

The optimal patch test concentration for ascaridole as a sensitizing component of tea tree oil

Wietske Andrea Christoffers¹, Brunhilde Blömeke², Pieter-Jan Coenraads¹ and Marie-Louise Anna Schuttelaar¹

¹Department of Dermatology, University Medical Center Groningen, University of Groningen 9700RB, Groningen, The Netherlands and ²Department of Environmental Toxicology, University of Trier, 54286 Trier, Germany

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Summary

Background. Tea tree oil is used as a natural remedy, but is also a popular ingredient in household and cosmetic products. Oxidation of tea tree oil results in degradation products, such as ascaridole, which may cause allergic contact dermatitis.

Objectives. To identify the optimal patch test concentration for ascaridole, and to investigate the relationship between a positive reaction to ascaridole and a positive reaction to oxidized tea tree oil.

Patients/materials/methods. Three hundred and nineteen patients with eczema were patch tested with ascaridole 1%, 2%, and 5%, and 250 patients were patch tested with oxidized tea tree oil 5%. Readings were performed on D3 and D7 according to a patch test calibration protocol.

Results. With an increasing ascaridole test concentration, the frequency of positive reactions increased: ascaridole 1%, 1.4%; ascaridole 2%, 5.5%; and ascaridole 5%, 7.2%. However, the frequencies of irritant and doubtful reactions also increased, especially for ascaridole 5%. A positive reaction to ascaridole was related to a positive reaction to tea tree oil.

Conclusions. This study is in support of ascaridole being a sensitizer. We recommend patch testing with ascaridole at 2%. The finding that every positive reaction to oxidized tea tree oil is accompanied by a positive reaction to ascaridole suggests that ascaridole might be a contact allergen in oxidized tea tree oil.

Key words: allergic contact dermatitis; ascaridole; *Melaleuca alternifolia*; tea tree oil.

Tea tree oil (CAS no. 68647-73-4) is an oil originally distilled from the leaves of *Melaleuca alternifolia*, which is cultivated in the states of New South Wales and Queensland in Australia. The indigenous Australian population used the oil as a herbal medicine for centuries.

Correspondence: Marie-Louise Anna Schuttelaar, Department of Dermatology, University Medical Center Groningen, Hanzeplein 1, PO Box 30.001, 9700 RB Groningen, The Netherlands. Tel: +31 50 3612520; Fax: +31 50 3619247. E-mail: m.l.a.schuttelaar@umcg.nl

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Nowadays, tea tree oil is used as a natural remedy for various conditions, such as acne and warts, and the oil is a popular ingredient in household and cosmetic products. Although consumers might assume that natural products are safer than synthetic products, tea tree oil is known to cause allergic contact dermatitis.

In 1991, the first cases of allergic contact dermatitis caused by tea tree oil were reported in patients who had used tea tree oil as a natural remedy and had become sensitized (1, 2). In these early reports, patch tests were performed with undiluted tea tree oil, sometimes even causing extreme bullous reactions. Over time, tea tree oil diluted 1:3 in alcohol, 1:5 in olive oil or 1% in alcohol was recommended, on the basis of a few case reports

(3, 4). This was supported by a study of Coutts et al. in 2002 in the United Kingdom, who patch tested 550 dermatitis patients with pure, oxidized tea tree oil (5). In total, 13 patients (2.4%) developed a positive reaction; however, 209 patients (38%) developed an irritant reaction to the pure patch test preparation. In 1999, a standardized variant of oxidized tea tree oil 5% was added to the North American Contact Dermatitis Group screening panel. After an initial rise in prevalence (6), the rate of sensitization to oxidized tea tree oil 5% in petrolatum was 1.0% in 4299 dermatitis patients in 2009–2010 in the United States (7). This is comparable with the results of other studies in Europe and Australia that reported prevalence rates of sensitization to oxidized tea tree oil that varied between 0.5% and 1.8% of the patch tested population (8–10).

Besides the search for the optimal patch test concentration of tea tree oil, the search for the sensitizing component was started, given the fact that tea tree oil contains > 100 different components. In 1999, Hausen postulated that the degradation products of monoterpenes are probably the sensitizers in tea tree oil (11). These monoterpenes develop through photo-oxidation of fresh tea tree oil within days to months. The most important sensitizers are probably the sesquiterpene α -phellandrene and the monoterpenes terpinolene, ascaridole, α -terpinene, 1,2,4-trihydroxy menthane, α -phellandren, and (+)-limonene (8, 11–13).

