


IDEA meeting on Pre- and Pro-haptens
October 2015


Update on clinical data
Johanna Bråred Christensson^{1,2}

**Discussion on relevance of cross reactivity
between fragrance hydroperoxides**
Ann-Therese Karlberg¹


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
Many common fragrance chemicals used today belong to the chemical family of terpenes




Linalyl acetate



Linalool




Limonene

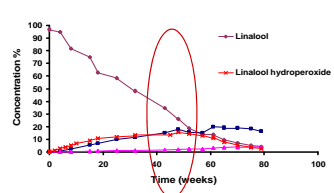


Geraniol

○ Unsaturated double bonds: prone to autoxidation




Oxidation of linalool



Oxidation is a process¹
Slowed by BHT². Different in different batches.
Instead of oxidation time: define composition of oxidation mixture (oxidized patch test material)


¹ Sköld et al. *Chem. Res. Toxicol.* 2004; 17, 1697-1705
² Karlberg et al. *Ann Occup Hyg.* 1994; 38: 199-207



Linalool and limonene are presently among the most common fragrance ingredients used in consumer products

Linalool	60-90% of examined products
Limonene	60-80%
Geraniol	40-80%
Linalyl acetate	27-60%

Rastogi et al. *Contact Dermatitis* 2001;45:221-5; and 1998;38:29-35
Buckley. *Br J Dermatol* 2007;157:295-300
de Groot AC, Frosch PJ *Contact Dermatitis* 1997;36:57-86



Linalool and limonene: tandem exposure in consumer products

Limonene and linalool were the most frequent coupled (tandem) exposures to fragrance chemicals
Uter W et al. *Contact Dermatitis.* 2013; 69: 335-41

Both among the highest exposure to high end users
RIFM Expert Panel, Belsito D et al. *Food Chem Toxicol.* 2008; 46 Suppl 12:S1-S71




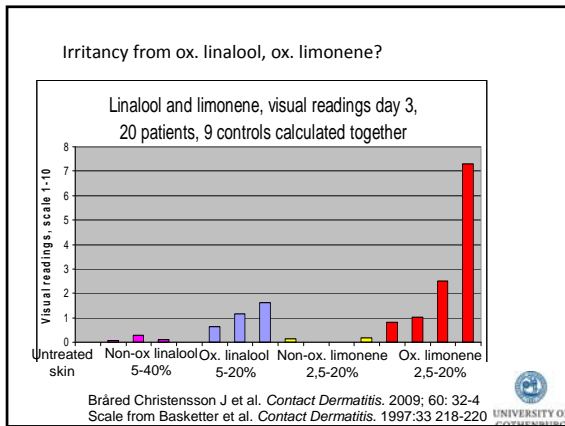
Linalool and limonene: tandem exposure in consumer products

Limonene and linalool were the most frequent coupled (tandem) exposures to fragrance chemicals
Also among the highest exposure to high end users

Clinical studies cannot give information of cross reactivity since no control of the exposure

Uter W et al. *Contact Dermatitis.* 2013; 69: 335-41
RIFM Expert Panel, Belsito D et al. *Food Chem Toxicol.* 2008; 46 Suppl 12:S1-S71.





Contact allergy to linalool vs oxidized linalool

Linalool	Ox linalool
0.2% positive ¹ (10% pet.) • (unstated purity, not intentionally oxidized)	1.3% positive ² (2% pet.) Dose-response: 2-11% pet.: recommended ox linalool 6% pet. → 5.3% positive ³
0.3% positive ⁴ (10% pet.)	5.9% positive ox linalool 6% pet ⁴

¹ Schnuch et al. *Contact Dermatitis*. 2007; 57: 1-10
² Matura et al. *Contact Dermatitis*: 2005; 52(6): 320-328.
³ Bråred Christensson et al. *Contact Dermatitis*: 2010; 62: 32-4
⁴ Audrain et al *British Journal of Dermatology* 2014: 171: 292-297

Non-ox. vs ox. limonene (citrus fragrance)

Limonene * (unstated purity, not intentionally oxidized)	Ox limonene
0.1% positive ¹ (*2% pet.)	1.6-2.8% positive ² (3% pet.)
0.2% positive ³ (10% pet.)	5.0% positive ³ (3% pet.)

1. Schnuch et al. *Contact Dermatitis*. 2007; 57: 1-10
2. Karlberg et al. *Contact Dermatitis* 1997; 36, 201-206
Matura et al. *Contact Dermatitis* 2003; 49, 15-21
Matura et al. *Contact Dermatitis* 2006; 55: 274-279
3. Audrain et al *British Journal of Dermatology* 2014: 171: 292-297

Prevalence of contact allergy to ox linalool and ox limonene in an international study

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Ana Gimenez Arnau, Dept of Dermatology, Hospital del Mar, Universitat Autònoma, Barcelona, Spain
Chee Leok Goh, National Skin Centre, Singapore
Rosemary Nixon, Occupational Dermatology Research and Education Centre, Skin and Cancer Foundation, Victoria, Australia
Ian R White, Department of Cutaneous Allergy, St John's Institute of Dermatology, London, UK

Declaration of interests: Magnus Bruze is a member of Rexpa, an independent expert panel to Research Institute for Fragrance Materials (RIFM).
The other authors have no interests to declare.

