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Review

A review of the toxicity of *Melaleuca alternifolia* (tea tree) oil

K.A. Hammer [a,*], C.F. Carson [a], T.V. Riley [a,b], J.B. Nielsen [c]

[a] *Microbiology and Immunology (M502), School of Biomedical and Chemical Sciences, The University of Western Australia, 35 Stirling Hwy, Crawley, WA 6009, Australia*
[b] *Division of Microbiology and Infectious Diseases, Western Australian Centre for Pathology and Medical Research, Queen Elizabeth II Medical Centre, Nedlands, WA 6009, Australia*
[c] *Environmental Medicine, University of Southern Denmark, Winsløwparken 17, DK-5000 Odense, Denmark*

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Abstract

The essential oil of *Melaleuca alternifolia*, also known as tea tree or melaleuca oil, is widely available and has been investigated as an alternative antimicrobial, anti-inflammatory and anti-cancer agent. While these properties are increasingly well characterised, relatively limited data are available on the safety and toxicity of the oil. Anecdotal evidence from almost 80 years of use suggests that the topical use of the oil is relatively safe, and that adverse events are minor, self-limiting and occasional. Published data indicate that TTO is toxic if ingested in higher doses and can also cause skin irritation at higher concentrations. Allergic reactions to TTO occur in predisposed individuals and may be due to the various oxidation products that are formed by exposure of the oil to light and/or air. Adverse reactions may be minimised by avoiding ingestion, applying only diluted oil topically and using oil that has been stored correctly. Data from individual components suggest that TTO has the potential to be developmentally toxic if ingested at higher doses, however, TTO and its components are not genotoxic. The limited ecotoxicity data available indicate that TTO is toxic to some insect species but more studies are required.

Keywords: Essential oil; Terpene; Allergy; Ecotoxicity

Contents

Abbreviations: bw, body weight; LC_{50}, lethal concentration 50%; LD_{50}, lethal dose 50%; LT_{50}, lethal time 50%; NOAEL, no observed adverse effect level; ppm, parts per million; TTO, tea tree oil.

* Corresponding author. Tel.: +61 8 9346 1986; fax: +61 8 9346 2912.
E-mail address: khammer@cyllene.uwa.edu.au (K.A. Hammer).

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1. Oil provenance

Before discussing the toxicity of TTO it is worth considering the provenance of oils marketed as TTO. TTO is a complex mixture of terpene hydrocarbons and tertiary alcohols distilled mainly from plantation stands of the Australian native plant *Melaleuca alternifolia* (Maiden and Betche) Cheel of the Myrtaceae family. A series of standards has attempted to define and limit the variation seen in this heterogeneous mixture, since it is subject to considerable batch-to-batch variation depending on growth conditions at the plantations. Currently the composition of TTO is regulated by an international standard for "Oil of *Melaleuca* terpinen-4-ol type" which sets maxima and/or minima for 14 components of the oil (International Organisation for Standardisation, 1996) (Table 1). Notably the standard does not dictate the species of *Melaleuca* from which the TTO must be sourced but large-scale commercial TTO is produced from *M. alternifolia*. However, oils which clearly fail to meet the appropriate standard have regrettably been described in the literature as TTO or *Melaleuca* oil and biological properties (Shin, 2003; Mills et al., 2004) including adverse reactions (De Groot and Weyland, 1992; van der Valk et al., 1994) have been erroneously ascribed to TTO. In many reports on the safety and toxicity of TTO, the compositional characteristics of the oil are not stated. As TTO is a natural product with an inherent heterogeneity, future studies describing the biological properties of TTO, particularly those describing its safety and toxicity, should address these oversights and focus on oils that meet the international standard.

Table 1
Composition of *M. alternifolia* (tea tree) oil

Component	Composition (%)	
	ISO 4730 range[a]	Typical composition[b]
Terpinen-4-ol	$\geqslant 30$[c]	40.1
γ-Terpinene	10–28	23.0
α-Terpinene	5–13	10.4
1,8-Cineole	$\leqslant 15$[d]	5.1
Terpinolene	1.5–5	3.1
ρ-Cymene	0.5–12	2.9
α-Pinene	1–6	2.6
α-Terpineol	1.5–8	2.4
Aromadendrene	Traces—7	1.5
δ-Cadinene	Traces—8	1.3
Limonene	0.5–4	1.0
Sabinene	Traces—3.5	0.2
Globulol	Traces—3	0.2
Viridiflorol	Traces—1.5	0.1

[a] International Organisation for Standardisation (1996).
[b] Brophy et al. (1989).
[c] No upper limit is set although 48% has been proposed.
[d] No lower limit set.

