Six years in IDEA – what is our progress in understanding and managing the pre-hapten question?

The clinical picture and the breath of the problem

Johanna Bråred Christensson

Dermatochemistry and Skin Allergy, Department of Chemistry and Molecular Biology, University of Gothenburg, Gothenburg, Sweden

Citysjukhuset +7, Gothenburg, Sweden

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- Linalool and limonene, fragrance terpenes, autoxidation
- Clinical studies on oxidized linalool and oxidized limonene
- What do we know on
  - Reproducibility of the reactions?
  - How to interpret high rate of irritant/doubtful reactions?
  - How to interpret concomitant reactions to other allergens?
- Clinical picture – what does it tell us? How do we explain the high rates of positive reactions?
- Breath of the problem: What do we know clinically on other molecules next to linalool and limonene? (eg oxidized linalyl acetate, oxidized geraniol)

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Autoxidation (air oxidation)

- Autoxidation - spontaneous reaction with oxygen in air at normal temperatures
- Many fragrance terpenes are very low sensitizing compounds in their basic form BUT when exposed to air → form oxidation products which can be strong allergens
- Hydroperoxides (primary oxidation products) are main allergens in the oxidation mixtures

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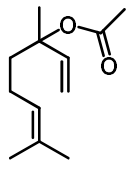
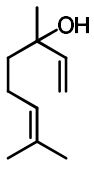
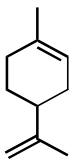
Many common fragrance chemicals used today belong to the chemical family of terpenes



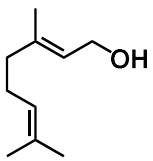
Linalyl acetate



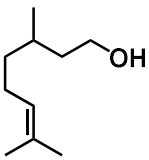
Linalool



Limonene



Geraniol



Citronellol

…and are easily autoxidized at air exposure forming allergenic oxidation products

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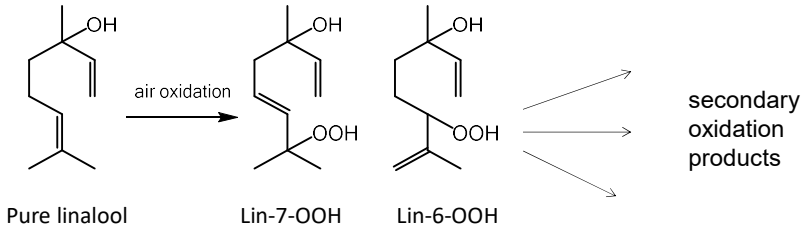
Autoxidation of linalool at air exposure in room temperature

Linalool → linalool hydroperoxides (main allergens) → secondary ox. products

OOH fraction: 19% in the oxidation mixture



Pure linalool — Lin-7-OOH — Lin-6-OOH

LLNA: EC3 46.2

LLNA hydroperoxide fraction: EC3 1.6

- Allergens accumulate in oxidation mixture, composition will change over time
- Oxidized linalool (oxidation mixture) after 40 w: **EC3 9.4**

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Sköld M. Contact Allergy to Autoxidized Fragrance Terpenes Thesis 2005

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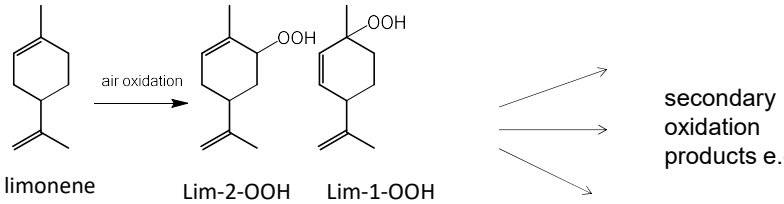
Autoxidation of limonene at air exposure in room temperature

Limonene→ limonene hydroperoxides (main allergens) → secondary ox. products

6% 1% in the oxidation mixture



Pure limonene — Lim-2-OOH — Lim-1-OOH

Carvone

LLNA: EC3 30% **EC3 0.83 %** **EC3 0.19 %**

- Allergens accumulate in oxidation mixture, composition will change over time
- Oxidized limonene (oxidation mixture) after 10 w: **EC3 3.0 %**

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Karlberg AT, Magnusson K, Nilsson U. *Contact Dermatitis.* 1992 2 6: 332-40.
Karlberg AT, Shao LP, Nilsson U, Gäfvert E, Nilsson JL. *Arch Dermatol Res.* 1994; 286: 97-103.
Karlberg A-T, Dooms-Goossens A. *Contact Dermatitis* 1997: 36: 201-206

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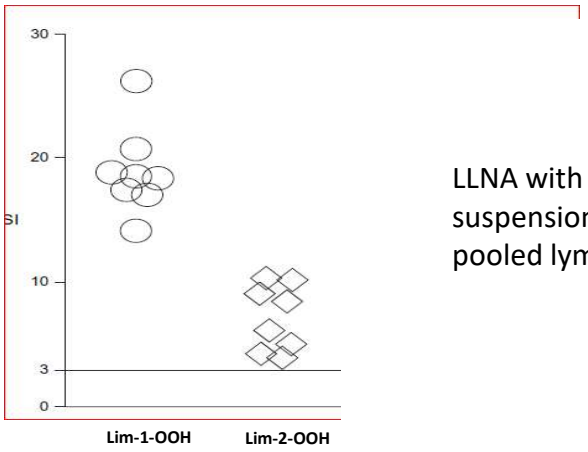
Lim-1-OOH is a more potent sensitizer compared to Lim-2-OOH

Lim-1-OOH more potent to induce lymph node cell proliferation compared with Lim-2-OOH

significant difference (*P* = 0.0008) between the groups.