Ascaridole (1,4-epodioxy-*p*-menth-2-ene; CAS no. 512-85-6) (Fig. 1) is a potential sensitizer in oxidized tea tree oil (8, 11). It is a monoterpene, derived from oxidized α -terpene. In a previous study, we patch tested 602 patients with ascaridole 1%, and found 9 (1.5%) positive reactions (14). Only 1 positive reaction to ascaridole 1% was judged to be clinically relevant, because the patient recalled the use of pure tea tree oil followed by the development of dermatitis. Furthermore, ascaridole 1% caused 15 (2.5%) doubtful reactions, which led to the assumption that the concentration of ascaridole 1% was too low to produce positive reactions. We therefore patch tested ascaridole 5% in 144 patients in the same study; this caused 21 positive reactions, but also 14 (9.7%) doubtful and 5 (3.5%) irritant reactions. Only in 1.4% of these patients was the reaction clinically relevant. One patient recalled the use of pure tea tree oil, and the other patient had used a shaving soap containing tea tree oil (15). Interestingly, positive and irritant reactions caused by ascaridole 5% were remarkably similar, especially with regard to bullous reactions, making it difficult to differentiate between positive and irritant reactions. The substantial number of irritant reactions warranted a

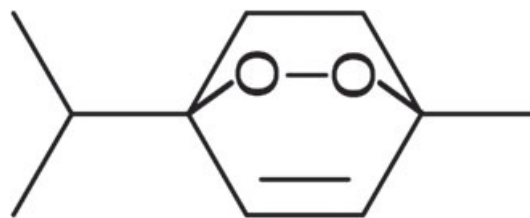


Fig. 1. Chemical structure of ascaridole (1,4-epodioxy-*p*-menth-2-ene).

decrease in the patch test concentration. Thereupon, patch testing with ascaridole 2% was initiated, and the results of this are described in this article. In addition, the clinical relevance of the positive reactions was assessed more thoroughly. Finally, we added tea tree oil to our cosmetic patch test series, to investigate the relationship between a positive reaction to ascaridole and a positive reaction to oxidized tea tree oil.

Materials and Methods

Chemicals

Ascaridole (99.8% purity) was provided by the Institute of Pharmacology, University of Bonn, Germany. It was diluted in pet. in-house by the pharmacy of the University Medical Center Groningen, at concentrations of 1%, 2%, and 5%. The preparations were used for a maximum of 3 months, and were stored in a refrigerator (7°C) during this period.

Oxidized tea tree oil 5% pet. was purchased from Chemotechnique Diagnostics, Vellinge, Sweden. To oxidize the tea tree oil, pure tea tree oil was mixed with air for 5 min daily in an Erlenmeyer flask. The flask was exposed to a daylight fluorescent lamp for 12 hr daily for 3 days (Chemotechnique Diagnostics, pers. comm. 2013).

Patch test procedure

Patch tests were applied to the upper back for 48 hr of occlusion with van der Bend[®] square chambers (Van der Bend BV, Brielle, The Netherlands), and fixed with Fixomull[®] Stretch (BSN Medical, Hamburg, Germany). Approximately 25 µg of the test preparation was applied to each chamber. Results were read on D3 and D7. In order to improve the quality of the readings and to better distinguish the morphology of irritant and allergic reactions, a patch test calibration protocol was used (16, 17). Exclusion criteria were pregnancy, an angry back in the past, and the use of oral immunosuppressive drugs in the 2 weeks prior to the patch test procedure.

Outpatients

From November 2011 to March 2013, ascaridole 1%, 2% and 5% was added to the cosmetic patch test series at the Dermatology Department of the University Medical Center Groningen. A total of 290 consecutive outpatients with eczema were patch tested with the three different concentrations of ascaridole.

In February 2012, oxidized tea tree oil 5% was added, and was patch tested in 221 of the 290 patients.

Re-patch tested patients

Twenty-nine patients who were patch tested with only ascaridole 1% or 5% between March 2008 and October 2011 and had developed a positive, doubtful or irritant reaction in the past were re-patch tested with the complete series of ascaridole 1%, 2% and 5% and oxidized tea tree oil between November 2012 and February 2013 (14). This study was approved by the Medical Ethics Committee of the University Medical Center Groningen, and informed consent was obtained from all participants.