2. Toxicity following oral exposure

TTO can be toxic if ingested, as evidenced by experimental studies in rats and from cases of human poisoning. The oral LD_{50} for TTO in the rat is 1.9–2.6 ml/kg (Russell, 1999). Rats dosed with the lesser amount of $\leqslant$1.5 g TTO/kg body weight appeared lethargic and ataxic and showed depressed activity levels 72 h post dosing (Kim et al., 2002). By day 4, however, all but one animal given this lower dose had regained all locomotor functions. Although values determined in animal models are not necessarily directly related to human toxicity, the experimental data do indicate that TTO is orally toxic.

Limited data are available describing the frequency of human poisoning with TTO. The American Association of Poison Control Centers surveillance system recorded 7310 exposures to essential oils in 2003, of which 787 were registered as TTO, second only to clove oil with 870 reported cases (Watson et al., 2004). The identity of the essential oil was unknown in 4647 cases. Of the 787 cases identified as TTO, 518 (65.8%) occurred in children less than 6 years of age, 57 occurred in those aged 6–19 and 212 were in adults older than 19 years. The reason for exposure was described as unintentional in 737 cases and intentional in 20 cases. An additional 28 exposures were reported because they resulted in an adverse reaction after normal, labelled or recommended use of the product, suggesting that these individuals were sensitive to the oil or that indications for the product use were inappropriate. A total of 385 cases had known outcomes and these were categorised as none ($n = 238$), minor (including skin irritation) (134), moderate (12), major (1) and death (0). No deaths due to TTO have been reported in the literature.

Published cases of oral poisoning in humans tend to be more dramatic in children because of their low body weight compared to an adult. One such case report involved a 23-month-old child who drank less than 10 ml of 100% pure TTO (Jacobs and Hornfeldt, 1994). After a nap of approximately 30 min, he was unsteady on his feet and appeared as

if 'drunk'. The child was taken to a hospital and treated with activated charcoal and sorbitol via a naso-gastric tube, and approximately 5 h later he appeared to be asymptomatic. All other signs (such as respiratory rate, oxygen saturation, pupil reactivity, electrolytes and blood glucose) were normal throughout (Jacobs and Hornfeldt, 1994). The authors attributed the clinical symptoms to a central nervous system depression caused by the ingested TTO. Similar symptoms were reported in a 17-month-old boy beginning 10 min after the ingestion of an unknown but less than 10 ml volume of 100% pure TTO (Del Beccaro, 1995). Under observation in hospital, complete resolution of symptoms occurred after approximately 5 h. In a third case, the ingestion of 2 teaspoons of 100% pure TTO by a 4-year-old boy led to symptoms of ataxia within 30 min followed by unconsciousness and unresponsiveness requiring intubation (Morris et al., 2003). The boy's neurologic status improved gradually over 10 h and he was discharged from hospital 24 h after admission without respiratory or neurologic sequelae.

A case of poisoning in an adult occurred when a patient drank approximately half a tea cup of TTO corresponding to a dose of approximately 0.5–1.0 ml/kg body weight (Seawright, 1993). The patient was comatose for 12 h, and semi-conscious and hallucinatory for the following 36 h. Symptoms of abdominal pain and diarrhoea continued for approximately 6 weeks after this. In another incident, a 60-year-old man who swallowed one and a half teaspoonfuls of TTO as a preventative for a cold presented with a red rash which covered his feet, knees, upper body and arms including his palms and elbows (Elliott, 1993). His hands, feet and face were also swollen. The rash and other symptoms gradually disappeared and approximately one week later he had more or less recovered.

Apart from these reports, there are no data on the systemic toxicity of TTO in humans. However, the available knowledge clearly demonstrates the toxicity of TTO following oral exposure and the ingestion of TTO should not be recommended. Despite this, deliberate ingestion is occasionally suggested (Belaiche, 1985; Blackwell, 1991) or reported (Elliott, 1993; Seawright, 1993). TTO is categorised as a Schedule 6 poison in Australia. According to the Drugs, Poisons and Controlled Substances Act 1981, substances classed within this category have "a moderate potential for causing harm, the extent of which can be reduced through the use of distinctive packaging with strong warnings and safety directions on the label". To this end, 100% pure TTO is labelled that it must be kept out of the reach of children, is packaged with a child-resistant cap and is labelled 'not to be taken internally'. It is noteworthy that of the three specific cases of poisoning in children discussed above, at least two of the products were packaged without a child-resistant cap.