LLNA with single cell suspension from non-pooled lymph nodes

Bråred Christensson J & Johansson S. et al. Contact Dermatitis 2008:59:344-52

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Lim-1-OOH and Lim-2-OOH show specific patch test reactions in patients

- 763 consecutive dermatitis patients: oxidized limonene, Lim 1 OOH 0.5%, Lim 2 OOH 0.5%
- In total 25/763 patients showed pos. reactions (3.2 %) to any of lim-prep.

- Lim-1-OOH 0.50 % (200 μg/cm^2): **2.4 % pos.**
- Lim-2-OOH 0.50 % (200 μg/cm^2): **1.7 % pos.**
- Ox. limonene 3.0 % (200 μg/cm^2): **1.2 % pos**.

- **18 pos to Lim-1-OOH**, 8/18 pos also to Lim-2-OOH, **10/18 pos only to Lim-1-OOH**
- **13 pos to Lim-2-OOH**, 8/13 pos also to Lim-1-OOH, **5/13 pos only to Lim-2-OOH**

Ox. limonene used contained Lim-1-OOH 1 % + Lim-2-OOH 6 % and + limonene 55 %
Patch test prep.3.0 % pet. contained 0.03 % Lim-1-OOH + 0.18 % Lim-2-OOH (0.21 % Lim-OOHs)

Bråred Christensson et al. Contact Dermatitis 2014: 70:291-99

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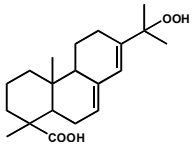
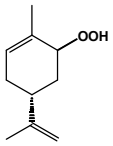
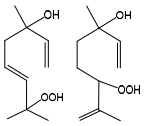
Patch test reactions in colophony- allergic individuals – no cross reactivity between hydroperoxides

29 indviduals tested with :

- ✓15-Hydroperoxyabietic acid
  (most important sensitizer in colophony)
- ✓Limonene-2-OOH
- ✓Linalool-6,7-OOHs







Only 1/29 individuals reacted to more than one hydroperoxide

Bråred-Christensson J. et al. Contact Dermatitis 2006: 55: 230-237

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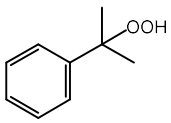
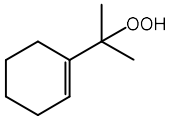
Experimental studies of cross reactivity between hydroperoxides in guinea pig tests: 15-HPA, Lim-OOH, cyclohexene OOH and Cumene OOH

- No general cross reactivity demonstrated
- Cross reactivity between cumene-OOH and cyklohexene-OOH due to over all structural similarity



cumene hydroperoxide



cyklohexene hydroperoxide

Bråred-Christensson J. et al. Contact Dermatitis 2006: 55: 230-237

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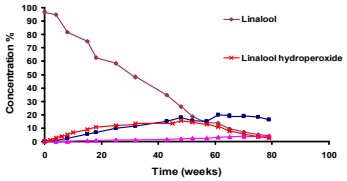
Background –*clinical studies* on oxidized limonene and oxidized linalool

Studies made using:

- Oxidized linalool 2%, 4%, 6%, 11% in pet. → **oxidized linalool 6% pet.** selected, containing **1% Lin OOH**
- Oxidized limonene 3% and 5% pet. → **oxidized limonene 3% pet.** selected containing **0.3% Lim OOH**

Standardized patch test material: ***Hydroperoxides of Limonene 0.3%®*** *and* ***Hydroperoxides of linalool 1%®*** *from* ***Chemotechnique Diagnostics, Vellinge, Sweden.***

- So… ***oxidized limonene 3% pet.*** **and** ***oxidized linalool 6% pet*** **correspond to** ***Hydroperoxides of Limonene 0.3%®*** **and** ***Hydroperoxides of linalool 1%®***



Very important to use standardized patch test material

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Contact allergy to linalool vs oxidized linalool/Hydroperoxides of linalool®

Linalool	Oxidized linalool 6%/Hydroperoxides of linalool 1%®
0.2% positive[1] *(10% pet.)* 0.1% positive[3] *(10% pet.)* 0.3% positive[6] *(10% pet.)*	5.3% positive[4] *(6% pet, 1% Lin-OOHs)* 6.9% positive[5] *(6% pet, 1% Lin-OOHs)* 5.9% positive[6] *(6% pet, 1% Lin-OOHs)* 4.9% pos [7] *(1.0% OOHs®)*

[1] Schnuch et al. *Contact Dermatitis*. 2007; 57: 1-10
[2] Matura et al. *Contact Dermatitis:* 2005: 52(6): 320-328.
[3] Heisterberg et al *Contact Dermatitis* 2011, 65, 266–275
[4] Bråred Christensson et al. *Contact Dermatitis:* 2010; 62: 32-41
[5] Bråred Christensson et al. *Contact Dermatitis*. 2012;67:247-59
[6]Audrain et al *British Journal of Dermatology* 2014: 171: 292–297
.7Deza et al. *Contact Dermatitis*. 2016 Nov 292–297

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Contact allergy to limonene vs oxidized limonene/ Hydroperoxides of limonene®