Combining the 290 outpatients with the 29 re-patch tested patients gives 319 patients who were patch tested with the three concentrations of ascaridole. In total, 250 of these 319 patients were tested with oxidized tea tree oil in addition to the three ascaridole concentrations.

Clinical relevance

The clinical relevance of a positive patch test result was evaluated in relation to the history of exposure and the subsequent dermatitis pattern. A positive patch test reaction was judged to be clinically relevant when a patient had been exposed to a product containing tea tree oil, and the presence of tea tree oil was confirmed by the product label. In addition, the patient had to develop dermatitis after the exposure, and a causative relationship had to be suspected. A repeated open application test was not conducted to establish clinical relevance.

The potential source of exposure and the clinical relevance were assessed for patients with a positive reaction to ascaridole or tea tree oil by means of a questionnaire. Specific questions were asked about the current and past use of pure tea tree oil and natural products, and visits to beauticians, in relation to dermatitis to investigate potential sources of exposure to tea tree oil or other 'natural' products. In addition, patients were provided with a list of products that might contain tea tree oil, such as pure tea tree oil, and well-known applications such as body and facial creams, shampoos, shower gels, massage oils, and aromatherapy materials. After a literature study, less obvious products, such as

shoe cream and anti-parasite products for pets, were also included in this list (8, 10, 13). Supported by a list of synonyms provided by us, patients were requested to inspect their kitchen and bathroom cabinets to identify products containing, among others, 'tea tree oil' or 'melaleuca alternifolia'.

Statistics

Analyses were performed with IBM SPSS™ version 20. The chi-square test and Mann–Whitney *U*-test were used to compare differences between groups. Fisher's exact test was used to investigate the relationship between a positive reaction to ascaridole and a positive reaction to oxidized tea tree oil.

Results

Patch test results

Of the 290 consecutive patch tested outpatients, 89 (30.7%) were male and 143 (49.3%) were aged > 40 years. In 68 (23.4%) patients, occupational factors were suspected to play a role in general. The primary location of the dermatitis was the face in 137 (47.2%) patients, the hands in 90 (31.0%) patients, and the legs in 5 (1.7%) patients. In 122 (42.1%) patients, a history of atopic dermatitis was reported by the patient.

The MOAHLFA index for this population was: M30.7, O23.4, A42.1, H31.0, L1.7, F47.2, and A49.3.

The results of the patch testing in terms of total numbers of tested patients, and the frequency of positive, doubtful and irritant reactions, are shown in Table 1.

An increase in the number of positive reactions with increasing ascaridole test concentration was recorded: ascaridole 1% caused positive reactions in 1.4% (4/290); ascaridole 2% caused positive reactions in 5.5% (16/290); and ascaridole 5% caused positive reactions in 7.2% (21/290). However, the higher frequencies of positive reactions with increasing concentrations of ascaridole were accompanied by higher frequencies of irritant reactions; ascaridole 1% caused irritant reactions in 0.3% (1/290); ascaridole 2% caused irritant reactions in 1.7% (5/290); and ascaridole 5% caused irritant reactions in 9.0% (26/290). Also, the number of doubtful reactions increased with concentration: ascaridole 1% caused doubtful reactions in 3.1% (9/290); ascaridole 2% caused doubtful reactions in 4.1% (12/290); and ascaridole 5% caused doubtful reactions in 10.3% (30/290).

Of the 9 patients with doubtful reactions to ascaridole 1%, 5 patients showed positive reactions and 4 patients showed negative reactions to ascaridole 2%.

Table 1. Patch test results for ascaridole at 1%, 2% and 5% and oxidized tea tree oil, patch tested from February 2012 to March 2013 in consecutive dermatitis patients of the Department of Dermatology. The strongest result on either D3 or D7 is registered. Two hundred and twenty-one of the 290 patients were patch tested with ascaridole 1%, ascaridole 2%, ascaridole 5%, and oxidized tea tree oil 5%. MOAHLFA index: M30.7, O23.4, A42.1, H31.0, L1.7, F47.2, and A49.3

	No. tested	+, n (%)	++, n (%)	+++, n (%)	Irritant reaction, n (%)	?, n (%)	Total +, n (%)	Relevant, n (%)
Ascaridole 1%	290	4 (1.4)	–	–	1 (0.3)	9 (3.1)	4 (1.4)	1 (0.3)
Ascaridole 2%	290	15 (5.2)	1 (0.3)	–	5 (1.7)	12 (4.1)	16 (5.5)	5 (1.7)
Ascaridole 5%	290	20 (6.9)	1 (0.3)	–	26 (9.0)	30 (10.3)	21 (7.2)	4 (1.4)
Oxidized tea tree oil 5%	221	2 (0.9)	–	–	–	1 (0.5)	2 (0.9)	–