3. Toxicity following dermal exposure

A moderate amount of data on the acute dermal toxicity of TTO is available, while the effects of chronic exposure remain uncharacterised. A caveat that needs to be attached to several studies on irritancy and allergic reactions relates to the inclusion criteria for study participants. Thus, studies on irritancy often preclude participants with a previous history of allergies to cosmetic products, a history of active skin disease or dark skin. This selection potentially excludes the more susceptible fractions of the human population with regard to irritancy and allergy, which may cause an underestimate of the true potential for TTO to cause dermal toxicity. Other studies, however, use outpatients visiting dermatological clinics, which may result in the opposite bias by potentially overestimating the true prevalence of allergy and irritancy. Accordingly, the study population needs to be considered when planning as well as evaluating human studies on the prevalence of toxicity following dermal exposure to TTO.

3.1. Irritant reactions

Irritant reactions are often concentration dependant and are not reliant on previous exposure to the irritating agent. Irritant reactions may usually be avoided through the use of lower concentrations of the irritant and this bolsters the case for discouraging the use of 100% TTO and promoting the use of well-formulated products.

The irritant capacity of TTO has been investigated using a protocol based upon the Draize human sensitisation test (Draize, 1955) with occlusive patch testing using Finn chambers (Southwell et al., 1997; Aspres and Freeman, 2003). In the first of these studies, TTO was prepared at concentrations ranging from 5% to 100% in different formulations, and these mixtures were applied in patch tests on the backs or upper arms of volunteers (Southwell et al., 1997). After 24 h, patches were removed and the skin was examined for any reactions. A new chamber was then applied to the same area, and examined again 24 h later. This was repeated at 24 h intervals for a total of 21 days. Of the 28 patients, 25 did not demonstrate any irritant reactions but the remaining three showed allergic type reactions. The TTO component 1,8-cineole, which has a reputation as a skin irritant, was also tested at concentrations up to and including 28% and did not produce any irritant reactions (Southwell et al., 1997). Using a similar protocol, Aspres and Freeman (2003) tested 5%, 25% and 100% TTO in cream, ointment and gel bases on 311 volunteers. The mean irritancy scores for 306 evaluable subjects were very low, the highest being 0.250 for neat TTO (Aspres and Freeman, 2003). A smaller study where 20 patients were patch-tested with 1% TTO found no irritant reactions (Knight and Hausen, 1994). In a recent Danish study, 217 patients consecutively sampled in a dermatology clinic were patch tested with 10% TTO without any irritant reactions recorded (Veien et al., 2004). The same group of patients was also exposed to a lotion containing 5% TTO that caused weak irritant reactions in 44 (20%) of the patients. This part of the study was later repeated on 160 new patients exposed to four newly formulated lotions

containing 5% TTO. In this second trial, only four had weak but apparently TTO-related irritant reactions (Veien et al., 2004). The latter trial with no irritant reactions to new formulations points to a generic problem related to the formation of oxidized and potentially irritant products in most natural oils, a problem that relates not only to irritant reactions, but also to allergic reactions (see below), and needs to be addressed by oil producers and vendors.

3.2. Contact allergy

Contact allergy is defined as a cutaneous reaction caused by direct contact with an allergen to which the patient has become sensitised (Hensyl, 1990). Once an allergic reaction to TTO has occurred, it is likely that all subsequent exposures to TTO, regardless of concentration, will elicit further allergic reactions. Numerous case reports of contact allergy due to the topical use of TTO have appeared in the medical literature during recent decades (Apted, 1991; De Groot and Weyland, 1992; Selvaag et al., 1994; van der Valk et al., 1994; De Groot, 1996; Bhushan and Beck, 1997). The reactions have been in response to 100% pure TTO as well as lower concentrations of TTO in various formulations.