Limonene	Ox. limonene 3%/Hydroperoxides of limonene 0.3%®
0.1% positive [1] (*2% pet.)* 0% positive [3] (*2% pet.)* 0.2% positive[5] *(10% pet.)*	1.6-2.8% positive [2] (*3% pet.*) 5.2% positive [4] (*3% pet, 0.3% Lim-OOH*) 5.0% postitive[5] (*3% pet, 0.3% Lim-OOH*) 5.1% pos [6] *(0.3% Lim-OOH)*

1. Schnuch et al. *Contact Dermatitis*. 2007; 57: 1-10
2. Karlberg & Dooms Goossens. *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.Heisterberg et al *Contact Dermatitis* 2011, 65, 266–275
4.Bråred Christensson et al. *Contact Dermatitis*. 2013;68:214-23
5. Audrain et al *British Journal of Dermatology* 2014: 171: 292–297
6. Deza et al. *Contact Dermatitis*. 2016 Nov 292–297

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2016: Dose response study Spain, 3639 patients in 22 departments

Hydroperoxides of limonene®	Hydroperoxides of linalool ®
0.1% pet. → 1.4% pos	0.25% pet. → 1.3% pos
0.2 pet. → 3.4% pos	0.50% pet. → 2.9% pos
0.3% pet. → 5.1% pos	**1.0% pet. → 4.9% pos**

Deza et al. *Contact Dermatitis*. 2016 Nov 292–297

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2017: Dose response muticenter study UK: 4563 patients in 12 UK centres


Hydroperoxides of limonene ®	Hydroperoxides of linalool®
0.1% pet. → 1.3% pos	0.25% pet. → 2.5% pos
0.2 pet. → 3.2% pos	0.5% pet. → 5.1% pos
0.3% pet. → 5.3% pos (n=241)	**1.0% pet. → 7.7% pos (n=352)**

"The majority of patients reacting to limonene/linalool did not react to any fragrance marker in the baseline series."

Wlodek et al. *British Journal of Dermatology* 2017: 1708-1715.

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2017: USA 103 patients


Hydroperoxides of limonene 0.3%®	Hydroperoxides of linalool 1%®
8% pos	**20% pos**

Nath et al. Dermatitis. 2017 Sep/Oct;28(5):313-316.

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2018: Study Netherlands: 821 patients, tertiary referral centre

Hydroperoxides of limonene 0.3%®	Hydroperoxides of linalool 1%®
0.3% pet. → 9.4% pos (n=77)	**1.0% pet. → 11.7% pos (n=96)**

- 38 of these patients (4.6%) reacted to both.

Dittmar D and Schuttelaar M. Contact Dermatitis. 2019; 80:101–109.

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Relevant exposure contributing to patients dermatitis?
As found by clinician, past or present: multicentre study 9 centres Europe, Singapore, Australia

Relevant exposure found by clinician, past or present										Over-all
Test centre	1	2	3	4	5	6	7	8	9	
Pos to ox limonene (n=152)	75%	71	43	40	20	17	15	12	0%	36%
Pos to ox linalool (n=200)	80%	70	60	50	40	35	29	20	10%	42%

Why differences between test centres in our study?:
- Some protocols were filled at visit, some at later date
- Different approach to discussion about exposure
- Difference in interpretation “relevant exposure”
  - labelling with limonene/linalool vs proven content of hydroperoxides after chemical analysis

Brared Christensson et al. *Contact Dermatitis*. 2013; 68: 214-23
Brared Christensson et al. *Contact Dermatitis,*: 2014 71: 264-7

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Which products were implicated?

- Domestic and occupational products
- Perfumes, shampoos, soaps, body creams, deodorants
- Creams for massage, sunscreens , detergents and domestic cleaners
- Antiseptic tea tree oil-based products, fragrances for candle-making
- Several masseurs, laboratory technician (occupation limonene)

Bråred Christensson et al. *Contact Dermatitis*. 2013; 68: 214-23
Bråred Christensson et al. *Contact Dermatitis,:* 2014 71: 264-7

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Products implicated, UK study

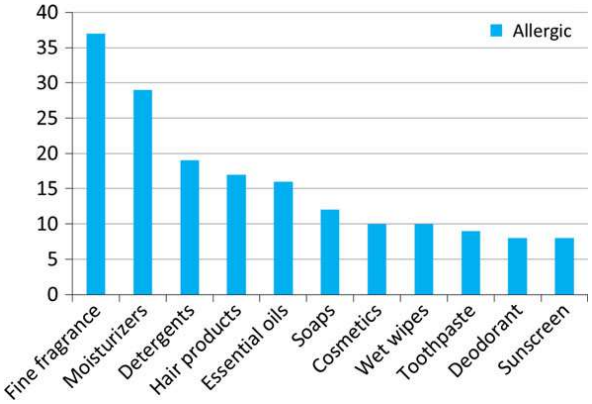

Fig 4. Products giving allergic reactions in 152 of the 411 patients with positive reactions to hydroperoxides of limonene and/or linalool.

Audrain et al *British Journal of Dermatology* 2014: 171: 292–297

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2018: Study Netherlands, tertiary referral centre

- Possibly or probably clinically relevant reactions: 66.3% and 68.8%, to limonene and linalool respectively
- Certain clinical relevance: 18.2% and 19.8%, to limonene and linalool respectively.