Overall results

	No. tested	No. pos	% pos	Range
Ox linalool	2900	200	6.9%	3-13%
(Audrain et al)	4731	281	5.9%	
Ox limonene	2900	152	5.2%	2-12%
(Audrain et al)	4731	237	5.0%	


Bråred Christensson et al. *Contact Dermatitis*. 2013;68:214-23.
Bråred Christensson et al. *Contact Dermatitis*. 2012;67:247-59
Audrain et al *British Journal of Dermatology* 2014: 171: 292-297

Support for true fragrance allergy?

Ox linalool:
Positive, doubtful and negative, concomitant reactions to fragrance markers


	FM I	FM II	HICC	Myroxylon perillae	Colo-phonium	≥1 fragrance marker a/o colophonium	
Positive	26%	20%	8%	15%	8%	79/200	40%
Doubtful	8%	10%	3%	6%	5%	51/271	19%
Negative	5%	2%	1%	3%	2%	230/2388	10%

Ox limonene:
Positive, doubtful and negative, concomitant reactions to fragrance markers

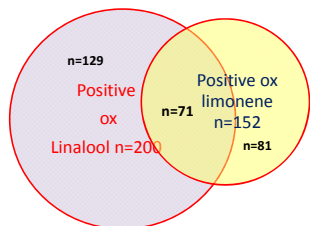


	FM I	FM II	HICC	<i>Myroxylon perelirae</i>	Colophonium	≥1 fragrance marker a/o colophonium	
Positive	26%	14%	7%	21%	10%	64/152	42%
Doubtful	12%	9%	4%	9%	5%	40/204	20%
Negative	6%	3%	2%	3%	2%	267/2519	11%


Concomitant contact allergies are common



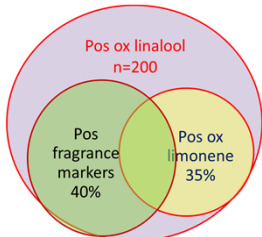
Concomitant reactions ox linalool and ox limonene




46% of pos to ox limonene also positive to ox linalool
35% of pos to ox linalool also positive to ox limonene



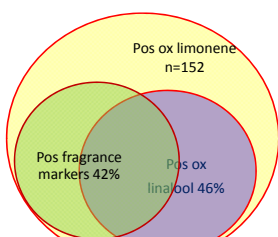
Concomitant reactions ox linalool




40% positive to other fragrance markers
35% positive to ox limonene
56% positive to other fragrance marker a/o ox limonene



Concomitant reactions ox limonene

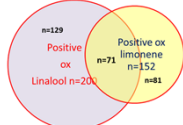


42% positive to other fragrance markers
46% positive to ox linalool
65% positive to other fragrance marker a/o ox linalool




Concomitant reactions ox linalool and ox limonene

Concomitant reactions ox linalool and ox limonene



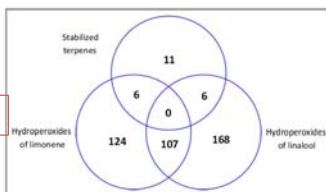
46% of pos to ox limonene also positive to ox linalool
35% of pos to ox linalool also positive to ox limonene

281 positive to any ox mixture
25% reactions to both: tandem exposure common
75% reacted only to one:
29% only ox limonene
46% only ox linalool



Allergy to ox. Limonene and Linalool in the U.K.


411 reacted to either or both ox mixtures, 107 to both
26% reacted to both -74% reacted only to one

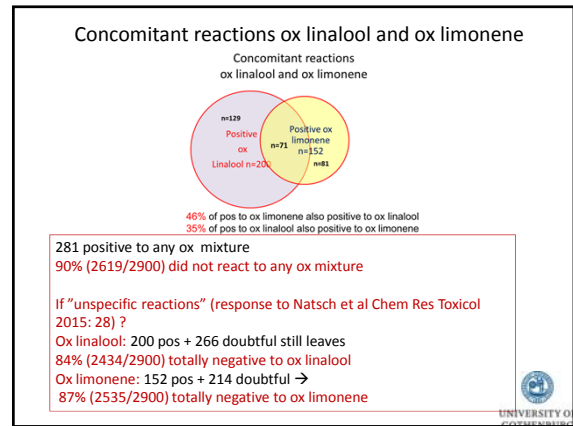
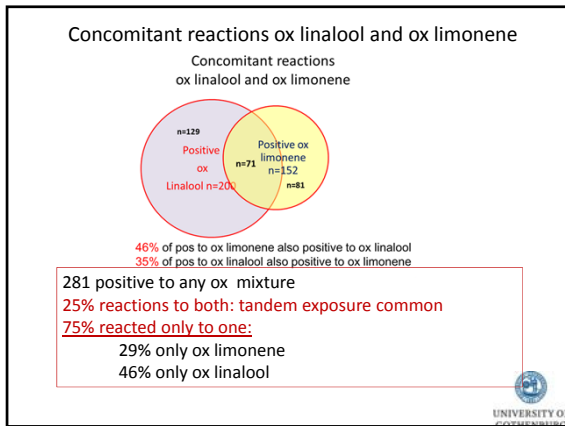


