation in 3 patients. Positive results were interpreted as preliminary results useful in understanding *Demodex* pathogenicity in causing several ocular surface diseases. Retrospective nature and the lack of using a standardized format to grade symptoms as well as randomisation with lid scrub using baby shampoo and small number of patients were recognised as a limitation of the value of this study (Gao *et al.* 2007).

Finally the results of a case study were described by Millar 2008 where 100% TTO was used for the topical treatment of multiple warts, due to human papilloma virus, on the hand of a seven year old girl. Salicylic acid (12%) and lactic acid (4%) was previously used on this condition but only resulted in the temporary removal of the warts and they recurred in greater numbers. After five days treatment with undiluted TTO, all warts were reduced in size. After a further 7 days, there was no evidence of warts and complete reepithelialisation of the area. No recurrence has been reported.

A summary of these studies is presented in Table 5.

Table 5: Clinical studies on humans

Indication	Reference	Method	Participants	Posology	Interventions	Outcomes	Comments
Treatment of mild to moderate acne	Bassett *et al.* 1990	RCT, investigator blinded	124 patients (119 evaluable) with mild to moderate acne; 60F/64M; 12-35yrs Treatment: 58 patients Control: 61 patients	Topical application	Treatment: 5% TTO gel Control: 5% benzoyl peroxide (BP)	Both significantly reduced inflamed lesions but BP better than TTO; Treatment with TTO had less scaling, pruritus, dryness; BP better at reducing oiliness; Treatments equivalent for non-inflamed lesions, erythema Adverse reactions: 44% in TTO group, 79% in BP group (e.g. dryness, stinging, burning, redness); significantly fewer events in TTO group	5% TTO gel showed to ameliorate acne lesions
Treatment of mild to moderate acne vulgaris	Enshaieh *et al.* 2007	Double blind placebo controlled RCT. Randomisation by software allocation	30 patients in each study arm (15-25 years) with mild to moderate facial acne vulgaris. No significant difference in characteristics between groups	Application to affected area twice daily for 20 minutes then washing off with tap water. Continue treatment for 45 days.	Treatment: 5% TTO gel Control: vehicle gel placebo. Same colour, texture, pack size, different labels	Significant reduction in total lesion count (TLC) and ASI with Treatment group. Significant difference between Treatment and Control group in improvement in TLC and ASI (Treatment 3.55 times and 5.75 times more effective than Control respectively). Treatment group also had significant reduction in comedones number, papules number and pustules number and was significantly more improved than Control group. Adverse reactions: Side effects similar and tolerable between both groups (minimal pruritus, little burning, minimal scaling	Study insufficient to support the use of 100% TTO in furunculosis
Furunculosis	Feinblatt 1960	Case series	35 patients with furunculosis, three complicated by carbuncles and 3 with diabetes. (9F/26M; 17-57 years) 25 treated with TTO and 10 untreated controls	Paint surface freely with TTO 2-3 times daily after thoroughly cleaning the site	Treatment: 100% TTO Controls: untreated	10 controls – 5 cases had boils finally incised; 5 cases had infection remaining after 8 days. 25 treatment – 1 case had boil requiring incision; 15 cases had infected site removed completely in 8 days; Remaining cases had infection reduced Adverse reactions: No toxic effects. Three patients complained of slight temporary stinging.	Study insufficient to support the use of 100% TTO in furunculosis

Indication	Reference	Method	Participants	Posology	Interventions	Outcomes	Comments
Decolonisation of MRSA positive wounds and wound healing	Edmondson *et al.* 2011	Uncontrolled open-label pilot study	11 patients colonised with MRSA	wound cleaning at each dressing change 3 times a week or daily as deemed necessary	Treatment: 3.3% TTO shaken in water	No participant MRSA negative after treatment 8 began to heal and reduced in size as measured by computer planimetry Adverse reactions: 2 participants experienced pain during cleaning procedure that may or may not have been because of TTO irrigation	3.3% TTO can influence positively wound healing through its antimicrobial activity. Limit of the study is the small number of participants
Resolution of culture-positive toenail onychomycosis	Buck *et al.* 1994	RCT, double blind, multicenter	117 patients with culture-positive onychomycosis; 64 TTO group; 53 in comparator group	Both treatments applied to affected nail twice daily for 6 months	100% TTO; 1% clotrimazole	TTO: Full or partial resolution for 60% Clotrimazole: Full or partial resolution for 61% After 6 months of therapy, both treatments comparable and TTO as effective as conventional treatment Adverse reactions: 7.8% in TTO and 5.7% in clotrimazole group (erythema, irritation, edema)	Efficacy of 100% TTO comparable to clotrimazole in treatment of onychomycosis
Treatment of onychomycosis	Syed *et al.* 1999	RCT, double blind	60 patients with fungal infection of large toenail and clinical diagnosis of onychomycosis (21F/39M, 18-80yrs) with clinical diagnosis of onychomycosis Treatment 1: 40 patients Treatment 2: 20 patients	Three times daily topically apply treatment to large toenail and cover with occlusive plastic dressing.	Treatment 1: 2% butenafine hydrochloride with 5% TTO cream Treatment 2: TTO unspecified concentration cream	Treatment 1: 80% cured Treatment 2: 0% cured Significant difference at 36 weeks Adverse reactions: 10% in Treatment 1 group had mild inflammation.	The response to treatment 2 is unexpected and perhaps 8 weeks treatment period is insufficient. The concentration of TTO in the control (Treatment 2) cream is unspecified
Treatment of fluconazole-refractory oral candidiasis in patients with AIDS	Jandourek *et al.* 1998	Prospective open-labelled	13 patients (12 evaluable) with AIDS and fluconazole-refractory oral candidiasis	15 ml mouthwash 4 times daily. Solution swished in mouth for 30-60s then expelled with no rinsing for 30	Branded non-prescription TTO mouth wash preparation	Clinical response rate of 67% after 4 weeks (cure in 2 patients, improvement in 6 patients, no response in 4 patients, 1 deterioration) Adverse reactions: No serious adverse events. 8/12	4 patients were non-compliant with study regimen or did not attend scheduled visits

Indication	Reference	Method	Participants	Posology	Interventions	Outcomes	Comments
				mins		patients reported mild to moderate oral burning when solution in contact with mucosa, primarily in first week.	No information on the con-centration of TTO in the preparation used. Small number of patients.
Treatment of fluconazole-refractory oropharyngeal candidiasis (OPC) in patients with AIDS	Vazquez et al. 2002	Single centre, open-label prospectiv e trial; Patients randomly assigned to two treatment arms	27 patients with AIDS and fluconazole-refractory OPC Treatment 1: 13 patients (12 evaluated) Treatment 2: 14 patients (13 evaluated)	Treatment 1: 15 ml of solution 4 times daily for 14 days; Treatment 2: 5 ml of solution 4 times daily for 14 days Solution swished in mouth for 30-60s then expelled with no rinsing for 30 mins	Treatment 1: Branded non-prescription alcohol based TTO oral solution Treatment 2: Branded non-prescription non-alcohol based TTO oral solution	7 patients clinically cured and 8 patients improved after 28 days of treatment. Six patients unchanged but stable. Two patients deteriorated. Overall clinical response of 60% after 4 weeks.	No information on the concentration of TTO in the preparations used. Small number of patients.
Treatment of denture stomatitis (Type II – diffuse inflammation with generalized hyperemia of the denture-supporting tissue)	Catalán *et al.* 2008	3 arm controlled study (9 participant s randomise d to each)	27 non-smoking, non-diabetic, non-hytertense, not on antibiotics, exhibiting clinical evidence of DS Type II (26 W/1M, 50 to 77 yrs)	Intervention placed on maxillary prosthesis which was placed intraorally. Patient slept with condi-tioned prosthesis and cleaned the denture using only cold water rinse. Reapplication to prosthesis at day 4 and day 8	Control: Coe-Comfort (CC) tissue conditioner Treatment 1: TTO 1 ml mixed with 4 ml CC Treatment 2: Nystatin 2 ml mixed with 3 ml CC	Examination on day 12 showed similar clinical healing for both treatment groups (8 out of 9). Both treatment groups showed a statistically significant improvement in palatal inflammation and inhibition of *Candida albicans* compared to control but no difference between the treatment arms at day 12, however the data suggest the decrease in *Candida albicans* was faster with Treatment 1 than with Treatment 2.	Small number of patients
Treatment of tinea pedis	Satchell *et al.* 2002a	RCT double blind, 3 arm study	158 patients enrolled (137 evaluated; 54F/104M; 17-83 yrs with culture-positive tinea pedis; 54 in treatment 1 group, 51 in treatment 2 group, 53 in placebo group	Apply solution to affected areas twice daily for 4 weeks	Treatment 1: 25% TTO in placebo base Treatment 2: 50% TTO in placebo base; Placebo (20% ethanol, 80% polyethylene glycol)	Effective cure (mycological cure and marked clinical response) was significantly improved for both TTO treatments compared to placebo (48% of Treatment 1 group, 50% of Treatment 2 group, 13% of placebo). Both TTO treatments had significantly better mycological cure rate and improved clinical score than placebo	The study supports efficacy of 50% and 25% TTO versus placebo in the treatment of interdigital tinea pedis.