- ”In the majority (71.5%) of the patients judged as having ”certain relevance”, the responsible product types were rinse-off products such as soaps and shampoos”

Dittmar D and Schuttelaar M. Contact Dermatitis. 2019; 80:101–109.

2017: Study UK

“In almost two-thirds of patients with positive patch tests to limonene and linalool the reaction was clinically relevant.”

Wlodek et al. *British Journal of Dermatology* 2017: 1708-1715.

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2013: Repeated open application test (ROAT) oxidized linalool

ROAT: **simulate use of a product:** low concentrations of allergen applied repeatedly: will it cause allergic contact dermatitis?

ROAT in ox linalool-allergic patients: ox linalool in perfume- and cream base

→**eczematous reactions down to 0.3% oxidized linalool (corresponding 560 µg/g or 5.4 µg/cm^2 linalool hydroperoxides)**

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

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2018: Repeated open application test (ROAT) oxidized limonene

- Patients with allergic reactions, with doubtful reactions, negative controls
- 3-week double-blind vehicle-controlled ROAT: application 2 times daily with "fine fragrance" containing Lim-OOHs
  - **1260, 420 and 140 µg/g → dose/area per application of *Lim-OOHs* of 3.0, 0.99 and 0.33 µg/cm²**
- *Patch test* dilution series limonene hydroperoxides:
  - **5922 µg/g (152 µg/cm²) → 24 µg/g (0.65 µg/cm²)**

Bennike et al. *Contact Dermatitis*. 2018;1–9.

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Results: ROAT ox limonene:

- Among **11 allergic subjects** to Lim-OOHs, in ROAT:
  - **11 (100%), 7 (64%), and 3 (27%)** reacted to Lim-OOHs of **1260, 420 and 140 µg/g**
- **No reactions in 17 controls** to the highest dose. sensitized vs controls ($P < 0.0001$).

- **2 of 13 doubtful patients** had **positive ROAT reactions** to the highest Lim-OOHs dose applied **1260 µg/g** (**3.0 µg/cm²**) **(ns), 1 of doubtful reacted to 140 µg/g** .

- **In the patch test dilution series: 3 allergic patients reacted to 24 µg/g (0.65 µg/cm²) Lim-OOHs**

Conclusions:

- **Contact allergy to Lim-OOHs is of clinical relevance in patients with positive patch test reactions**
- **Doubtful patch test reaction to Lim-OOHs 0.3% pet. can be clinically relevant**

Bennike et al. Contact Dermatitis. 2018;1–9.

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Results: ROAT ox limonene:

TABLE 1 Overview of the used concentrations and doses of oxidized limonene, including content of limonene hydroperoxides, and proportions of observed reactions in allergic and doubtful allergic participants

Test solution (vehicle)	Concentration		Dose		Observed reactions, n (%)	
	Concentration of oxidized limonene (%)	Content of limonene hydroperoxides, % (ppm)	Dose per application of oxidized limonene (μg/cm²)	Content of limonene hydroperoxides per application (μg/cm²)	Allergic subjects (n = 11)	Doubtful allergic subjects (n = 13)
Confirmatory patch test (pet.)[a]	3	0.3 (3000)	1200	120		
Patch test dilution series (80% ethanol)	4.7	0.59 (5922)	1206	152	11 (100)	13 (100)
	1.6	0.20 (1974)	400	50	10 (91)	8 (62)
	0.52	0.066 (658)	133	17	10 (91)	5 (38)
	0.17	0.022 (219)	44	5.5	6 (55)	1 (7.7)
	0.058	0.0073 (73)	15	1.9	4 (36)	1 (7.7)
	0.019	0.0024 (24)	5.1	0.65	3 (27)	0
	Vehicle	-	-	-	0	0
ROAT solutions (80% ethanol)	1.0	0.13 (1260)	24	3.0	11[b] (100)	2 (15)
	0.33	0.042 (420)	7.9	0.99	7 (64)	1 (7.7)
	0.11	0.014 (140)	2.6	0.33	3 (27)	1 (7.7)
	Vehicle	-	-	-	0	0

Bennike et al. Contact Dermatitis. 2018;1–9.

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2019: ACD from ox linalool in a deodorant

60-year-old non-atopic woman
Problems with scented deodorant
Now only uses unscented - healed

Patch testing:

- The Swedish baseline series **NEG**
- The separate fragrances in fragrance mix I and II **NEG**
- HP of limonene® 0.3% (ox. lim. 3% with 0.3% OOHs) **NEG**
- HP of linalool® 1% (ox. linal. 6% with 1% OOHs) **POS (++)**
- Distilled linalool 6.0%, 2.0%, 0.67% in pet. **POS (+, (+), (?))**

Use test:

- The deodorant labelled to contain linalool, right armpit **POS**
- An unscented deodorant, left armpit **NEG**

Isaksson M et al. Contact Dermatitis. 2019;81:213–214

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ACD from ox linalool in a deodorant

Right armpit before
Start of use test

Use test D 10: ACD with
smarting and pruritus

Chemical analyses:

- Deodorant: linalool 650 µg/g, linalool OOHs **14 µg/g** (Lin-6-OOH 8.4 µg/g, Lin-7-OOH 5.6 µg/g)
- Distilled linalool 6.0% in pet.: linalool OOHs **48 µg/g*** (Lin-6-OOH 3.4 µg/g, Lin-7-OOH 45 µg/g)**
  ***1.9 µg/cm²** of LinOOHs at the patch test site **OOHs most probably formed at test preparation