Patch testing in consecutive patients attending dermatology clinics has been done on a number of occasions and may give some indication of the frequency of allergy to TTO. In a study of 725 consecutive patients presenting to a patch test clinic and tested with 5%, 1% and 0.1% TTO, only one patient had a positive patch test to 5% and 1% oil (Lisi et al., 2000). No patient reacted to 0.1% oil. In another series, 550 patients were tested with 100% pure oil and allergic reactions were recorded in 13 (2.4%) of the patients (Coutts et al., 2002). Veien et al. (2004) patch tested 217 consecutive patients with 10% TTO and a commercially available lotion containing 5% TTO, and found one (0.5%) and three patients (1.4%), respectively, had positive patch tests. In a follow-up on 160 other patients, none demonstrated allergic reactions toward four newly formulated lotions containing 5% TTO (Veien et al., 2004). In the study by Southwell et al. (1997) described above, three (10.7%) of the original 28 participants showed distinct allergic reactions. Similarly, in the study by Aspres and Freeman (2003), three (0.97%) of the 309 participants completing the study developed grade 3 skin reactions during the initial induction period, indicating an allergic reaction.

More targeted testing of patients visiting dermatology clinics with a history of TTO use has also been done. Of 1216 patients presenting to a dermatology department, 14 reported using products containing TTO and were patch tested to these products at 5%, 10%, 50% and/or 100% (Fritz et al., 2001). Seven patients showed positive patch tests to their TTO products. Of 50 patients presenting to a dermo-gynaecology clinic for investigations into vulval and vaginal disorders, 13 patients reported using TTO products, and were patch tested and tested negative (Brenan et al., 1996).

In a few instances, patients with a history of contact allergy towards TTO have been investigated in detail, providing more insights into the components responsible for allergy and/or positive patch tests. A series of seven such patients was described in a report by Knight and Hausen (1994). All patients reacted to 1% TTO after patch testing using Finn chambers. In addition, these patients also reacted to one or more of the components D-limonene, α-terpinene, aromadendrene, terpinen-4-ol and α-phellandrene at 1%, 5% or 10%. Furthermore, patch testing of three allergic participants with TTO components showed that they reacted mostly to the sesquiterpenoid fractions but not the pure monoterpenes (Southwell et al., 1997).

It has been suggested that the oxidation products formed within TTO during prolonged storage are the main allergens (Hausen et al., 1999; Hausen, 2004). Newly distilled TTO appears to have a relatively low sensitising capacity, whereas TTO kept for prolonged periods is a moderate to strong sensitiser and has a significantly increased peroxide value (Hausen et al., 1999). This same study also suggests that the most important allergens formed could be terpinolene, α-terpinene, ascardiole and 1,2,4-trihydroxymethane (Hausen et al., 1999; Hausen, 2004). However, the cause of allergy is still controversial and no single allergen responsible for all allergies has been identified.

3.3. Systemic reactions

Toxicity following dermal application of inappropriately high doses of melaleuca oil to cats or dogs treated for fleas has been described. Animals had typical signs of depression, weakness, uncoordination and muscle tremors. However, the treatment of clinical signs has been sufficient to achieve recovery without sequelae within 2–3 days (Villar et al., 1994). For the same reason (fleas) three cats each had 120 ml of 100% pure TTO applied to their shaved but intact skin (Bischoff and Guale, 1998). All three cats experienced severe symptoms (hypothermia, uncoordination, dehydration, trembling), and one died after three days. The other two cats recovered within 24 and 48 h, respectively. The authors noted that the cat that died had elevated blood urea and persistent dehydration, which suggests that the animal may have had pre-existing renal damage unrelated to the TTO poisoning (Bischoff and Guale, 1998). There has also been one report of a systemic hypersensitivity reaction to TTO in a 38-year-old man (Mozelsio et al., 2003). Following dermal application of TTO he suffered immediate flushing, pruritus, a constricted throat and lightheadedness. His reaction, however, was not due to an IgG or IgE response.

4. Ototoxicity

TTO has been suggested as an effective treatment for a number of microorganisms commonly associated with otitis externa and otitis media, but its possible ototoxicity has only been evaluated in a single study by Zhang and

Robertson (2000). The ototoxicity of TTO was examined in guinea pigs by measuring the thresholds of the compound auditory nerve action potential (CAP) to tone bursts before and after instillation of the oil into the middle ear (Zhang and Robertson, 2000). After 30 min of instillation, 100% pure TTO caused a partial CAP threshold elevation at 20 kHz. A lower concentration of oil (2% TTO in saline with 0.5% Tween 80 detergent) did not cause any significant lasting threshold change. While this suggests that concentrations of 2% TTO or less may be safe for use within the ear, a high concentration of TTO applied to the round window for a relatively short time was to some extent ototoxic to the high-frequency region of the cochlea (Zhang and Robertson, 2000).