Table 2. Co-sensitization to oxidized tea tree oil 5% and any ascaridole concentration. The table includes the patch test results of 250 patients who were all patch tested with ascaridole 1%, ascaridole 2%, ascaridole 5%, and oxidized tea tree oil. Two hundred and twenty-one of the 250 were outpatients patch tested between February 2012 and March 2013, and 29 were re-patch tested patients. MOAHLFA index: M31.0, O22.6, A48.9, H30.7, L1.6, F47.3, and A50.5

	Tea tree oil-positive	Tea tree oil-negative	Total
Any ascaridole concentration positive	6	24	30
All ascaridole concentrations negative	0	220	220
Total	6	244	250

Of the 12 patients with doubtful reactions to ascaridole 2%, only 1 patient showed a positive reaction to ascaridole 5%. Three reactions remained doubtful for ascaridole 5%. Three of 12 patients with doubtful reactions to ascaridole 2% showed irritant reactions to ascaridole 5%, and in 5 patients with doubtful reactions to ascaridole 2%, no reaction was caused by ascaridole 5%.

All of the patients with positive reactions to ascaridole 1% also showed reactions to ascaridole 2%.

In the unselected 221 consecutive outpatients we found 2 (0.9%) positive reactions to oxidized tea tree oil and 1 (0.5%) doubtful reaction; no irritant reactions were registered (Table 1).

Co-sensitization to ascaridole and tea tree oil

Combining the 221 outpatients consecutively tested with ascaridole 1%, 2% and 5% and tea tree oil 5% with the 29 re-patch tested patients gives a total of 250 patients who were patch tested with this entire series (Table 2).

Six patients developed a positive reaction upon patch testing with oxidized tea tree oil. These 6 patients also showed a positive reaction to ascaridole (Tables 2 and 3).

The association between a positive reaction to oxidized tea tree oil and a positive reaction to ascaridole was statistically significant for all concentrations (ascaridole 1%, $p < 0.001$; ascaridole 2%, $p < 0.001$; ascaridole 5%, $p < 0.001$) and ascaridole and tea tree oil overall ($p < 0.001$).

An increased number of patients reacting to other fragrances, such as linalool, eugenol, or isoeugenol, was not observed, and neither did the number of sensitizations to other oils such as peppermint oil or eucalyptus oil deviate from that in the general patch tested population (Table 3).

Aspect of irritant reactions

The difference between an irritant and allergic reaction was scored according to a patch test calibration protocol (16). The irritant reactions caused by ascaridole 1% had a silk-like (or cigarette paper-like) appearance. For ascaridole 2%, the morphology of the irritant reactions was also silk-like in most cases. One patient had a positive reaction to ascaridole 1%, but papulo-pustular irritant reactions to ascaridole 2% and ascaridole 5% (Fig. 2d).

The irritant reactions caused by ascaridole 5% showed a wide range of different morphologies: silk-like skin, sharp demarcated redness, pustules, bullae, and erosions (Fig. 2). Some irritant reactions caused by ascaridole 5% closely resembled very strong positive reactions, especially with regard to the erosions and potential remnants of bullae.

Positive reactions in the total study population

In our total study population ($n = 319$), we identified 37 positive reactions to either tea tree oil or ascaridole (Table 3). The mean age of patients with a positive reaction to ascaridole was 47 years, ranging from 20 to 69 years (Table 3). A minority of patients were male ($n = 11$, 29.7%), and 14 (37.8%) patients had atopic dermatitis diagnosed by a physician. Sixteen (43.2%) patients suffered from facial dermatitis, and 3 of these

Table 3. Overview of patch test results from all patients reacting to tea tree oil and/or ascaridole. The strongest result on D3 or D7 is shown. Exposure was established by the patients themselves by means of a questionnaire and a checklist