Isaksson M et al. Contact Dermatitis. 2019;81:213–214

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2017: Eyelid dermatitis from ox linalool

7-year-old atopic girl
Pruritic, burning, oozing eruption on her eyelids since 6-months
Patch testing:

- **In total** 60 haptens **NEG**
- North American Comprehensive Series **NEG**
- HP of limonene® 0.3% (ox. lim. 3% with 0.3% OOHs) **NEG**
- HP of linalool® 1% (ox. linal. 6% with 1% OOHs) **POS (+)**

Healed when stopped using linalool-containing shampoo

Chemical analyses of the shampoo:
GC/MS: linalool and linalool oxide (non-sens. secondary ox. product from Lin-7-OOH)
LC/MS: linalool 87 µg/g, linalool oxide 0.8 µg/g, **Lin-OOHs 0.2 µg/g**

Conclusion:
The results strongly suggest Lin-OOHs in the shampoo as a critical factor contributing to this patient's eyelid dermatitis.

Elliott JF, et al. *Contact Dermatitis 2017:* **76**, 114–128

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What do we know on reproducibility of the reactions?

- No specific information
- Reproducibility of contact allergic reactions in general is 60-95%
- When same allergens on either side of back: discordant in 5%

- Variations over time, biological variability, medication, UV exposure, immunological memory

- Many persons avoid fragranced products
  - Bennike *et al* ROAT study: 2 of 11 allergic patients were exposed to fragranced products in daily life (p=0.002 compared to controls)

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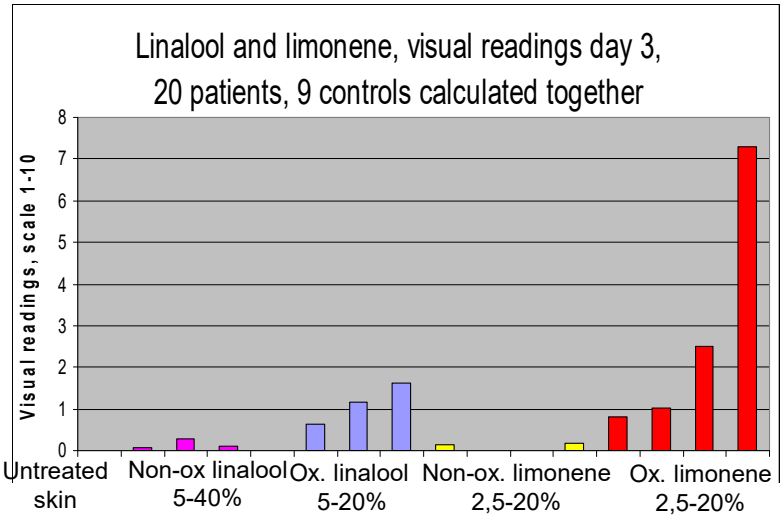
Irritancy from oxidized linalool and/or oxidized limonene?



Bråred Christensson J et al. *Contact Dermatitis.* 2009; 60: 32-4
Scale from Basketter et al. *Contact Dermatitis.* 1997:33 218-220

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How to interpret high rate of irritant/doubtful reactions?

- How are reactions scored?
  - Visual and tactile reading according to IDCRG criteria

Table 1 Scoring of patch tests according to the International Contact Dermatitis Research Group[7]

Score	Reaction
0	Negative
?+	Doubtful: erythema only
1+	Weak (nonvesicular) positive allergic reaction; erythema, infiltration and possibly papules
2+	Strong (vesicular) positive allergic reaction; erythema, infiltration, papules and vesicles
3+	Extreme positive allergic reaction; bullous reaction

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How to interpret high rate of irritant/doubtful reactions?

Doubtful: ”erythema not covering the whole test area… Infiltration not covering the whole test area …. few papules but no erythema or infiltration over the whole test area”

IRR/Irritation: ”irritant reaction of different types: dry skin, scaling, pustules…shiny skin, cigarette paper stucture ….bullae, erosion”

Patch test is read on day 2 or 3 or 4 after 48 hours of occlusion

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Are all test centres reading in the same way?
Results oxidized linalool 6% (Lin-OOHs 1%)

Test centre	no	Positive		Doubtful		Irritant	
Barcelona	299	*11*	**4%**	*0*	**0%**	*0*	**0%**
Copenhagen	440	*21*	**5**	*68*	**15**	*24*	**5**
Gothenburg	397	*15*	**4**	*11*	**3**	*1*	**0.2**
London	271	*14*	**5**	*9*	**3**	*0*	**0**
Malmö	300	*10*	**3**	*13*	**4**	*0*	**0**
Melbourne	289	*24*	**8**	*7*	**2**	*1*	**0.3**
Odense	298	*24*	**8**	*108*	**36**	*7*	**2**
Seville	300	*43*	**14**	*6*	**2**	*3*	**1**
Singapore	306	*38*	**12**	*44*	**14**	*3*	**1**
TOTAL	2900	*200*	6.9%	*266*	9.2%	*39*	1.3%

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Results Oxidized limonene 3% (Lim-OOHs 0.3 %)