Indication	Reference	Method	Participants	Posology	Interventions	Outcomes	Comments
						Adverse reactions: One patient in Treatment 1 group and 3 patients in Treatment 2 group reported moderate to severe dermatitis. Mild stinging reported in two patients in Treatment 1 group and placebo group. No serious adverse events.	Potential development of dermatitis during TTO treatment.
Treatment of tinea pedis	Tong *et al.* 1992	RCT, double blind, 3 arm study	121 patients (104 evaluable) with clinically diagnosed tinea pedis; 16-65yrs; 25F/79M 37 in treatment 1 group, 33 in treatment 2 group, 34 in placebo group	Apply cream topically twice daily for four weeks	Treatment1: 10% TTO in sorbolene; Treatment 2: 1% tolnaftate; Placebo group: sorbolene	Clinical efficacy (reduction of signs and symptoms – scaling, inflammation, itching, burning) improved significantly for both Treatments compared to placebo. Mycological efficacy for Treatment 2 was significantly better than Treatment 1 and placebo. Mycological cure and clinical improvement in 46% (Treatment 2), 22% (Treatment 1), 9% (placebo). For this combined measure, Treatment 2 significantly better than placebo but no significant difference between Treatment 1 and Treatment 2. Adverse reactions: No adverse events. Skin tolerance excellent. One patient in Treatment 2 group reported mild erythema.	The study supports efficacy of cream containing 10% TTO in improving symptoms of tinea pedis but with no significant effects against the basic cause of pathology
Various skin infections	Belaiche 1985b	Case series	3 cases of intertrigo infected with *Candida albicans*; 4 cases of angular stomatitis infected with *Candida albicans* and Streptococci; 2 cases of staphylococcal and streptococcal impetigo in children; 6 cases of staphylococcal acne; 11 cases of nail infections by *Candida*	Topical application for 6 weeks - 2 months twice a day application twice a day application local treatment twice a day application for 3 months, twice a day application	100% TTO	positive effects successful in 3 out of 4 patients improvement in 10-15 hours amelioration of the lesions, without a complete healing, acting on the infection and not on the sebaceous glands activity successful in 8 patients with the first positive result in the first week; no significant improvement in 3 patients. event controlled after 20 hours	

Indication	Reference	Method	Participants	Posology	Interventions	Outcomes	Comments
			albicans; 1 case of pityriasis versicolor [tinea versicolour caused by the yeasts Malassezia and/or Trichophytum				
Various skin conditions	Shemesh & Mayo, 1991	Case series	50 patients, with various skin conditions: Mild facial and back acne, thrush, skin rashes, dermatitis, eczema, infected pustules, oral canker sores (aphthous stomatitis), *herpes simplex*, tinea cruris, tinea pedis, tinea barbae	1 to 4 weeks treatment depending on the severity	Treatment: 100% TTO; Lozenges with 1% TTO + 2.5 mg ground leaf; 5% TTO cream	Substantial improvement of the conditions. Adverse reactions: One patient stopped treatment due to mild erythematous skin sensitivity to 100% TTO.	
Treatment of recurrent herpes labialis (RHL / cold sores)	Carson *et al.* 2001	Randomised placebo-controlled, investigator blinded trial;	20 patients (18 evaluated) with self-reported history of RHL. 9 in each arm. 18-70 years.	Applied gel 5 times daily	Receive either 6% TTO in aqueous gel base or placebo gel	The median time to re-epithelialization after treatment with TTO was 9 days compared with 12.5 days after placebo. 8/9 patients in TTO group compared to 6 in placebo group commenced treatment at vesicle stage and beyond Adverse reactions: 1 patient in TTO did not develop RHL and was withdrawn due to unspecified adverse event.	
Halitosis (Antimicrobial activity against oral micro-organisms)	Groppo *et al.* 2002	Randomised three-arms controlled	30 subjects randomly divided into the three groups G1, G2 and G3.	Week 1: no treatment or standard dentifrice Week 2: 1 min mouthwashes 30 mins after the last toothbrushing of the day using 10 ml of control solution and standard dentifrice. Week 3: 1 min	Week 1: baseline Week 2: control solution (vehicle solution: distilled water, 5% spearmint essence and 2% sorbitol) Week 3: G1- 0.12% chlorhexidine in a vehicle solution; G2 - 2.5% solution of a garlic (Allium	G1 and G2 showed antimicrobial activity against mutans *streptococci*, but not against other oral micro-organisms. TTO group showed antimicrobial activity against mutans *streptococci* and other oral microorganisms. Maintenance of reduced levels of micro-organisms was observed only for garlic and TTO during the two consecutive weeks (4^{th} and 5^{th}). Unpleasant taste (chlorhexidine 40%,	

Indication	Reference	Method	Participants	Posology	Interventions	Outcomes	Comments
				mouthwashes for 7 days using 10 ml of one of the treatment solutions (G1, G2 and G3) 30 mins after the last tooth brushing of the day. Week 4 and 5: treatment discontinued	sativum L.) aqueous extract 1:1; and G3-0.2% TTO in vehicle solution and 0.5% Tween 80. Receive either 6% TTO in aqueous gel base or placebo gel	TTO 30%, garlic 100%), burning sensation (chlorhexidine 40%, TTO 60%, garlic 100%), bad breath (chlorhexidine 40%, TTO 20%, garlic 90%), and nausea (chlorhexidine 0%, TTO 10%, garlic 30%) were reported.	
Prevention of dental plaque growth	Arweiler *et al.* 2000	Three arm cross over study, non-randomised	8 subjects 23-34 years	Rinse twice daily for 2 minutes with 15 ml of solution using no mechanical brushing warm water	Week 1: water (placebo) Week 2: 0.1% Chlorhexidine (positive control) Week 3: 0.34% TTO dispersed in milk and diluted with water	TTO reduced neither the plaque index nor the plaque area relative to the placebo, although reduction of vital bacteria compared to placebo. Chlorhexidine significantly reduced plaque area Adverse reactions: All subjects complained about intensive and unpleasant taste of TTO. Study may have dropped off particularly as in 3rd week patients had to mix the TTO solution themselves.	No significant efficacy of TTO was detected on the amount of vital bacteria although there was a reduction compared to placebo.
Effect on plaque and chronic gingivitis	Soukoulis & Hirsch 2004	3 arm, double-blind, longitudinal, non-cross-over study. Gels randomly distributed	58 subjects recruited with 49 subjects evaluated (24F/25M; 18-60 years) with moderate to severe gingivitis, non-smokers.	For 8 weeks, gel applied along entire length of toothbrush and twice daily used as dentifrice in contact with gingival tissues adjacent to teeth for min of 2 mins. No rinsing, eating, drinking for 30 mins following gel application.	Treatment: 2.5% TTO gel Positive control: 0.2% chlorhexidine gel Negative control: Placebo gel	TTO had significant reduction in Papillary Bleeding Index (PBI) and Gingival Index (GI) but did not reduce plaque scores which tended to increase towards end of study No adverse reactions	
Clearance of MRSA colonisation	Dryden *et al.* 2004	Randomised controlled trial; Balanced	236 colonised with MRSA (224 evaluable) Standard treatment: 114 patients TTO treatment: 110	Nasal application 3 times per day for 5 days; Body wash applied all over body at	Standard treatment: Mupirocin 2% to anterior nares; chlorhexidine	No significant difference between treatments for clearing MRSA. Mupirocin significantly more effective at clearing nasal carriage. TTO more effective at clearing superficial skin	