Age (years)	Sex	TTO 5%	Asc 1%	Asc 2%	Asc 5%	Location	Source of exposure	Beautician, complaints	Natural products, complaints	Homeopathic, complaints	Aromatherapy	Concomitant reactions
57	F	+	+	IR	IR	Facial	–	Never	Current: no	Never	Never	CAPB, Ni
59	M	+	+	++	+	Hand	–	Never	Never	Never	Never	Parthenolide, sesquiterpene lactone mix, sorbic acid
60	F	+	+	+	++	Hand	–	In the past: yes	Never	Never	Never	FMI, MDBGN, MBT, mercapto mix, Ni
28	M	+	+	+	+	Periorbital	Williams shaving oil*	Never	In the past: yes	Never	Never	–
23	F	+	?	+	+	Hand	–	Current: no	Never	Never	Never	Eugenol, formaldehyde, isoeugenol
38	F	+	–	+	+	Hand	–	Never	Current: yes	Never	Never	Co, colophonium, Cr, Ni, PTBFR, MDBGN, CAPB
49	F	?	+	+	IR	Periorbital	Baby soap Etos* Eczeffleur cream*	Current: no	Current: yes	Never	Never	–
43	F	?	?	+	+	Facial	PhD wax*	In the past: yes	Never	Never	Never	CAPB, captan, FMI, glyceryl monothio-glycolate, MDBGN, MHC, oak moss absolute, PTBFR, thiolactic acid
58	M	–	IR	IR	+	Facial	–	Never	Never	Never	Never	–
24	F	–	+	+	IR	Hand	–	Current: yes	In the past: no	Never	Never	CAPB, iodoproponyl butylcarbamate
55	F	–	+	+	+	Facial	–	Never	Never	Never	Never	CAPB
58	M	–	+	+	+	Hand	–	Never	Current: no	Never	Never	MCI/MI
52	F	–	+	+	++	Hand	–	Never	Never	Never	Never	Lanolin (wool alcohols), <i>Myroxylon pereirae</i>
69	F	–	+	+	+	Periorbital	Pure tea tree oil for onychomycosis Babor cosmetics*	Current: yes	Current: yes	Current: yes	Never	Co, HMBA, Ni, thiomersal
55	F	–	+	+	++	Feet	–	Never	Never	Never	Never	Co, colophonium, Cr, FMII, PTBFR, thiomersal, anise alcohol
22	F	–	?	+	+	Hand	de Tuinen tea tree oil facial mask*	Never	Current: yes	Never	Never	–
65	M	–	?	+	+	Facial	–	Never	In the past: no	Never	Never	Epoxy resin
49	F	–	?	+	+	Hand	Pure tea tree oil as disinfectant	In the past: no	Never	Current: yes	Never	Sesquiterpene lactone mix, Laurel oil
68	F	–	–	+	+	Generalized	–	Current: no	Never	Never	Never	Carba mix, MDBGN, oak moss
58	M	–	–	+	+	Hand	–	Never	Never	Never	Never	Isoeugenol, <i>Myroxylon pereirae</i>
28	M	–	–	+	+	Hand	–	Never	Never	Never	Never	–
20	F	–	–	+	+	Trunk	–	Current: yes	Current: no	Current: no	Never	Clove oil, FMI, FMII, lemon grass oil, lanolin, <i>Myroxylon pereirae</i>
66	M	–	–	+	?	Hand	–	Never	Never	Never	Never	FMI, MDBGN, lanolin
34	F	–	–	+	IR	Facial	Obagi cosmetics*	Never	In the past: no	Never	Never	Budesonide, FMII, MCI/MI

Table 3. Continued

Age (years)	Sex	TTO	Asc 5%	Asc 1%	Asc 2%	Asc 5%	Location	Source of exposure	Beautician, complaints	Natural products, complaints	Homeopathic, complaints	Aromatherapy	Concomitant reactions
65	F	—	—	IR	+		Facial	—	In the past: no	Never	Never	Never	Diazolidinyl urea, imidiazolidinyl urea, Ni, oleamidodipropylidimethylamine, PPD
50	M	—	—	—	+		Generalized	—	Never	In the past: no	Never	Never	MCI/MI
39	F	—	—	—	+		Hand	—	In the past: no	Current: no	Never	Never	Co, FMI, Ni, peppermint oil, clove oil
40	F	—	—	—	+		Hand	—	Current: no	Never	Never	Never	MCI/MI
51	F	—	—	—	+		Feet	—	Never	In the past: no	In the past: no	Never	Ni
66	F	—	—	—	+		Facial	Data missing	—	—	—	—	Benzyl benzoate, caine mix, <i>Myroxylon pereirae</i> , Ni, phenyl salicylate, PTBFR
49	F	NT	+	+	?		Facial	—	Current: yes	Never	Never	Never	CAPB, formaldehyde, MCI/MI
59	F	NT	—	+	+		Facial	—	Current: no	Never	Never	Never	—
52	F	NT	—	+	+		Facial	—	Never	Never	Current: yes	Never	BHA, benzyl salicylate, cinnamyl alcohol, farnesol, FMI, hexyl cinnamal, butyl phenyl methyl propional, linalool, <i>Myroxylon pereirae</i> , α -amyl cinnamal
24	F	NT	—	+	IR		Facial	—	In the past: no	In the past: no	Never	Never	MDBGN, FMII
57	M	NT	—	?	+		Trunk	—	Never	Never	Never	Never	—
24	M	NT	—	—	+		Hand	—	Never	Never	Never	Never	CAPB
24	F	NT	—	—	+		Facial	—	Never	Current: no	Never	Never	Black rubber mix, caine mix, disperse blue, FMII, Ni, PPD, thiuram mix