Test centre	No	Positive		Doubtful		Irritant	
Barcelona	299	*13*	**4%**	*0*	**0%**	*4*	**1%**
Copenhagen	440	*16*	**4**	*53*	**12**	*17*	**4**
Gothenburg	397	*10*	**2**	*1*	**0.2**	*0*	**0**
London	271	*8*	**3**	*9*	**3**	*0*	**0**
Malmö	300	*7*	**2**	*11*	**4**	*0*	**0**
Melbourne	289	*18*	**6**	*11*	**4**	*0*	**0**
Odense	298	*23*	**8**	*73*	**24**	*3*	**1**
Seville	300	*20*	**7**	*6*	**2**	*0*	**0**
Singapore	306	*37*	**12**	*40*	**13**	*1*	**0.3**
TOTAL	**2900**	**152**	**5.2%**	**204**	**7%**	**25**	**1%**

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Test centre	Total no. tested	Lim-OOHs 0.3% pet. No. of positive patch test reactions (%)	Lim-OOHs 0.3% pet. No. of doubtful patch test reactions (%)	Lim-OOHs 0.3% pet. No. of irritant patch test reactions (%)	Lin-OOHs 1.0% pet. No. of positive patch test reactions (%)	Lin-OOHs 1.0% pet. No. of doubtful patch test reactions (%)	Lin-OOHs 1.0% pet. No. of irritant patch test reactions (%)
Alcorcon	228	1 (0.4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Alicante	177	7 (4.0)	0 (0)	17 (9.6)	5 (2.8)	0 (0)	11 (6.2)
Badalona	168	1 (0.6)	0 (0)	0 (0)	3 (1.8)	0 (0)	0 (0)
Barcelona (Hospital del Mar)	296	11 (3.7)	0 (0)	0 (0)	18 (6.1)	0 (0)	0 (0)
Barcelona (Hospital Sant Pau)	166	5 (3.0)	0 (0)	0 (0)	7 (4.2)	0 (0)	0 (0)
Cádiz	77	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Canarias	107	3 (2.8)	0 (0)	0 (0)	5 (4.7)	0 (0)	1 (0.9)
Fuenlabrada	162	6 (3.7)	0 (0)	3 (1.9)	2 (1.2)	0 (0)	6 (3.7)
Guadalajara	211	10 (4.7)	1 (0.5)	3 (1.4)	27 (12.8)	6 (2.8)	16 (7.6)
León	191	9 (4.7)	3 (1.6)	0 (0)	9 (4.7)	2 (1.0)	0 (0)
Madrid (Fundación Jiménez Díaz)	227	18 (7.9)	2 (0.9)	1 (0.4)	13 (5.7)	0 (0)	1 (0.4)
Madrid (Hospital 12 Octubre)	107	2 (1.9)	1 (0.9)	0 (0)	2 (1.9)	0 (0)	0 (0)
Madrid (Hospital La Princesa)	130	5 (3.8)	0 (0)	3 (2.3)	7 (5.4)	0 (0)	2 (1.5)
Murcia (Hospital Massager)	83	11 (13.3)	3 (3.6)	1 (1.2)	11 (13.3)	2 (2.4)	1 (1.2)
Murcia (Hospital Virgen Arrixaca)	82	0 (0)	1 (1.2)	2 (2.4)	8 (9.8)	0 (0)	0 (0)
Navarra	128	3 (2.3)	0 (0)	1 (0.8)	9 (7.0)	0 (0)	1 (0.8)
Santiago de Compostela	269	10 (3.7)	0 (0)	5 (1.9)	4 (1.5)	0 (0)	9 (3.3)
Sevilla	214	53 (24.8)	0 (0)	3 (1.4)	26 (12.1)	2 (0.9)	2 (0.9)
Toledo	183	21 (11.5)	3 (1.6)	0 (0)	10 (5.5)	5 (2.7)	0 (0)
Valencia	113	1 (0.9)	0 (0)	3 (2.7)	1 (0.9)	0 (0)	2 (1.8)
Vigo	97	2 (2.1)	0 (0)	5 (5.2)	1 (1.0)	0 (0)	3 (3.1)
Vitoria	223	8 (3.6)	0 (0)	8 (3.6)	11 (4.9)	0 (0)	15 (6.7)
Total	*3639*	*187 (5.1)*	*14 (0.4)*	*55 (1.5)*	*179 (4.9)*	*17 (0.5)*	*70 (1.9)*

Deza et al. *Contact Dermatitis*. 2016 Nov 292–297

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Table 2 Total number of patients patch tested at each centre and the number and percentage of irritant, doubtful and positive reactions to hydroperoxides of limonene 0·3% and linalool 1·0% at day 4/5[a]