Indication	Reference	Method	Participants	Posology	Interventions	Outcomes	Comments
		randomisation using software allocation	patients	least once per day for 5 days; Application to skin lesions, wounds and ulcers once per day for 5 days	gluconate 4% soap over body; silver sulfadiazine 1% cream to skin lesions, wounds, leg ulcers. TTO Treatment: 10% TTO cream to anterior nares; 5% TTO body wash over body; 10% TTO cream to skin lesions, wounds, leg ulcers and as alternative to body wash for axillae, groins	sites and skin lesions. TTO preparations were safe and well tolerated.	
Clearance of colonised or infected MRSA	Caelli *et al.* 2000	Randomized, controlled pilot study	30 hospital inpatients colonised or infected with MRSA (15 in each study arm; 32-82 years for TTO group)	Minimum three days treatment	4% TTO nasal ointment + 5% TTO body wash vs. Standard treatment: 2% mupirocin nasal ointment + triclosan body wash	TTO; 33% cleared, 20% chronic, 47% incomplete; for standard treatment 13% cleared, 53% chronic, 33% incomplete (not significant) Adverse reactions: No adverse events. Mild swelling of nasal mucosa to acute burning reported for TTO nasal ointment (number not reported). One patient in standard treatment reported skin tightness	Pilot study. Small number of patients
Treatment of mild to moderate dandruff	Satchell *et al*. 2002b	RCT, investigator blinded	126 patients with mild to moderate dandruff (> 14 yrs); 63 TTO group, 62 placebo group	For 4 weeks, wash hair daily, leaving shampoo in for 3 mins before rinsing	5% TTO shampoo; placebo shampoo	Whole scalp lesion score significantly improved in TTO group (41.2%) compared to placebo group (11.2%). Total area of involvement score, total severity score and itchiness and greasiness had statistically significant improvement in TTO group compared to placebo.	
Treatment of ocular *Demodex*	Gao *et al.* 2007	Case series	11 patients (6F/6M: 60.2±11.6yrs) with ocular *Demodex* not using topical or systemic anti-	Weekly lid scrub: 3 times a cotton tip wetted in 50% TTO to scrub lash roots from one end to	Weekly lid scrub: 50% TTO diluted with mineral oil Daily lid scrub: 0.5 ml TTO	*Demodex* count dropped to zero for 2 consecutive visits in less than 4 weeks in 8 patients. 10/11 patients showed different degrees of symptomatic relief and notable reduction of inflammatory	Small number of patients

Indication	Reference	Method	Participants	Posology	Interventions	Outcomes	Comments
			inflammatory and antibacterial medications before TTO scrub.	other as 1 stroke. 6 strokes applied. Dry cotton tip used to remove excess 5 mins later. Reapplication after 5 mins. Daily lid scrub: With eyes closed, lids massaged with TTO shampoo and water for 3-5 minutes, medium pressure then rinsed with water. Twice daily for 1 month, then once daily.	shampoo mixed with tap water (Kato Sales, Florida).	signs. Significant visual improvement in 6 of 22 eyes was associated with stable lipid tear film Adverse reactions: The weekly office lid scrub with 50% TTO resulted in mild irritation in 6 patients and moderate irritation in 3 patients. Patients' symptoms were relieved, ocular surface inflammation resolved and lipid tears film stability improved.	
Treatment of various gynaecological conditions	Peña 1962	Open, uncontrolled	96 trichomonal vaginitis, 4 *Candida albicans* vaginitis, 20 nulliparous cervicitis from *Trichomonas vaginalis*, 10 chronic endocervicitis	vaginal canal washed for 30 sec then tampon left in place for 24 hours – weekly treatment	Treatment: TTO 40% in solution	Cured and healed cervicitis in 10 patients after 4 weekly treatments Effective concentration found to be 20% solution of TTO Adverse reactions: No irritation, mild drying effect.	
Treatment of vaginal discharge typical of anaerobic vaginosis	Blackwell 1991 2	Case study	40 year old woman	5 day application in form of TTO vaginal pessaries	Vaginal pessary containing TTO in a vegetable oil base	Vaginal secretions normal	
Vaginal infections associated with *Candida albicans*	Belaiche 1985a	Open, non-controlled		90 days daily application in form of a TTO vaginal capsule every night before sleeping;	Vaginal capsule containing TTO 0.2 g	23 out of 27 patients showed a complete cure. Remaining patients had moderate improvement of discharge. *Candida albicans* disappeared in 21 patients, 4 of them had to continue the treatment due to the persistence of leucorrhea. Biological examinations showed the disappearance of *Candida*	

Indication	Reference	Method	Participants	Posology	Interventions	Outcomes	Comments
						albicans in 21 patients. Adverse reactions: One out of 28 patients experienced vaginal burning sensation and withdrew from study.	
Treatment of warts on finger	Millar & Moore 2008	Case study	Seven yr old girl	TTO applied with sterile cotton wool swabs to each lesion, each evening after bathing and prior to sleep.	100% TTO. Previously used salicylic acid (12%w/w) and lactic acid (4% w/w) resulting in temporary removal of warts but they recurred in greater numbers	After 5 days, all warts reduced in size. After a further 7 days, no evidence of warts and complete re-epithelialisation. No recurrence to date.	

TTO – Tea Tree Oil, RCT – Randomised Controlled Trial, F – female, M – male

4.2.2.3. Clinical studies conducted with combinations containing TTO

Mycosis

Treatment of toenail onychomycosis with 2% butenafine and 5% TTO in cream

The objective of a randomised, double-blind, placebo-controlled study was to examine the clinical efficacy and tolerability of 2% butenafine hydrochloride and 5% TTO incorporated in a cream to manage toenail onychomycosis in a cohort. Sixty outpatients (39 M, 21 F) aged 18–80 years (mean 29.6) with 6–36 months duration of disease were randomised to two groups (40 and 20), active and placebo. Patients were shown how to apply the trial medication at home three times a day topically for 7 days. After 16 weeks, 80% of patients using medicated cream were cured, as opposed to none in the placebo group. Four patients in the active treatment group experienced subjective mild inflammation without discontinuing treatment. During follow-up, no relapse occurred in cured patients and no improvement was seen in medication-resistant and placebo participants (Syed *et al*. 1999).

Assessor's comment: this is randomised, double-blind, placebo-controlled study showing efficacy of a combination of TTO (5%) with 2% butenafine hydrochloride incorporated in a cream in management of toenail onychomycosis.