5. Developmental toxicity

No studies of potential developmental toxicity following exposure to TTO have been published yet. However, a study of the embryofoetotoxicity of α-terpinene, which is present at approximately 9% in TTO, has demonstrated significant toxicity in a rat model (Araujo et al., 1996). The offspring of dams given 60 mg/kg bw from day 6 to day 15 of pregnancy had delayed ossification and skeletal malformations. At 30 mg/kg bw no effects were seen on either dams or offspring. Effects at doses higher than 60 mg/kg bw were accompanied by maternal toxicity. Experiments were close to the given guidelines for this kind of study. The authors suggested a NOAEL for embryofoetotoxicity of 30 mg/kg bw for oral exposure of rats to α-terpinene (Araujo et al., 1996). Two studies with β-myrcene, present at approximately 0.5% in TTO have indicated NOAELs for toxic effects on fertility and general reproductive performance of 250 mg/kg bw (Delgado et al., 1993) and 300 mg/kg bw (Paumgartten et al., 1998). These limited data suggest that TTO is potentially embryofoetotoxic, although only if ingested at relatively high levels.

6. Toxicity against cell lines in vitro

6.1. Cell toxicity

The testing of human or animal cells in vitro is seen as a potential alternative to animal testing to determine toxicity. Several studies have evaluated the toxic effects of TTO and/or components on (human) cell lines in vitro. The amounts of TTO that reduced the growth of cells by 50% as compared to controls (IC_{50}) after 24 h ranged from 20 to 2700 μg/ml for HeLa, K562, CTVR-1, Molt-4, Hep G2, HL-60, fibroblast and epithelial cells (Söderberg et al., 1996; Hayes et al., 1997; Mikus et al., 2000; Schnitzler et al., 2001). In addition, TTO produced toxic effects against human monocytes at concentrations of $\geqslant$0.004% (Hart et al., 2000) or $\geqslant$0.016% (Brand et al., 2001) and at $\geqslant$0.016% against human neutrophils (Brand et al., 2001). Studies assessing the cytotoxicity of TTO components against several cell lines have demonstrated that terpinen-4-ol, α-terpineol, terpinolene, α-phellandrene, aromadendrene, sabinene and α- and β-pinene were all more active than whole TTO (Hayes et al., 1997; Mikus et al., 2000), whereas 1,8-cineole appeared to be less active than TTO (Hayes et al., 1997). In vitro cytotoxicity data have been used to predict or estimate skin irritancy and correlations between in vitro cytotoxicity data and in vivo skin irritancy have been made for various compounds (Korting et al., 1994). Although TTO and components display cytotoxic activity, any relationship between this and in vivo effects remains unknown. A further drawback for the use of these in vitro TTO cytotoxicity data is that they pertain to target cell concentrations of TTO. Thus, for a topically applied product like TTO, data on percutaneous penetration and in vivo disposition including metabolism are needed for any relevant assessment of a potential in vivo risk.

6.2. Mutagenicity

TTO was non-mutagenic in the bacterial reverse mutation assay using *Salmonella typhimurium* strains TA98 and TA100 (Evandri et al., 2005; Fletcher et al., 2005) and *Escherichia coli* strain WP2*uvr* (Evandri et al., 2005), both with and without metabolic activation. Furthermore, the following components were non-mutagenic in the *Salmonella*/microsome (Ames) test or the *Bacillus subtilis* rec- assay: terpinen-4-ol (Fletcher et al., 2005), α-terpinene (Gomes-Carneiro et al., 2005), 1,8-cineole (Oda et al., 1978; Yoo, 1985; Gomes-Carneiro et al., 1998), α-terpineol (Oda et al., 1978; Florin et al., 1980), cymene (Rockwell and Raw, 1979), limonene (Florin et al., 1980; Watabe et al., 1981; Connor et al., 1985), α-pinene (Rockwell and Raw, 1979; Florin et al., 1980; Connor et al., 1985; Gomes-Carneiro et al., 2005), β-pinene (Florin et al., 1980), linalool (Rockwell and Raw, 1979; Oda et al., 1978; Ishidate et al., 1984) and β-myrcene (Gomes-Carneiro et al., 2005). 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 (+/− metabolism). 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 (Gomes-Carneiro et al., 1998).