Site	Total tested	Limonene 0·3% day 4/5 Irritant n	Irritant %	?+ n	?+ %	1+/2+/3+ n	1+/2+/3+ %	Linalool 1·0% day 4/5 Irritant n	Irritant %	?+ n	?+ %	1+/2+/3+ n	1+/2+/3+ %
Centres seeing 500+ patients per year													
Leeds	758	0	0·0	5	0·7	26	3·4	0	0·0	5	0·7	34	4·5
Oxford	695	13	1·9	0	0·0	46	6·6	13	1·9	5	0·7	43	6·2
Dundee	557	24	4·3	8	1·4	50	9·0	61	11·0	14	2·5	78	14·0
Newport	550	4	0·7	1	0·2	18	3·3	4	0·7	4	0·7	37	6·7
Sheffield	531	36	6·8	33	6·2	23	4·3	64	12·1	43	8·1	53	10·0
Subtotal	3091	77	2·5	47	1·5	163	5·3	142	4·6	71	2·3	245	7·9
Centres seeing < 500 patients per year													
Leicester	341	6	1·8	2	0·6	9	2·6	8	2·3	1	0·3	12	3·5
Cardiff	281	0	0·0	1	0·4	14	5·0	0	0·0	1	0·4	21	7·5
Birmingham	250	0	0·0	23	9·2	0	0·0	0	0·0	24	9·6	0	0·0
Swansea	191	0	0·0	11	5·8	32	17	0	0·0	8	4·2	32	16·8
Bath	165	10	6·1	3	1·8	4	2·4	28	17·0	1	0·6	10	6·1
East Kent	140	0	0·0	5	3·6	11	7·9	0	0·0	6	4·3	26	18·6
Cork	104	0	0·0	18	17·3	8	7·7	0	0·0	20	19·2	6	5·8
Subtotal	1472	16	1·1	63	4·3	78	5·3	36	2·4	61	4·1	107	7·3
All	4563	93	2·0	110	2·4	241	5·3	178	3·9	132	2·9	352	7·7

Wlodek et al. *British Journal of Dermatology* 2017: 1708-1715.

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How to interpret high rate of irritant/doubtful reactions?

- Doubtful reactions—rather than irritant reactions
- Different rates in different test centres
- Analyses of reading traditions
- Compare allergens, ex MBDGN

Table 2. Patch test results in 2661 consecutive dermatitis patients tested simultaneously with 4 methyldibromo glutaronitrile (MDBGN) preparations in petrolatum

	Female ($n=1684$)			Male ($n=977$)			Total ($n=2661$)		
Test preparation MDBGN (%)	Positive (%)	Doubtful (%)	IR (%)	Positive (%)	Doubtful (%)	IR (%)	Postive (%)	Doubtful (%)	IR (%)
1.0	4.1	7.6	1.4	5.0	9.1	1.6	4.4	8.2	1.5
0.5	2.7	5.1	0.8	3.5	6.2	1.1	3.0	5.5	0.9
0.3	1.7	1.5	0	2.4	1.5	0.1	1.9	1.5	0.04
0.1	0.8	0.5	0	1.4	0.7	0	1.1	0.5	0

Gruvberger et al. Contact Dermatitis 2005: 52:14-18

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Concomitant reactions: international multicentre study
Fragrance markers (FM, FMII, Myroxylon pereirae, colophonium)

	≥1 fragrance marker a/o colophonium			≥1 fragrance marker a/o colophonium	
Positive ox limonene	64/152	**42%**	ox linalool	79/ 200	**40%**
Doubtful ox limonene	40/204	**20%**	ox linalool	51/271	**19%**
Negative ox limonene	267/2519	**11%**	ox linalool	230/2388	**10%**

Bråred Christensson et al. *Contact Dermatitis*. 2013;68:214-23
Bråred Christensson et al. *Contact Dermatitis*. 2012; 67: 247-59

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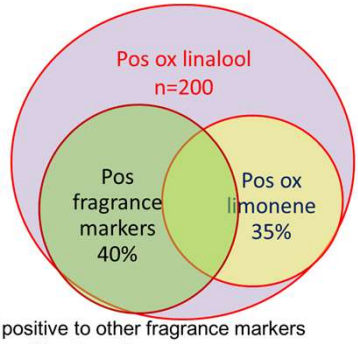
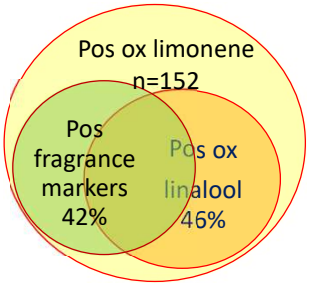
Concomitant reactions: international multicentre study



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



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

Bråred Christensson et al. *Contact Dermatitis* 2016: 74: 273-280

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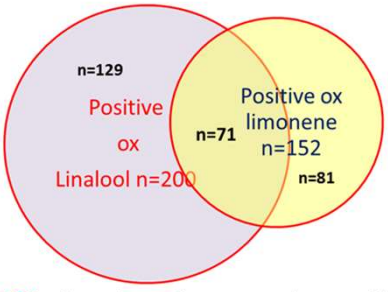
2016: Concomitant reactions ox linalool and ox limonene

Concomitant reactions
ox linalool and ox limonene



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

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

Bråred Christensson et al. *Contact Dermatitis* 2016: 74: 273-280

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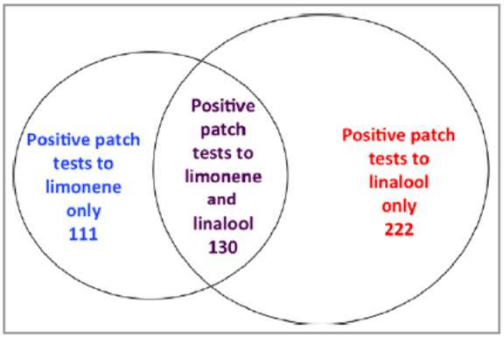
2017: Dose response muticenter study UK: 4563 patients in 12 UK centres



463 positive to any of ox limonene or linalool
28% reactions to both: tandem exposure common
72% reacted only to one:
24% only ox limonene
48% only ox linalool

Wlodek et al. *British Journal of Dermatology* 2017: 1708-1715.