Halitosis

Reduction of Mouth Malodour and Volatile Sulphur Compounds in Intensive Care Patients using an Essential Oil Mouthwash

A study was carried out to explore the effect of an essential oil solution on levels of malodour and production of volatile sulphur compounds (VSC) in patients nursed in intensive care unit. Thirty two patients received 3 min of oral cleaning using an essential oil solution (mixture of TTO, peppermint, *Mentha piperita* and lemon, *Citrus limon*) on the first day, and benzydamine hydrochloride on the second day. Two trained nurses measured the level of malodour with a 10 cm visual analogue scale (VAS) and VSC with a Halimeter before (Pre), 5 min after (Post I) and 1 h following treatment (Post II). The level of oral malodour was significantly different following the essential oil session, and differed significantly between two sessions at Post I ($p < 0.005$) and Post II ($p < 0.001$). Differences between the two sessions were significant (benzydamine hydrochloride, $p < 0.001$; essential oil, $p < 0.001$) in the level of VSC and significantly lower in the essential oil session than benzydamine hydrochloride at the Post II ($p < 0.05$). These findings suggest that mouth care using an essential oil mixture of diluted TTO, peppermint and lemon may be an effective method to reduce malodour and VSC in intensive care unit patients (Hur *et al*. 2007).

Assessor's comment: These studies suggests that TTO, alone or in combination, probably due to its antimicrobial activity against oral microorganisms, can be useful to fight halitosis.

A Clinical Study: *Melaleuca*, Manuka, *Calendula* and Green Tea Mouth Rinse

A mouthwash (IND 61,164) containing essential oils and extracts from four plant species (*Melaleuca alternifolia*, *Leptospermum scoparium*, *Calendula officinalis* and *Camellia sinensis*) was tested. The study aimed to evaluate the safety, palatability and preliminary efficacy of the rinse. Fifteen subjects completed the Phase I safety study. Seventeen subjects completed the Phase II randomised placebo-controlled study. Plaque was collected, gingival and plaque indices were recorded (baseline, 6 weeks, and 12 weeks). The relative abundance of two periodontal pathogens (*Actinobacillus actinomycetemcomitans*, *Tanerella forsythensis*) was determined utilizing digoxigenin-labelled DNA probes. ANCOVA was used at the $p = 0.05$ level of significance. Two subjects reported a minor adverse event. One subject withdrew from the study. Several subjects objected to the taste of the test rinse

but continued treatment. Differences between gingival index, plaque index or relative abundance of either bacterial species did not reach statistical significance when comparing nine placebo subjects with eight test rinse subjects. Subjects exposed to the test rinse experienced no abnormal oral lesions, altered vital signs, changes in liver, kidney, or bone marrow function. The authors concluded that larger scale studies would be necessary to determine the efficacy and oral health benefits of the test rinse (Lauten *et al*. 2005).

Assessor's comment: a preliminary study on a small number of patients showing positive effects of mouth rinse containing TTO in combination with Manuka, Calendula and Green Tea.

Pediculosis

An ex vivo, assessor blind, randomised, parallel group, comparative efficacy trial of the ovicidal activity of three pediculicides after a single application - TTO and lavender oil, eucalyptus oil and lemon TTO, and a "suffocation" pediculicide

Components to the clinical efficacy of pediculicides are: (i) efficacy against the crawling stages (lousicidal efficacy); and (ii) efficacy against the eggs (ovicidal efficacy). Lousicidal efficacy and ovicidal efficacy are confounded in clinical trials. A trial was specially designed to rank the clinical ovicidal efficacy of pediculicides. Eggs were collected, pre-treatment and post-treatment, from subjects with different types of hair, different coloured hair and hair of different length.

Subjects with at least 20 live eggs of *Pediculus capitis* (head lice) were randomised to one of three treatment-groups: a TTO and lavender oil pediculicide (TTO/LO); an eucalyptus oil and lemon TTO pediculicide (EO/LTTO); or a "suffocation" pediculicide. Pre-treatment: 10 to 22 live eggs were taken from the head by cutting the single hair with the live egg attached, before the treatment (total of 1,062 eggs). Treatment: The subjects then received a single treatment of one of the three pediculicides, according to the manufacturers' instructions. Post-treatment: 10 to 41 treated live eggs were taken from the head by cutting the single hair with the egg attached (total of 1,183 eggs). Eggs were incubated for 14 days. The proportion of eggs that had hatched after 14 days in the pre-treatment group was compared with the proportion of eggs that hatched in the post-treatment group. The primary outcome measure was % ovicidal efficacy for each of the three pediculicides.

Seven hundred twenty two subjects were examined for the presence of eggs of head lice. Ninety two of these subjects were recruited and randomly assigned to: the "suffocation" pediculicide (n = 31); the TTO/LO (n = 31); and the EO/LTTO (n = 30 subjects). The group treated with EO/LTTO had an ovicidal efficacy of 3.3% (SD 16%) whereas the group treated with TTO/LO had an ovicidal efficacy of 44.4% (SD 23%) and the group treated with the "suffocation" pediculicide had an ovicidal efficacy of 68.3% (SD 38%).

Ovicidal efficacy varied substantially among treatments, from 3.3% to 68.3%. The "suffocation" pediculicide (68.3% efficacy against eggs) and the TTO/LO (44.4% efficacy against eggs) were significantly more ovicidal than EO/LTTO (3.3%) ($P < 0.0001$). The "suffocation" pediculicide and TTO/LO are also highly efficacious against the crawling-stages. Thus, the "suffocation" pediculicide and TTO/LO should be recommended as first line treatments (Barker & Altman 2011).

Assessor's comment: this study shows the efficacy of a combination of TTO with lavender oil as pediculicide.

4.3. Clinical studies in special populations (e.g. elderly and children)

No significant study has been performed in special populations.

Combination of Essential Oil of Melaleuca alternifolia and Iodine in the treatment of Molluscum Contagiosum in children.

A randomized double blinded placebo controlled three arm study, with intention to treat analysis, was performed in children for the treatment of molluscum contagiosum viral infection, which is a common benign childhood condition and is increasingly found as a sexual transmitted disease in adults. Fifty-three children (mean age 6.3+5.1 years) were randomised and treated with twice a day topical application of either a combination of 75% V/V TTO, canola oil and organically bound iodine in a proprietary formulation (TTO-I – 19 patients), TTO an canola oil in the same proportion (18 patients), or the same organically bound iodine alone in a vehicle of canola oil as control (I - 16 patients). The concentration of iodine both in the control preparation and in the TTO-I was 35 µmolar. The treatment consisted in the application of 4 µl medication on each molluscum lesion twice a day for 30 days or until all lesions had resolved, if this required less than 30 days, and was considered successful if lesions completely cleared or were reduced in number by greater than 90%. Forty-eight children were available for follow up at the end of 30 days, being lost 2 children in both the I and TTO group and 1 in the TTO-I group. Best results were shown in the TTO-I group were 11 children had total resolution and 5 had a reduction in the number of lesions greater than 90% with a total of 16 patients meeting the study criteria for the treatment success. In the TTO group 3 patients met the criteria and only 1 in the I group. Since adverse effect were limited to a small amount of redness around the base of some lesions, with no discontinued treatment due to adverse reaction, results of the study suggest a synergistic safe use of TTO and organically bound iodine in the treatment of molluscum contagiosum (Markum & Baillie 2012).

4.4. Overall conclusions on clinical pharmacology and efficacy

TTO has been widely investigated in several clinical studies, which showed its efficacy as an antiseptic in various conditions.

Two RCT conducted in different countries support the ability of a 5% TTO gel to ameliorate lesions in the treatment of mild to moderate acne vulgaris (Enshaieh *et al*. 2007, Bassett *et al*. 1990). Another study conducted by Feinblatt (1960) is insufficient to show the efficacy of 100% TTO for the treatment of furunculosis (boils) despite the positive findings.