In tests with mammalian cells, γ-terpinene did not increase DNA strand breakage in human lymphocytes at 0.1 mM but did at 0.2 mM (Aydin et al., 2005). 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 (Sasaki et al., 1989). Another study showed linalool to be non-mutagenic using a Chinese hamster fibroblast cell line (Ishidate et al., 1984). β-Myrcene did not have mutagenic activity when tested with human lymphocytes (Kauderer et al., 1991) and was not genotoxic in bone marrow cells of rats administered β-myrcene orally (Zamith et al., 1993). Overall, the

available data on the mutagenicity of TTO and its individual constituents indicate low mutagenic potential, using both bacterial and mammalian test systems.

7. Ecotoxicity

Ecotoxicology can be loosely defined as the effects of pollutants on natural ecosystems. Although data from acute toxicity testing of single animal or insect species may be regarded as overly simplistic, they are often the starting point for assessing ecotoxicity. Data describing the ecotoxicity of TTO are very limited. The toxicity of TTO against fish, amphibians, insects, worms or other aquatic and terrestrial species, or ecosystems, has not been assessed to any great extent.

7.1. Acute toxicity of TTO to aquatic organisms

Two publications have assessed the potential for TTO to be used as an antifungal agent in fish aquaculture (Marking et al., 1994; Campbell et al., 2001). Whilst both studies tested the efficacy of TTO against aquatic fungi, Marking et al. (1994) also assessed the toxicity of TTO to rainbow trout eggs. They found that TTO was non-toxic to rainbow trout eggs at a concentration of 1500 ppm. Furthermore, TTO yielded an LC_{50} of approximately 500 ppm in the brine shrimp assay, a widely used ecotoxicity indicator, although no information other than the result is given (McCage et al., 2002).

Ecotoxicity data for several components of TTO are shown in Table 2. Limonene and cymene are classified as slightly toxic (LC_{50} between 10 and 100 mg/l), α-terpineol is moderately toxic (LC_{50} between 1 and 10 mg/l), α-pinene appears to be practically non-toxic (LC_{50} between >100 mg/l) and data for β-pinene are equivocal. Notably absent are any data for the TTO components terpinen-4-ol or γ-terpinene, the two components present in the highest proportions in TTO. Whilst ecotoxicity data for the components of TTO can only be used as a guide together with knowledge on expected concentrations of the specific component in the essential oil, they suggest that TTO is probably non-toxic for most relevant aquatic exposure scenarios. However, new knowledge on the ecotoxicity of the two major components of TTO, terpinen-4-ol and γ-terpinene, may change this evaluation.

7.2. Acute toxicity of TTO to terrestrial insects

The acute toxicity of essential oils and components has most commonly been evaluated in the context of using essential oils as crop fumigants and protectants. Data describing the toxic effects of TTO on insects are limited. TTO is active against the whitefly *Trialeurodes vaporariorum*, causing 91% mortality to adults at a concentration of 0.0023 ml/l of air, 97% mortality to nymphs at 0.0093 ml/l of air and 88% mortality to eggs at 0.0047 ml/l of air (Choi et al., 2003). Varroa mites, which are parasitic to honey bees, have been shown to be susceptible to TTO. Mites were exposed to 40 μl of a 50% solution of TTO on filter paper and, after 6 h treatment, 59.4% of mites were dead, compared to only 20% of control mites (Sammataro et al., 1998). TTO also has activity against *Pediculus humanus capitis*, the human head louse (Yang et al., 2004). Using a contact bioassay, the treatment of female lice with 0.25 mg/cm^3 TTO caused 50% of insects to die (LT_{50}) after 31.5 min. In contrast, TTO had no obvious effects against the rice weevil *Sitophilus oryzae* (L.), with an LD_{50} of >0.15 ml/l of air (Lee et al., 2001). In addition to toxicity tests with whole oils, the toxicity of essential oil components has also been determined (Table 3). Overall, these studies indicate that TTO is likely to be toxic to insects. Activity may be due, in part, to the anticholinesterase activity of TTO and components (Mills et al., 2004). However, it has been stated that the acute toxicity of