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How to interpret concomitant reactions to other allergens

- Patients reacting to Hydroperoxides of linalool® or Hydroperoxides of limonene®
  - React to other fragrance markers in about 40%
  - 30-40% react to the other respective hydroperoxide preparation
- Most of the allergic patients react only to one of the hydroperoxide patch test preparations
- The vast majority of patients DO NOT react in any way to any of the hydroperoxide markers

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Clinical picture – what does it tell us? How do we explain the high rates of positive reactions?

- Similar figures from many countries, single centres and multicentre studies: reproducible results
- Male : female ratio about 1:2, 1:4 etc: if random reactions this would be 1:1
- Most persons react to one of the two: specificity of reactions
- Repeated judgement of clinical relevance in relation to exposure
- ROAT studies confirm that low concentrations will cause eczema in allergic patients
- Massive exposure to limonene and linalool in very diverse products

→ Evidence of specific, true, allergic reactions
→ We need to identify the exposure

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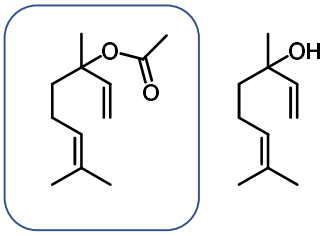
Oxidized linalyl acetate



- When products have been analyzed, linalyl acetate has been found in **30-90%** of the products but not declared

- Clinical study: 1717 patients were patch tested with oxidized linalyl acetate at 6.0% in petrolatum
- **2.2% of patients showed positive reactions to oxidized linalyl acetate**
- 43% of the positive cases also had positive patch test reactions to other fragrance markers

Hagvall L, Berglund V, Bråred Christensson J. Contact Dermatitis. 2015 Apr;72(4):216-23

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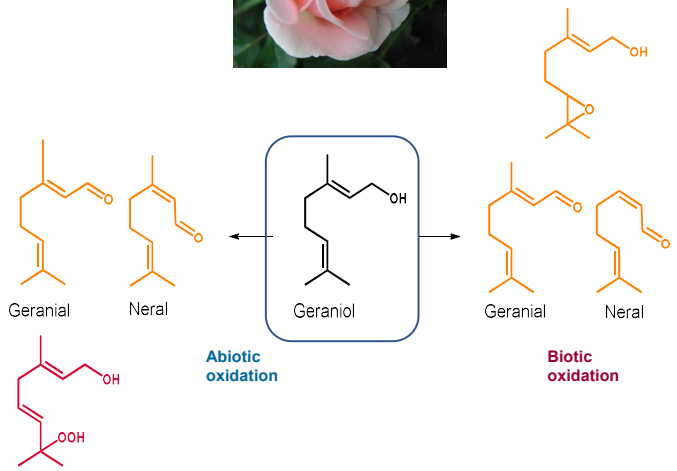
Geraniol

- Geraniol is both a pre- and a prohapten
- A hydroperoxide and sensitizing aldehydes are formed in autoxidation of geraniol[1]
- Sensitizing aldehydes and epoxides are formed in metabolic activation of geraniol in the skin[2]

Citral = Geranial + Neral (2: 1)



1. Hagvall et. al. Chem Res Toxicol. **2007**, *20*, 807-814
2. Hagvall et. al. Toxicol Appl Pharm. **2008**, *233*, 308-313

Contact allergy to geraniol vs oxidized geraniol

Geraniol	Ox. geraniol
4% pet. **0.1% pos**	*4% pet.* **0.9% pos**
6% pet. **0.5% pos**	*6% pet.* **2.3% pos**
11% pet. **1.1% pos**	*11% pet.* **4.6% pos**

Hagvall et al. *Contact Dermatitis*. 2013; 68: 224-31

2019: Sweden multicentre study: oxidized geraniol, geranial, neral, citral in 1476 patients (Swedish Contact Dermatitis Research Group)

Total tested 1476 at five test centres	Positive reactions (%)	Doubtful reactions (%)
Oxidized geraniol 11.0% pet.	8.2%	4.9%
Geraniol 6.0%	1.0%	0.7%
Geranial 3.5% pet	3.4%	4.2%
Neral 3.5% pet.	1.9%	4.2%
Citral 3.5% pet.	2.9%	3.1%

- Together, citral and geranial gave 4.2% positive patch test reactions in consecutive dermatitis patients.
- In patients with positive reactions to citral or its components, 25-34% reacted to FM II and 61% reacted to oxidized geraniol.

Hagvall et al. Accepted for publication *Contact Dermatitis* 2019

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Autoxidation occurs in essential oils

- **Petitgrain oil**: linalyl acetate hydroperoxides, linalool hydroperoxides[1]
- **Sweet orange oil:** limonene hydroperoxides[1]
- **Lavender oil**: linalyl acetate hydroperoxides, linalool hydroperoxides[2]

- Hydroperoxides identified <u>at delivery from producer</u> and, in increasing amounts <u>after storage also dark in the refrigerator</u>

1. Rudbäck J *J Sep Sci*. 2014; 37: 982-9.
2. Hagvall L *Contact Dermatitis*. 2008; 59:143-50

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Oxidation products in commercial products?

- Remember broad range of products
- Companies selling, companies buying
- Check every batch in large companies?
- Small companies?