Clinical trials support the efficacy versus placebo of 50% and 25% TTO solutions in the treatment of interdigital tinea pedis (Satchell *et al*. 2002a) and the traditional use of a cream containing 10% TTO to improve symptoms of tinea pedis, but with no significant effects against the basic cause of the pathology (Tong *et al*. 1992).

A RCT showed that 100% TTO has an effect comparable to that of clotrimazole for the treatment of onychomycosis (Buck *et al*. 1994). Another RCT (Syed *et al*. 1999) did not show effects of TTO in onychomycosis, but information are lacking on the TTO concentration of the cream used in the study.

The use of TTO for the reduction of yeast and fungal infections was studied in various clinical trials conducted by different investigators, but in some studies information on the TTO content of the preparation used is not provided (Jandourek *et al*. 1998, Vazquez & Zawawi 2002) and in the other studies the number of patients or the study design cannot be considered supportive for the well-established use (Catalán *et al*. 2008, Belaiche 1985a, Belaiche 1985b).

Two RCT (Dryden 2004, Caelli *et al*. 2000) and one open controlled pilot study (Enshaieh *et al*. 2007, Bassett *et al*. 1990) conducted by different investigators showed that different concentrations (3.3-10%) of TTO may influence positively wound healing through its antimicrobial activity and clearance of MRSA.

Clinical studies for the relief of the symptoms associated with a variety of oral cavity diseases or for the prevention of dental plaque growth support the use and antimicrobial activity of various TTO preparations (TTO commercial oral solutions, 6% TTO in aqueous gel, 0.34% TTO dispersed in milk and diluted with water, 2.5% TTO gel) but they were performed in a too small number of patients or showed no significant results (Jandourek *et al*. 1998, Vazquez & Zawawi 2002, Catálan *et al*. 2008, Groppo *et al*. 2002, Arweiler *et al*. 2000, Soukoulis & Hirsch 2004).

The clinical study on the use of TTO for the treatment of ocular *Demodex* (Gao *et al*. 2007) provides an interesting hypotesis for further investigation.

Clinical investigations on the use in vaginitis, cervicitis and endocervicitis gives only a very low level of evidence, insufficient to support the use of any formulation tested (Peña 1962, Blackwell 1991, Belaiche 1985a).

5. Clinical Safety/Pharmacovigilance

5.1. Overview of toxicological/safety data from clinical trials in humans

Most of the clinical studies in which skin irritations and allergies were demonstrated utilized 1% TTO preparations thus indicating that commonly used topical concentrations are likely to elicit allergic responses in susceptible individuals. Because of demonstrated systemic toxic effects, TTO should never be used internally. In 2005, Nielsen reviewed the reported toxicity of TTO and its major components and derived an estimated NOAEL for whole TTO of 330 mg/kg b.w. based on component data with a worst case scenario of 117 mg/kg b.w. (Nielsen 2005).

Skin Irritation

In a recent review, Hammer *et al.* (2006) reported the results of a number of publications on human patch testing with TTO. The results of these studies are summarised in the **Table 6**. Undiluted TTO has been reported to cause skin irritation in a small proportion of subjects (generally <5%). The irritation potential of TTO may be related to the age of the oil, with aged oils (presumably containing higher levels of peroxides and degradation products such as ascaridol) displaying a greater incidence of irritation.

Table 6: Skin irritation potential of TTO in humans

Test substance	No of subjects	Results	Study
Ten different samples of undiluted TTO applied under occlusive conditions for 48 hours.	219	The prevalence of marked irritancy to 100% TTO ranged from 2.4% to 4.3%. Any level of irritancy (mild and marked) ranged from 7.2 to 10.1%.	Greig *et al.* 1999
Undiluted TTO and 25% TTO in cream, 25% TTO in ointment, 25% TTO in gel, 5% TTO in cream and 5% TTO + 5% synergist in cream. Applied under occlusive conditions for 48 hours.	311	Subjects were treated daily for a three week period during the induction phase of a sensitisation study. Mean irritancy score of 0.25 for undiluted TTO. The incidence of irritation with undiluted TTO was 5.5%. Formulations containing 25% or lower of TTO were non-irritating.	Altman 1991 Aspres & Freeman 2003

Test substance	No of subjects	Results	Study
TTO at 10% (in pet.) and 5% in a commercial lotion and 4 other formulations. Applied under occlusive conditions for 48 hours.	217	10% TTO (in pet.) did not cause irritation. The 5% lotion caused irritation in 44 subjects (20%). The 4 formulations tested on 160 subjects caused 5 weak reactions (3.1%). All test samples contained the same source of TTO. The other components in the formulation influence the incidence and severity of irritation.	Veien *et al.* 2004

Sensitisation

Greig *et al.* (2002) investigated the allergic reaction threshold using occluded patch testing in eight subjects previously confirmed to be sensitised to TTO. The reaction threshold concentrations for TTO were highly variable and were found to occur at 0.5% in one subject, while still being somewhat doubtful at 10% in one other subject. The lowest concentration able to induce a level 1-3 response in the other volunteers fell between these: 1% (one person), 2% (three people) and 5% (two people). In the same subjects, 11 individual components of TTO were also tested. The TTO components that caused reactions in pre-sensitised individuals were p-cymene, terpinolene, α-terpinene and γ-terpinene. The authors commented that they had concerns that the oil samples may have become oxidised within the duration of the study.

Elicitation

The elicitation studies generally demonstrate that the threshold for elicitation of allergic reactions in subjects sensitised to tea tree are >2% in the majority of sensitised subjects. Friedman & Moss (1985) suggested that when induction conditions are severe then the elicitation threshold is low. When induction occurs under mild conditions (as is the case with TTO) much higher exposures are required to elicit an allergic reaction and allergic reaction may not occur as long as exposure remains low.

Induction

A test on human volunteers using a low dose but highly maximized conditions failed to produce sensitisation reactions. A Kligman Human Maximization test was conducted on 1% TTO in petrolatum in 22 healthy male and female volunteers. The test material was applied under occlusion to the same site on the volar forearm of all subjects for 5 alternate-day 48-hour periods. The patch site was pre-treated for 24 hours with 5% aqueous SLS under occlusion for the initial patch only. Following a 10-14 day rest period, a challenge patch of the test material was applied to a fresh site for a 48-hour period under occlusion. Prior to challenge, 5% SLS was applied to the test site for 30 minutes under occlusion on the left side of the back whereas the test materials were applied without SLS treatment on the right side. A fifth site challenged with petrolatum served as a control (RIFM 1802).

Clinical Diagnostic Studies

Two cases of contact dermatitis associated with the application of TTO have been reported by Apted (1991). The use of a vehicle and other aspects of the patch testing were not discussed however, positive patch tests were apparently obtained.

A TTO hand-wash was provided for staff in the intensive care unit of a major hospital. A 45-year-old nurse developed raised red lesions at sites of contact within 5 min of application. This reaction occurred on 3 separate occasions, the lesions persisting for at least 36 h. Previously, she had regularly used a shampoo containing TTO at home without adverse effects. Patch testing was performed (using IQ chambers) on 3 separate occasions over several months, firstly on the outer upper arm and then on the upper back. There was no response to 10 different samples of 10% TTO tested at 10%. When the

TTO used in the manufacture of the handwash was tested at the concentration in the product (3%) there was no reaction. When tested at 100% however, the 10 samples of TTO produced reactions on 2 occasions. Mild erythema and pruritus also occurred with 6 of the 10 oils on 1 occasion and with 4 on the other. On the 2nd occasion, one oil caused erythema and oedema. She also gave vesicular responses to 3 metals (potassium dichromate, cobalt chloride, and nickel sulfate) (Greig *et al.* 1999).