Table 2
Acute toxicity of TTO components to aquatic species

Component	Aquatic species (life stage)	Data	References
ρ-Cymene	*Daphnia magna*[a]	LC_{50} = 9.4 mg/l	LeBlanc (1980)
	Cyprinodon variegatus[b]	LC_{50} = 56 ppm	Heitmuller et al. (1981)
Limonene	*Leuciscus idius melanotus*[c]	LC_{50} = 34 mg/l LC_{100} = 43 mg/l	Juhnke (1978)
	Artemia salina[d]	LD_{50} = 706 ppm	Hogg et al. (2001)
α-Pinene	*D. magna*	LC_{50} = 68 mg/l	LeBlanc (1980)
	A. salina	LD_{50} = 494 ppm	Hogg et al. (2001)
β-Pinene	*A. salina*	LD_{50} = 491 ppm	Hogg et al. (2001)
	Onchorhynchus mykiss[e] (fry)	LC_{50} = 1.2 mg/l	Passino-Reader et al. (1995)
α-Terpineol	*O. mykiss* (fingerlings)	Toxic dose range: 10–100 mg/l	Webb et al. (1976)
	Onchorhynchus kisutch[f]	LC_{50} = 6.8 mg/l	Stroh et al. (1998)
	O. mykiss	LC_{50} = 6.7 mg/l	Stroh et al. (1998)

[a] Water flea.
[b] Sheepshead minnow.
[c] Carp.
[d] Brine shrimp.
[e] Rainbow trout.
[f] Coho salmon.

Table 3
Selected acute toxicity data for TTO components and terrestrial insects

Component	Insect species (life stage)[a]	LD_{50}or LC_{50}	References
1,8-Cineole	*Sitophilus oryzae*[b]	23.5 μl/l of air	Lee et al. (2001)
	S. oryzae	22.8 μl/l of air	Lee et al. (2004)
	Tribolium castaneum[c]	15.3 μl/l of air	Lee et al. (2004)
	Rhyzopertha dominica	9.5 μl/l of air	Lee et al. (2004)
ρ-Cymene	*S. oryzae*	25.0 μl/l of air	Lee et al. (2001)
Limonene	*Apis mellifera*[d]	10.4 mg/l of air	Ellis and Baxendale (1997)
	Blatella germanica[e]	0.7 mg/insect	Karr and Coats (1988)
	Musca domestica[f]	0.37 μmol/fly	Grodnitzky and Coats (2002)
	M. domestica	0.09 mg/insect	Karr and Coats (1988)
	S. oryzae	61.5 μl/l of air	Lee et al. (2001)
	Spodoptera litura[g] (larva)	0.27 mg/larva	Hummelbrunner and Isman (2001)
Linalool	*B. germanica*	0.55 mg/insect	Coats et al. (1991)
	M. domestica	0.19 mg/fly	Rice and Coats (1994)
	M. domestica	6.8 mg/l of air	Rice and Coats (1994)
	S. oryzae	39.2 μl/l of air	Lee et al. (2001)
	T. castaneum	>1730 mg/l of air	Rice and Coats (1994)
Myrcene	*B. germanica*	>1.58 mg/insect	Coats et al. (1991)
	M. domestica	0.36 mg/fly	Coats et al. (1991)
α-Pinene	*M. domestica*	0.82 μmol/fly	Grodnitzky and Coats (2002)
α-Terpinene	*M. domestica*	0.86 μmol/fly	Grodnitzky and Coats (2002)
	S. oryzae	71.2 μl/l of air	Lee et al. (2001)
Terpinen-4-ol	*S. oryzae*	25.6 μl/l of air	Lee et al. (2001)
	S. litura (larva)	0.13 mg/larva	Hummelbrunner and Isman (2001)
α-Terpineol	*Apis mellifera*	0.02 mg/ml of air	Ellis and Baxendale (1997)
	B. germanica	1.07 mg/insect	Coats et al. (1991)
	M. domestica	1.29 μmol/fly	Grodnitzky and Coats (2002)
	M. domestica	0.31 mg/fly	Coats et al. (1991)
	M. domestica	0.20 mg/fly	Rice and Coats (1994)
	M. domestica	74.5 mg/l of air	Rice and Coats (1994)
	S. oryzae	69.1 μl/l of air	Lee et al. (2001)
	S. litura (larva)	0.14 mg/larva	Hummelbrunner and Isman (2001)

[a] Unless stated otherwise, data are for adult insects.
[b] Rice weevil.
[c] Red flour beetle.
[d] Honey bee.
[e] Cockroach.
[f] House fly.
[g] Tobacco cutworm.

monoterpenes to insects is relatively low, compared to conventional insecticides (Lee et al., 1997). Moreover, several studies have indicated that essential oils can have other effects, such as inhibition of larval growth, deterrence of feeding (Hummelbrunner and Isman, 2001) and inhibition of reproduction (Regnault-Roger and Hamraoui, 1995). Whether TTO also has these effects remains unknown.