Stabilized terpenes: 11
Hydroperoxides of limonene: 124
Hydroperoxides of linalool: 168
Intersection of all three: 0

Ox. limonene 3% containing Lim-OOHs 0.33% pet
Ox. linalool 6% containing Lin-OOHs 1%

Fig 1. Venn diagram showing the relationship between allergic reactions to the stabilized terpenes and hydroperoxides of linalool and limonene in 4731 patients tested (432 positive reactions in total).
Audrian et al. British Journal of Dermatology 2014; 171: 292-297





Methods, assessing relevance of positive patch test reaction

- Questionnaire: Experienced problems with scented products or perfumes (A-D)?
 - (A) certain** – has reacted with an itching dermatitis to at least one fine perfume or aftershave and also to other scented products;
 - (B) probable** – has reacted to one or more scented products, but a certain perfume has not been identified as the cause of a clinical reaction;
 - (C) questionable** – has reacted to various cosmetics with or without fragrances, other materials than fragrance constituents may be the cause of reaction;
 - (D) none** – has never reacted to scented materials

¹ Frosch et al. *Br J Dermatol* 1999; 141: 1076–1083

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Comparing responses A-D in those with positive reaction to ox limonene but no other fragrance markers -and those negative to all fragrances –asked before patch test

	Pos to ox limonene (n=152)	Pos to ox limonene- and no other fragrances, (n=86)	Neg to all fragrances (n=1737)
Responses A or B (certain or probable)	23%	18%*	8%*

* P<0.01, Fisher's exact test two tailed
→Significantly more patients experienced problems with fragrances in limonene-allergic group. Supports clinically relevant reactions.

Brared Christensson et al. *Contact Dermatitis*. 2013; 68: 214-23.
Brared Christensson et al. *Contact Dermatitis*. 2014; 71: 264-72

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Which products were implicated?

- Both domestic and occupational products containing limonene or linalool were used by patients on dermatitis areas
- Perfumes, shampoos, soaps, body creams, deodorants were frequently listed
- Creams for massage, sunscreens, detergents and domestic cleaners were also frequently implicated
- Antiseptic tea tree oil-based products, fragrances for candle-making
- A laboratory technician using limonene occupationally, several masseurs

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Autoxidation occurs in essential oils

- Petitgrain oil:** linalyl acetate hydroperoxides, linalool hydroperoxides¹
- Sweet orange oil:** limonene hydroperoxides¹
- Lavender oil:** linalyl acetate hydroperoxides, linalool hydroperoxides²
- Hydroperoxides identified at delivery from producer and, in increasing amounts after storage


- Rudbäck *J J Sep Sci*. 2014; 37: 982-9.
- Hagvall *Contact Dermatitis*. 2008; 59:143-70

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Linalool-allergic patients react in repeated open application test

Ecematous reactions to 1% and 0.3% oxidized linalool in perfume- and in cream base

Andersch Björkman et al 2013. *Contact Dermatitis*. 2014; 70: 129-38




Many fragrance terpenes oxidize or are metabolically altered

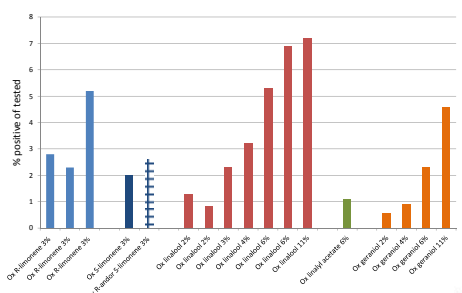
Table 1. Fragrance substances that have been experimentally shown to act as prehapten and/or prohaptens

Fragrance substance	Activation by air oxidation	Bioactivation (oxidation)
Cinnamyl alcohol	Yes	Yes
Eugenol	No	Yes
Geranial	Yes	No
Geraniol	Yes	Yes
Isoeugenol	No	Yes
Limonene	Yes	No
Linalool	Yes	No
Linalyl acetate	Yes	No
α -Terpinene	Yes	Yes

Karlberg A-T et al. *Contact Dermatitis*. 2013; 69: 323-34



Oxidized fragrance terpenes have been studied clinically in many studies



Substance	% Positive of Tested
Ox. linalool 2%	3.0
Ox. linalool 1%	2.5
Ox. linalool 0.5%	5.5
Ox. linalool 0.2%	2.0
Ox. linalool 0.1%	2.5
Ox. linalool 2%	1.5
Ox. linalool 1%	1.0
Ox. linalool 0.5%	2.5
Ox. linalool 0.2%	5.5
Ox. linalool 0.1%	7.0
Ox. linalool 1%	7.5
Ox. linalool 0.5%	1.0
Ox. geraniol 2%	0.5
Ox. geraniol 1%	1.0
Ox. geraniol 0.5%	2.5
Ox. geraniol 0.1%	5.0

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