Two professional aroma therapists with suspected allergic contact dermatitis after having handled a variety of essential oils in the course of their work were patch tested with a total of 60 and 22 oils, respectively. Occluded patches with the oils including TTO at 2% diluted in white petrolatum, were applied for 48 hours. In one of these patients a positive (+++) reaction was observed to this oil. It is not clear how many other oils produced positive reactions in this patient (Dharmagunawardena *et al.* 2002).

A 46-yr-old man applied pure TTO to a superficial abrasion on his left leg. Within a few days, the treated area became red and itchy. Applications of TTO were stopped, but the eruption became generalized, with urticarial plaques and atypical targets. A skin biopsy from a target-like lesion showed a spongiotic dermatitis. The patient then developed dermatitis under an Elastoplast® dressing used on the biopsy site. The lesions cleared with oral prednisone. Five months later, patch tests were done with the North American standard series and with TTO, hydroabietyl alcohol, abietic acid and turpentine peroxides. The patient was also tested to a drop of his own, old TTO. At day 4, the patient reacted to both TTO samples, with a stronger reaction to his own than to the fresh preparation. Positive reactions to colophony, hydroabietyl alcohol and Balsam of Peru were also noted (Khanna *et al.* 2000).

Open and closed tests on TTO at different concentrations in water were conducted on a 74-year-old man after the occurrence of blistering dermatitis from the use of a TTO containing wart paint. The patient reacted to a concentration of 1% at the closed site and at 100% at the open site. No effects were seen in 50 controls at 1% or 5% (Bhushan & Beck 1997).

A 64 year old woman with severe eczema of the ears, neck and upper chest following the use of Earex® ear drops was patch tested with the European standard, preservatives, cosmetics and the hairdressing series as well as her own products including Earex® ear drops which was positive. Further testing to the ingredients of Earex drops was conducted including 5% TTO to which she reacted. No further details provided (Stevenson & Finch 2003).

Tests were conducted on a 33-year-old woman after the occurrence of dermatitis from the use of undiluted TTO. Finn chambers and Scanpor tape were used. Reactions were assessed day 3. A positive reaction was observed (Selvaag *et al.* 1994).

In a study on the frequency of sensitisation to TTO in consecutive patients, patch tests were conducted in 10 dermatological departments. TTO gave positive reactions in 16/794 patients when tested at 5% in diethylphthalate. Of these 16 reacting patients, 12/16 pts had used TTO in the past, mainly as a treatment for herpes simplex, eczema and onychomycosis. 4/16 subjects denied any contact to TTO. 7/16 subjects also showed a positive patch test to oil of turpentine at 10% in petrolatum (Treudler *et al.* 2000).

A crystalline compound was isolated from oxidized TTO identified as 1,2,4-trihydroxymenthane by mass spectroscopy. Fifteen patients sensitive to TTO were tested epicutaneously with seven typical constituents of and two degradation products of TTO. Positive effects, 1,2,4-trihydroxymenthane was shown to be an important allergen as well as ascaridol, another degradation product of TTO. Besides 1,2,4-trihydroxymenthane and ascaridol, alpha-phellandrene, alpha-terpinene, and terpinolene were found to give positive reactions as well. The authors noted that TTO kept under practical daily conditions undergoes photo-oxidation within a short time, leading to the formation of peroxides and subsequently to the generation of degradation products. Compounds like ascaridol and 1,2,4-

trihydroxymenthane are formed. These degradation products are moderate to strong sensitizers and must be considered responsible for the induction of contact allergy developing in individuals having treated themselves with TTO (Harkenthal *et al.* 2000).

Seven male and female patients who had become sensitised to TTO were examined during a 3-year period in an outpatient dermatology clinic. They had been treating pre-existing skin conditions, which included foot fungus, dog scratches, "pimples" of the legs, insect bites and hand rashes. All patients initially had an eczematous dermatitis consisting of ill-defined plaques of erythema, oedema and scaling. In 3 patients vesciculation was also present. The patients were patch tested on their upper backs with Finn Chambers to a 1% solution TTO and solutions of 11 constituent compounds. The application time was 48 hours. Reactions were assessed at 50 hours. Control patches of ethanol, olive oil and a blank Finn Chamber were also applied. A total of 20 control patients with unrelated dermatoses were patch tested to the 1% TTO solution and 10 control patients were patch tested to solutions of 11 constituent compounds. 7 control patients were patch tested to the higher concentrations of the constituent compounds. The patch test vehicle was ethyl alcohol in all cases. All seven patients reacted to TTO at 1%. No effects were seen in 20 control subjects. Positive reactions were also seen with d-limonene, α-terpinene, aromadendrene, terpinen-4-ol, α-phellandrene, p-cymene, α-pinene and terpinolene (Knight & Hausen 1994).

Human Patch Tests

There are several human patch test studies with TTO reported in the literature. These have been summarised in **Table 7**. In total, patch tests have identified 151 subjects with positive reactions to TTO among 9367 subjects. The rate of allergic reactions varies from one study to another and is between 0.6% and 2.4% (mean 1.6%). The incidence and strength of the reactions was generally higher with oxidised TTO samples. Rutherford *et al.* (2007) concluded that oxidised TTO has a sensitising capacity three times stronger than fresh TTO. This is consistent with the finding of Hausen (Hausen *et al* 1999, Hausen 2004) and the relatively high rate of positive reactions observed in patch testing of a deliberately oxidised TTO sample (Coutts *et al.* 2002).

Nielsen (2005) concluded that the prevalence of positive findings following exposure of pre-sensitised dermatological patients in the clinical studies to TTO is generally around 0.4%-0.6% (Hammer *et al.* 2006). Thus, TTO has only a weak sensitising potential among pre-sensitised people, though the present known number may be an overestimate due to problems with aged TTO (unknown peroxide levels) and selection bias in some clinical studies.

While patch testing remains a useful diagnostic tool used by Dermatologists, it has some well recognised limitations. In most studies the researchers neglect to demonstrate clinical relevance of any positive patch testing results (Lachapelle 1997). Rutherford *et al.* (2007) observed positive patch tests with TTO in 41 out of 2320 patients. However when the patients were questioned regarding prior exposure to TTO products, only 17 out of 41 reactions were of possible clinical relevance, but none could be demonstrated to have probable or definite relevance. In other words, out of the 41 patients giving a positive patch test to TTO, 24 subjects had no identified prior exposure to TTO.

False positives in the patch tests are not uncommon. False positives can occur as a result of irritancy rather than a true allergic response, particularly as TTO can cause skin irritation both in animals (Beckmann & Ippen 1998) and humans (Aspres & Freeman 2003). Similarly, false positives may result from cross-reactions where patients react to a substance which is not the substance which initially induced the allergic state. TTO is an essential oil with components that are also found in other natural substances. The phenomenon of "excited skin syndrome" has also been suggested to contribute to false positives (Maibach In Ring & Burg 1981). This phenomenon occurs when a subject shows multiple positive patch tests which cannot be reproduced when the subject is retested.

It should also be noted that many of the Dermatological units obtain their samples of TTO from Chemotechnique Diagnostics have confirmed that their oil has been deliberately oxidised.