Asc, ascaridole; BHA, butylated hydroxyanisole; CAPB, cocamidopropyl betaine; Co, cobalt chloride; Cr, potassium dichromate; F, female; FM, fragrance mix; HMBA, 2-hydroxy 4-methoxy benzoic acid; IR, irritant reaction; M, male; MBT, mercaptobenzothiazole; MCI/MI, methylchloroisothiazolinone/methylisothiazolinone; MDBGN, methylidibromo glutaronitrile; MHC, methyl heptene carbonate; Ni, nickel sulfate; NT, not tested; PPD, *p*-phenylenediamine; PTBFR, *p*-tert-butylphenolformaldehyde resin; TTO, oxidized tea tree oil.

*Contains tea tree oil according to the product information.

had mainly periorbital dermatitis. Fifteen (40.5) patients suffered from hand dermatitis, and none of them had leg dermatitis.

We were unable to show a relationship between sex, location of dermatitis and atopic eczema and a positive reaction to ascaridole or tea tree oil. Ascaridole 5% caused significantly more irritant reactions in patients who reported a history of atopic dermatitis ($p = 0.016$).

Clinical relevance and potential exposure

The 37 patients with a positive reaction to tea tree oil and/or ascaridole were provided with a questionnaire to analyse potential exposure to tea tree oil and clinical relevance. One patient did not return the questionnaire.

Five of 36 patients admitted preferring natural treatments, and 2 of these patients recalled the use of pure tea tree oil to treat either onychomycosis or wound infections. None of the patients recalled using aromatherapy. Fifteen patients, all women, regularly visited a beautician. Six of them had ever developed a rash afterwards, and for 2 of them we were able to identify tea tree oil in the products used by the beauticians (PhD safewax[®] and Babor[™] cosmetics). These reactions were judged to be clinically relevant. Sixteen of the 36 patients admitted preferring natural products, and in 6 patients a natural cosmetic product turned out to contain tea tree oil and caused subsequent dermatitis.

In total, known exposure to a product containing ascaridole was shown in 7 of 36 patients (Table 3); 2