Although these ecotoxicological data illustrate that TTO causes mortality to insects and brine shrimp, differences in the units of measurement makes the drawing of meaningful conclusions about its overall toxicity difficult. Furthermore, to the best of our knowledge no long-term studies investigating the chronic and/or reproductive toxicity of TTO in animals or insects have been conducted.

7.3. Other

Two further studies offer insight into the ecotoxicity of TTO. An investigation of the degradation rate of cellulose sheets and dried plane tree (*Platanus* sp.) leaves suggested that those treated with neat TTO degraded significantly slower than those not treated with TTO (Boon and Johnstone, 1997). Furthermore, the TTO components terpinen-4-ol, cineol, linalool and α-terpinene have been shown to reduce seed germination and subsequent seedling growth in *Lactuca sativa* (Lettuce) at a concentration of approximately 25 ppm (Vokou et al., 2003). In particular, terpinen-4-ol completely inhibited germination. The components ρ-cymene and γ-terpinene did not have significant effects. These data imply that the leakage or spillage of large amounts of TTO into the environment could have negative effects on natural ecosystems. However, more data pertaining to the fate of TTO in soil and water, its effects on algae and other plants, and its biodegradability are required.

8. Conclusions

Based on present knowledge, it may be concluded that TTO is definitely toxic when ingested in higher doses, can

cause skin irritation at higher concentrations and may cause allergic reactions in predisposed individuals. However, since data indicate that the toxicity of TTO is dose-dependent, the majority of adverse events can be avoided through the use of lower concentrations. The exception to this is allergic reactions, which occur in only a very limited fraction of the human population and may be caused by oxidation products formed by exposure to light and/or air. There is little evidence to suggest that TTO or its components exhibit significant genotoxic potential. Limited data on two components of TTO (α-terpinene and β-myrcene) suggest that TTO itself could have potential for developmental toxicity, although only if ingested at relatively high levels. Finally, ecotoxicity data indicate some toxicity to specific terrestrial insects. However, firm conclusions pertaining to ecotoxicological effects must await further studies.

Conclusions about the overall toxicity of TTO are complicated by the fact that the oil contains over 100 components. In the absence of data for whole oil, insight may be gained from data for individual components. However, just as data for TTO may not necessarily apply to individual components, data for components may not apply to whole oil. The toxicity of TTO is a functional response to exposure to a mixture of different chemical compounds, which are absorbed and eventually reach their targets for toxicity, be it local or systemic. In the vast majority of toxicity reports, there is no information on the specific components responsible for the toxicity. Present knowledge is limited to knowing what reaches the outer boundary of the exposed organism. Although some data are available describing the absorption of several TTO components when administered individually (Parke et al., 1974; Cal and Sznitowska, 2003), no data are presently available describing the absorption (gastrointestinal or percutaneous) of each of the individual components when present as part of the mixture that constitutes TTO. Furthermore, the different components of TTO constitute from less than 1% to close to 40% of the oil and cover a wide range of solubilities and molecular weights. Considering the different physico-chemical characteristics of the TTO components, not all would be expected to have equal absorption rates. Thus, there is no information on the chemical composition of the mixture of chemicals being absorbed following exposure to TTO, and there is no knowledge of which individual chemicals are responsible for the observed toxicity. This lack of information is of less importance when evaluating observational studies on toxicity the of TTO, but will be a considerable challenge to toxicologists trying to identify specific perpetrators or potential health promoting substances within TTO as well as to regulators within different administrative organisations. This remains an important future focus area for the toxicological evaluation of TTO and its components. More data in this area would lead to an increased understanding of not only which TTO components may cause toxicity, but also those that may have health benefits, which is the ultimate reason that people use products containing TTO. Lastly, if oxidation products can be avoided, the available literature suggests that TTO can be used topically in diluted form by the majority of individuals without adverse effects.

Conflict of interest statement

There are no conflicts of interest.

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