Table 7: Summary of human patch test studies

Test substance	Number of subjects	Results	Study
Products containing TTO were tested concentrated or diluted	1216	Seven patients (0.6%) with an allergic contact dermatitis due to TTO were identified. Two of them also exhibited delayed type IV hypersensitivity towards fragrance-mix or colophony	Fritz *et al.* 2001
TTO formulations ranging from 5 to 100%	28	21-day RIPT resulted in 3 subjects (11%) showing allergic reactions to mixtures containing oxidised oils	Southwell *et al.* 1997
Undiluted TTO and 25% TTO in cream, 25% TTO in ointment, 25% TTO in gel, 5% TTO in cream and 5% TTO + 5% synergist in cream	311	Three (1%) subjects were sensitised to TTO.	Aspres & Freeman 2003
Ten different samples of undiluted TTO.	219	Five subjects (2.3%) exhibited confirmed sensitisation reactions.	Greig *et al.* 1999
Undiluted TTO, and 5%, 1% and 0.1% of TTO in petrolatum Stabilised by microencapsulation	725	Six subjects (0.8%) gave a definite reaction with undiluted TTO. Another 37 subjects presented equivocal to minimal reactions. Serial dilutions were positive until 1% concentration (one subject). There were no reactions at 0.1% concentration. The authors concluded that the sensitisation potential to TTO was "poor".	Lisi *et al.* 2000
Undiluted TTO which was deliberately oxidised	550	Thirteen (2.4%) subjects with 4 considered of relevance and 5 with possible relevance.	Coutts *et al.* 2002 (Abstract only)

TTO may be regarded as only a weak allergen, where it has any sensitising potential. Thus, normal in-use exposure may induce a sub-clinical allergic state which will not be elicited under normal exposure conditions but may become apparent only under occlusive patch test conditions. This is supported by the absence of any clearly documented epidemic of consumer complaints associated with TTO containing cosmetic products. This hypothesis has been proposed to explain some of the allergic responses seen in clinical studies for some fragrance ingredients (Hostynek & Maibach 2004). Furthermore, the relatively high volatility of TTO and the low dermal penetration may also explain the difference in the result obtained with diagnostic patch testing, where the dermal penetration is expected to be increased due to occlusion, and the lack of consumer complaints as demonstrated by company data.

5.2. Patient exposure

Aside from market presence and data from studies (see section 4), there are no concrete data concerning patient exposure.

5.3. Adverse events and serious adverse events and deaths

According to the data provided by ATTIA Ltd., since record keeping commenced in 1987, 23 adverse events for TTO have been recorded in Australia, corresponding to 0.8 incidents per year. As the estimated sale from 1987 is 25 million unit of bottle containing 100% TTO, the incidence appear

extremely low. Of the 23 events reported, 6 are of identified 100% TTO, 10 product are related to formulated product of less than 100% concentration of TTO, 7 are unidentified, no concentration is reported, but TTO is 'suspected'.

Cutaneous and mucosal reactions

Adverse skin reactions like smarting pain, itch, and allergic reactions have been reported. The frequency is not known (Swedish leaflet).

Burn-like skin reactions have been reported in Denmark. The frequency is rare (<1/1000).

It is likely that the irritation potential of tea tree oil may be related to the age of the oil, with aged oils (presumably containing higher levels of peroxides and degradation products such as ascaridol) displaying a greater incidence of irritation (Australian Government – Rural Industries Research and Development Corporation 2007).

Allergic reactions

Allergic skin reactions reported in Denmark are not common (≥1/1.000 and < 1/100).
Forty-six cases of allergic contact dermatitis with the use of TTO have been reported in the literature from 1991 to 2004, mostly limited to mild symptoms, such as erythema and pruritus, or eczematous plaque in the area of application; however bullous and erythema multiforme-like reactions have also been reported and, in one particular case of a 18 year female patient, linear Immunoglobulin A (IgA) disease appears to have been precipitated (Perrett *et al.* 2003, Crawford *et al.* 2004). Manifestation and location depend on the site of the application, duration of exposure and severity of the host immunological response (Crawford *et al*. 2004).

For example Varma *et al.* reported a case of vaginal application of TTO and lavender oil in a patient with concurrent severe eczema (Halcón & Milkus 2004). Bhushan & Beck (1997) reported a case of blistering dermatitis where a wart paint containing TTO had been used for a period of 4 months. The man had a positive patch test to 1% TTO, while 50 controls were negative on testing with 1% and 5% aqueous tea tree solutions. The case patient was treated with topical corticosteroids and recovered with no known sequelae (Halcón & Milkus 2004).

At the Skin and Cancer Foundation (Sydney, NSW, Australia), three of 28 normal volunteers tested strongly positive to patch testing with 25% TTO. Following further patch testing with TTO constituents, all three patients reacted strongly to two preparations containing sesquiterpenoid fractions of the oil, which supports the indication that sesquiterpenes hydrocarbons may be potent allergens and that the allergenic fraction may be reduced by removal of sesquiterpenes by fractionation and selection of genotypes with lower sesquiterpene contents. These adverse skin reactions were classified as allergic reactions rather than irritant, because erithema with market dermal oedema and itching appeared in the absence of the epidermal reaction usually seen in an irritant patch test reaction, where scaling and wrinkling of epidermis is evident (Rubel *et al.* 1998). Due to the widespread use of TTO, especially in Australia, prevalence rate for allergic contact dermatitis reactions are difficult to estimate and it seems that in Australia the prevalence is higher than in other Countries, such as for instance United States, due to the previous exposure to TTO (Crawford *et al*. 2004).

In the evaluation of patients with allergy to TTO it should be considered that they could have been exposed to several other essential oils with common chemical constituents known to be sensitizers (Crawford *et al*. 2004). Moreover, whereas fresh TTO seems to possess only a weak sensitizing potential, it is well known that oxidized constituents of TTO increase their ability to act as allergens (Harkenthal *et al*. 2000, Carson & Riley 2001, Norwegian Food Safety Authority 2012).In ten separate human patch test studies involving almost 9400 people, an average of 1.6 per cent of people showed some allergic reaction to TTO. It is known, however, that in several of the patch test studies degraded

tea tree oil was used to test for sensitisation. The incidence of sensitisation in the patch test studies may therefore be an overestimate due to peroxides and their degradation products in the oils tested (Australian Government – Rural Industries Research and Development Corporation 2007).

The studies generally have demonstrated that the TTO concentration at which an allergic response may be elicited is greater than 2% in the majority of sensitised subjects. Data collected by six companies that supply TTO products shows that the incidence of adverse reports is dependent on the concentration of oil, with most of the reports occurring with undiluted TTO. Overall, with records from more than 10 years covering 38 million products – many of which were full strength or high concentration tea tree oil, the incidence of adverse events reported for all tea tree oil-containing products is low (0.0016%) (Australian Government – Rural Industries Research and Development Corporation 2007.

Acute intoxications

Several cases of human TTO poisoning have been reported, mostly involving the ingestion of modest volumes (N 10-25 ml) of oil. In two cases, ingestion of TTO resulted in what appeared to be systemic contact dermatitis (Carson & Riley 1998).

It has been reported the case of a patient comatose for the first 12 h and then semi-conscious for the following 36 h after ingestion of approximately half a cup of TTO. Other cases reported that two children who ingested less than 10 ml TTO became ataxic and drowsy or disorientated. Both were treated supportively and recovered fully without further complications (Carson & Riley 1998).

Ingestion of significant quantities of TTO has been described in a 17-month-old male who ingested less than 10 ml of the pure oil (100%) and developed ataxia and drowsiness (Halcón & Milkus 2004).

Accidental poisonings following TTO ingestion demonstrate that at relatively high doses, TTO causes Central Nervous System depression and muscle weakness (Jacobs & Hornfeldt 1994, Del Beccaro 1995, Morris *et al.* 2003, Elliott 1993, Villar *et al.* 1994, Seawright 1993). However, these symptoms had generally resolved within 36 hours.

The 29th Annual Report of the American Association of Poison Control Centers ' National Poison Data System (NPDS) analyzed the data obtained in the year 2011 from 57-seven participating centres (PC) serving the entire population of the 50 states, American Samoa, the District of Columbia, Federated States of Micronesia, Guam, Puerto Rico, and the US Virgin Islands. Among 1,376 TTO exposure cases, no or minor outcome was reported in most cases, minor outcome in 192 cases, major in 5 cases and no death. In 30 cases exposures were intentional with adverse reactions in 37 cases (Bronstein *et al*. 2012)

5.4. Laboratory findings

No data available.