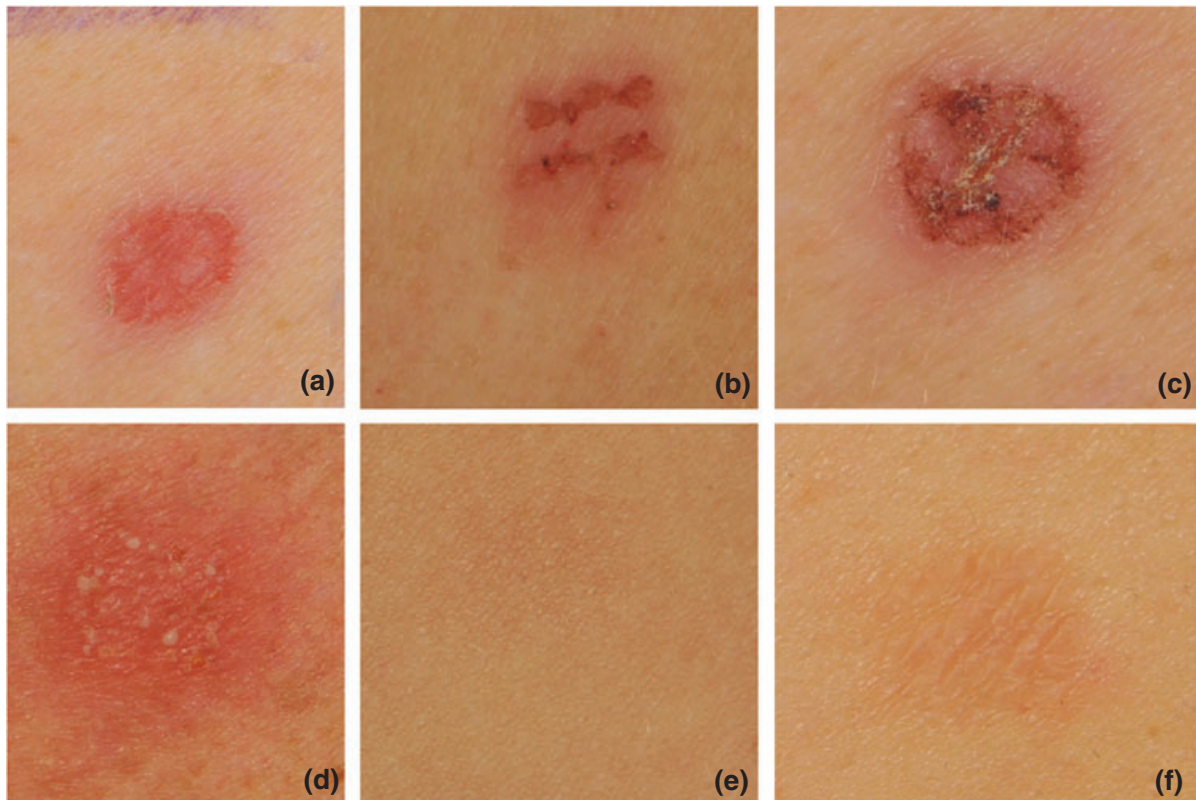


Fig. 2. A wide variety of irritant reactions caused by ascaridole 5%. (a, b, d–f) D3 readings. (c) D7 reading.

of these were exposed to multiple sources of tea tree oil. After exposure to tea tree oil, all of the patients developed a dermatitis pattern consistent with the use of the product, and all of these reactions were judged to be clinically relevant.

Considering only the 290 consecutively tested outpatients, ascaridole 1% caused a clinically relevant positive reaction in 0.3% (1/290), ascaridole 2% in 1.7% (5/290), and ascaridole 5% in 1.4% (4/290). We were unable to show a clinically relevant reaction caused by oxidized tea tree oil 5% in 221 outpatients. A relevant reaction to oxidized tea tree oil was found in 1 of the 29 re-patch tested patients (Table 3).

Discussion

Ascaridole is considered to be a moderate skin sensitizer, as it has the ability to induce antigen-specific cell proliferation in a murine local lymph node assay (18). *In vitro* investigations looking for activation of human monocyte-derived dendritic cells and monocytic cell lines (THP-1) as a model for dendritic cell activation showed augmentation of CD86 by ascaridole (19). The current

study supports the idea that ascaridole is a sensitizer, although it seems to express more irritant features at higher patch test concentrations. In the past, ascaridole 10% has been patch tested, and caused very strong positive, bullous reactions (20). These might have been irritant reactions.

Prevalence

In our unselected 221 outpatients, we found 2 (0.9%) positive reactions to oxidized tea tree oil 5% (Table 1). This is comparable with the number of sensitizations to oxidized tea tree oil of the North American Contact Dermatitis Group, which found a prevalence of 1.0% in 2009–2010 (7). This frequency is also comparable to those in studies from Germany, Austria, Denmark, and Australia, where the prevalence varied between 0.5% and 1.8% of the patch tested population (8–10).

In our population, the frequencies of sensitization to ascaridole were 1.4% for ascaridole 1%, 5.5% for ascaridole 2%, and 7.2% for ascaridole 5%. However, as ascaridole is not routinely tested, as far as we know, we are unable to compare our results with those obtained in other populations.

Patch test results

Ascaridole 2% seems to be the most favourable patch test concentration, on the basis of the ratio of positive (5.5%), irritant (1.7%) and doubtful (4.1%) reactions. Ascaridole 5% caused more irritant reactions (9.0%) and doubtful reactions (10.3%) than positive reactions (7.2%). Despite the patch test calibration protocol, it was difficult to differentiate between the irritant and positive reactions for ascaridole 5%. Therefore, we do not recommend testing ascaridole at 5%. Ascaridole 1% caused more doubtful than positive reactions (1.4% versus 3.1%). Although larger cohorts of patients need to be investigated to calculate a reliable reaction index, on the basis of these preliminary results we recommend a patch test concentration of 2%.

In this study, we found a total of 9 doubtful reactions to ascaridole 1%. In 5 of these, the reaction was positive upon patch testing with ascaridole 2%, whereas 4 doubtful reactions could not be reproduced (were negative) upon concomitant testing with ascaridole 2%. For ascaridole 2%, in only 1 patient was the doubtful reaction positive upon patch testing with ascaridole 5%; the remaining 11 doubtful reactions to ascaridole 2% were negative, remained doubtful or became irritant reactions upon patch testing with ascaridole 5%. A doubtful reaction becoming positive, becoming irritant or even disappearing upon patch testing with higher concentrations is also seen for other allergens, such as formaldehyde (21, 22).

Co-sensitization and clinical relevance

All 6 patients with positive reactions to oxidized tea tree oil showed positive reactions to ascaridole (Tables 2 and 3). This finding suggests that ascaridole is a contact allergen in oxidized tea tree oil, although we should not neglect the potential presence of other sensitizers in oxidized tea tree oil, such as α -phellandrene, terpinolene, α -terpinene, 1,2,4-trihydroxy menthane, α -phellandren, and (+)-limonene (8, 11–13). Twenty-four patients had a positive reaction to ascaridole without a positive reaction to oxidized tea tree oil. Recently, Sciarrone et al. investigated the components of tea tree oil, and found ascaridole at a concentration of only 0.2% (23). Rudbäck et al. investigated the auto-oxidation of α -terpinene in four bottles of tea tree oil, and in all of the bottles α -terpinene and oxidation products were found, but no ascaridole (18). The exact composition of the oxidized tea tree oil used in our study was unknown; it is possible that ascaridole was present at a concentration too low to elicit a reaction in all those who are sensitized to ascaridole, or, although unlikely, was not present at all.

Among the 6 patients with positive reactions to oxidized tea tree oil and ascaridole, there was only 1 patient in whom clinical relevance could be shown. A possible explanation is that patients are unaware of being exposed to tea tree oil, as different chemical names are used on product labels. Although we supplied the patients with a list of synonyms (15), in the future we can improve the identification by instructing patients to bring all of their suspected products.

It was remarkable that, in 6 patients with a positive reaction to ascaridole but without a positive reaction to tea tree oil, clinical relevance was shown, because there was exposure to products containing tea tree oil and the subsequent appearance of eczema after use of these products. This suggests that patch tests with ascaridole may provide clinically relevant positive reactions in patients that would be missed if they were only tested with oxidized tea tree oil.

An explanation for positive reactions to ascaridole unrelated to tea tree oil might be the presence of allergenic degradation products of ascaridole or other substances in the ascaridole preparation that are not present in the oxidized tea tree oil. We used 99.8% pure ascaridole for patch test preparations, although we did not verify whether this concentration changed over time. Perhaps we have patch tested with auto-oxidation products, although these should, in theory, be present in the oxidized tea tree oil as well.

In addition, the discrepancy between sensitization to ascaridole and the lack of exposure to tea tree oil could be explained by other, independent sources of ascaridole. The best known source of ascaridole is *Chenopodium ambrosioides* (24). The essential oil of *C. ambrosioides* contains 40–70% ascaridole, and is used as an antihelminthic. Because of its toxicity, this oil is no longer used in humans (25). The leaves of the plant are sometimes used, for example in South American cuisine or in order to produce medicinal tea. Another potential source, Boldo oil (*Peumus boldus* oil) (26), is used as a herbal remedy for various conditions. However, Boldo oil is banned from use in cosmetics as well (27), and its use as a herbal remedy is discouraged, in view of the potential risks associated with the toxicity of ascaridole (28). Therefore, these other sources of ascaridole are unlikely to be sensitizers in this population.

We collected a cohort of subjects with reactions to oxidized tea tree oil and/or ascaridole. For future studies, we suggest conducting thin layer chromatography on oxidized tea tree oil and performing patch tests with the chromatograms in this selected population, to gain further insights into other potential allergens in oxidized tea tree oil and their relationship with ascaridole.

In conclusion, the current study supports the idea that ascaridole might be a sensitizer. We recommend patch testing with ascaridole at a concentration of 2%. The finding that every positive reaction to oxidized tea tree

oil is accompanied by a positive reaction to ascaridole suggests that ascaridole might be a contact allergen in oxidized tea tree oil.

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