5.5. Safety in special populations and situations

In vitro pharmacological interactions between TTO and conventional antimicrobials (ciprofloxacin/amphotericin B) when used in combination were investigated. Interactions of TTO when combined with ciprofloxacin against *Staphylococcus aureus* indicate mainly antagonistic profiles. The interactions of TTO with amphotericin B indicate mainly antagonistic profiles when tested against *Candida albicans*. The authors concluded that the predominant antagonistic interactions noted, suggest that therapies with TTO should be used with caution when combined with antibiotics (van Vuuren *et al.* 2009).

Safety related to the use in pregnancy and lactation is unknown and therefore the use is not to be recommended.

5.5.1. Use in children and adolescents

The use in children under 12 years of age has not been established due to lack of adequate data.

5.5.2. Contraindications

Hypersensitivity to the active substance or to colophony as TTO cross-reacts with colophony (Norwegian Food Safety Authority 2012).

5.5.3. Special Warnings and precautions for use

Not to be used orally or as inhalation. Not to be used in eyes or in ears.

Not to be swallowed in case of use as a gargle or mouth wash.

If a rash develops discontinue use.

If fever or signs of exacerbating skin infection are observed, a doctor or a qualified health care practitioner should be consulted.

In cases of severe acne or for the eradication of fungal infection a doctor or a qualified healthcare practitioner shall be consulted.

If symptoms worsen during the use of the medicinal product, a doctor or a qualified health care practitioner should be consulted.

5.5.4. Drug interactions and other forms of interaction

None reported

5.5.5. Fertility, pregnancy and lactation

No fertility data available.

Safety during pregnancy and lactation has not been established. In the absence of sufficient data, the use during pregnancy and lactation is not recommended.

5.5.6. Overdose

None reported for the cutaneous use.

Accidental ingestion may cause central nervous system depression and muscle weakness. However, in adults these symptoms generally resolve within 36 hours (See "*Acute intoxications"* in section 5.3)

If ingestion occurs, the patient should be monitored and standard supportive treatment applied as required.

In children, ingestion of tea tree oil is a medical emergency requiring immediate hospital treatment and respiratory support.

5.5.7. Effects on ability to drive or operate machinery or impairment of mental ability

No studies on the effect on the ability to drive and use machines have been performed.

5.5.8. Safety in other special situations

Previous exposure to TTO or to several other essential oils with common chemical constituents known to be sensitizers may increase the possibility of allergic contact dermatitis reactions (Crawford *et al*. 2004).

Whereas fresh TTO seems to possess only a weak sensitizing potential, oxidized constituents of TTO increase their ability to act as allergens and as irritating agents (Harkenthal *et al*. 2000, Carson & Riley 2001, Norwegian Food Safety Authority 2012, Australian Government – Rural Industries Research and Development Corporation 2007). See also section *Allergic reactions in 5.3* Adverse events and serious adverse events and deaths.

5.6. Overall conclusions on clinical safety

Clinical studies and traditional use show that short-term use (not more than 1 month) of diluted TTO on skin or mucosa is safe, but it is not suitable to be used in the eye or ear.

Reported adverse events were minor and mostly limited to local irritation. A case of blistering dermatitis has been reported with a wart paint containing TTO used for a period of 4 months.

There is some evidence that 100% TTO can cause allergic reactions in some patients. The rate of allergic reactions reported in the literature in various patch testing studies ranges between 0.6% and 2.4% (mean 1.6%). The incidence and strength of the reactions is generally higher with oxidised TTO samples. Proper storage and handling of TTO and its formulated products are needed to avoid the development of these by-products and reduce the risk of skin irritation and sensitisation in sensitive individuals.

Oral use results in poisoning. Accidental ingestion of 10-25 ml, demonstrates that at these relatively high doses, TTO causes Central Nervous System depression and muscle weakness. However, these symptoms had generally resolved within 36 hours.

TTO was not genotoxic in *in vivo* mouse micronucleus test (up to 1750 mg/kg). Ames test data are incomplete.

Tests on reproductive toxicity and on carcinogenicity have not been performed.

6. Overall conclusions

Despite several studies show that the antiseptic properties of TTO in various conditions no herbal medicinal product used in clinical trials with positive outcome is currently authorised in Europe for a least 10 years and therefore the "well-established medicinal use" cannot be supported. However results of clinical studies reinforce the plausibility of the traditional uses of TTO preparations.

TTO has been used as a traditional medicine for more than 30 years in Europe and worldwide, particularly in Australia for a number of indications. Some of them are supported by pharmacological or clinical data which confirm the antibacterial activity, antifungal activity, antiviral activity and antiprotozoal activity under controlled conditions. TTO has a broad spectrum antimicrobial activity with little evidence for inducing tolerance and resistance. TTO products are a useful addition to the range of

skin hygiene and protection products. This type of product has a known safety profile with a long history of traditional medicinal use.

Overall, a monograph on *Melaleuca alternifolia* (Maiden and Betch) Cheel, *Melaleuca linariifolia* Smith, *Melaleuca dissitiflora* F. Mueller and/or other species of *Melaleuca*, aetheroleum radix is established with the following preparations and therapeutic indications.

1) Traditional herbal medicinal product for treatment of small superficial wounds and insect bites: liquid preparation containing 0.5% to 10% of essential oil to be applied to the affected area 1-3 times daily; 1-2 drops (0.033-0.066 ml) of undiluted essential oil to be applied to the affected area using a cotton bud 1-3 times daily.

2) Traditional herbal medicinal product for treatment of small boils (furuncles and mild acne): oily liquid or semi-solid preparations containing 10% of essential oil, to be applied to the affected area 1-3 times daily or 0,7-1 ml of essential oil stirred in 100 ml of lukewarm water to be applied as an impregnated dressing to the affected areas of the skin or undiluted essential oil to be applied to the boil using a cotton bud 2-3 times daily.

3) Traditional herbal medicinal product for the relief of itching and irritation in cases of mild athlete´s foot: oily liquid or semi-solid preparations containing 10% of essential oil, to be applied to the affected area 1-3 times daily; 0.17-0.33 ml of essential oil in a bowl containing an appropriate volume of warm water to cover feet. Soak feet for 5-10 minutes a day; undiluted essential oil to be applied to the affected area using a cotton bud 2-3 times daily until the condition is cleared up.

4) Traditional herbal medicinal product for symptomatic treatment of minor inflammation of oral mucosa: 0.17 – 0.33 ml of TTO to be mixed in 100 ml of water for rinse or gargle several times daily for symptomatic treatment of minor inflammation of oral mucosa.

Adverse skin reactions including smarting pain, mild pruritus, burning sensation, irritation, itching, stinging, erythema, oedema, allergic reactions and allergic contact dermatitis have been reported. The frequency is not known. Sensitization is more likely to appear with oxidized TTO and therefore human adverse reactions may be minimized by reducing exposure to aged, oxidized oil. Proper storage and handling are needed to avoid the formation of oxidation products which have greater potential for skin sensitisation. Thus TTO should be in air-tight containers, protected from light and heat and a shelf-life after opening should be stated in the label of formulated TTO products on the basis of appropriate studies. Burn-like skin reaction has been reported. The frequency is rare (<1/1.000).

There is insufficient data to support the safety of TTO during pregnancy and lactation or in children under 12 years and therefore the use in this population groups is not recommended as a precautionary measure.

The data on safety are considered sufficient to establish a list entry for the above mentioned preparations and indications.

Annex