Support program for gaining understanding on the clinical reactions observed by dermatologists

Background for support program: Clinical relevance, where are we today?

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- Linalool and limonene, fragrance terpenes
- Autoxidation
- Studies on oxidized linalool and oxidized limonene
- Clinical relevance
- Oxidized linalyl acetate, oxidized geraniol
- Exposure
- Future outlook

Linalool – lavender fragrance, mainly industrially produced. Common in fragranced products, main component in lavender oil, present in other essential oils

Limonene – citrus fragrance. Mainly a by-product of citrus juice industry. Common in fragranced products, used industrially as a solvent. Present in essential oils

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

Linalool in 60-90% of examined products
Limonene in 60-80% - " -

Rastogi et al.*Contact Dermatitis* 2001:45:221-5; 1998:38:29-35
Buckley. *Br J Dermatol* 2007:157:295-300
de Groot AC, Frosch PJ *Contact Dermatitis* 1997:36:57-86
Yazar et al *Contact Dermatitis*. 2011; 64: 265-72.

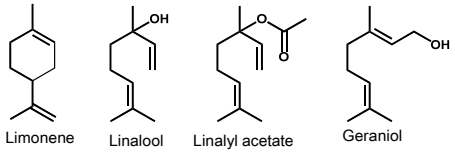
Linalool and limonene the most frequent coupled (tandem) exposures to fragrance chemicals- found together in products

Uter W et al. *Contact Dermatitis*. 2013; 69: 335-41

Linalool highest calculated exposure to high end users – from many sources daily

RIFM Expert Panel, Belsito D et al. *Food Chem Toxicol*. 2008; 46 Suppl 12:1-71

Limonene and linalool, like many fragrances belong to chemical family of terpenes



Limonene Linalool Linalyl acetate Geraniol

Terpenes: unsaturated double bonds →prone to autoxidation

- 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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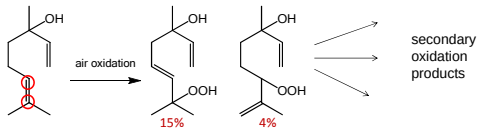
Autoxidation of linalool

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



- Allergens accumulate in oxidation mixture
- Oxidized linalool (oxidation mixture) much more sensitizing than non-oxidized (pure) linalool

Sköld M. Contact Allergy to Autoxidized Fragrance Terpenes Thesis 2005

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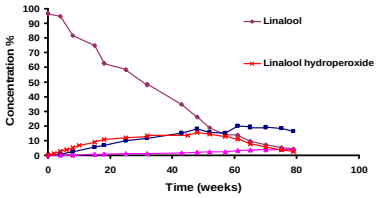
Autoxidation of linalool



- Composition of mixture will change over time
- Important to standardize patch test material

Sköld et. al. *Chem. Res. Toxicol.* 2004, 17, 1697-1705

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Autoxidation of limonene

- Autoxidizes
- Allergenic oxidation products formed in oxidation mixture
- Limonene hydroperoxides (main allergens), carvone[1]
- Oxidized limonene (oxidation mixture) much more allergenic than non-oxidized (pure) limonene

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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Contact allergy to linalool vs oxidized linalool

Linalool	Ox. linalool
0.2% positive[1] *(10% pet.)*	1.3% positive[2] *(2% pet.)*
0.1% positive[3] *(10% pet.)*	5.3% positive[4] *(6% pet, 1% OOH)*
	6.9% positive[5] *(6% pet, 1% OOH)*
0.3% positive[6] *(10% pet.)*	5.9% positive[6] *(6% pet, 1% OOH)*
	1.3% pos [7] *(0.25% OOH)*
	2.9% pos [7] *(0.5% OOH)*
	4.9% pos [7] *(1.0% OOH)*

[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

Limonene	Ox. limonene
0.1% positive [1] *(2% pet.)*	1.6-2.8% positive [2] *(3% pet.)*
0% positive [3] *(2% pet.)*	5.2% positive [4] *(3% pet, 0.3% OOH)*
0.2% positive[5] *(10% pet.)*	5.0% postitive[5] *(3% pet, 0.3% OOH)*
	1.4% pos[6] *(0.1% OOH)*
	3.4% pos[6] *(0.2% OOH)*
	5.1% pos [6] *(0.3% 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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Diagnosis of allergic contact dermatitis (allergic eczema)

1. Establishing contact allergy
2. Demonstration of present exposure to the sensitizer
3. Clinical relevance: the exposure should explain and/or contribute to the dermatitis
   exposure is correlated to localization, type, course of the dermatitis

   →identify sources of exposure
   May be obvious or difficult to assess
   Ex nickel, PPD

Bruze M. *Contact Dermatitis* 1990:23:224-225

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Assessing clinical relevance

- Difficult task if ubiquitous allergens, such as fragrances
- Bring in products or report labelling: products used on dermatitis areas
- Present, past, unknown exposure/relevance

- Few studies
- Heisterberg et al: 26 fragrance chemicals in EU directive: 76% of patients with positive patch test reactions had exposures judged clinically relevant

[1] Heisterberg et al. *Contact Dermatitis.* 2011; 65: 266-75

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Multicentre study: oxidized linalool, oxidized limonene

- 2900 consecutive dermatitis patients at 9 test centres
- Standardized patch test material, oxidized linalool 6% (LinOOH 1%), oxidized limonene 3% (LimOOH 0.3%)
- Frequencies of positive, doubtful and irritant reactions
- Study clinical relevance of positive patch test reactions and products implicated

Bråred Christensson et al. *Contact Dermatitis.* 2014; 71: 264-7
Bråred Christensson et al. *Contact Dermatitis.* 2012; 67: 247-59
Bråred Christensson et al. *Contact Dermatitis.* 2013;68:214-23
Bråred Christensson et al. *Contact Dermatitis.* 2016; 74: 273-80

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Clinical relevance of positive patch test reaction: three step approach in studies

1. Has patient experienced problems with scented products? (question before patch test)
2. Assessing relevant exposure: Is allergic patient using products containing limonene/linalool on dermatitis areas?
   - Past, present, unknown
   - Identify culprit products
3. Relation to other fragrance allergies?
   - Multiple sensitizations?

Bråred Christensson et al. *Contact Dermatitis.* 2014; 71: 264-7
Bråred Christensson et al. *Contact Dermatitis.* 2012; 67: 247-59

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Step 1: Questionnaire: Has patient experienced problems with scented products or perfumes?

(Asked before testing, questions from Frosch et al. *Br J Dermatol* 1999: 141: 1076–1083)

- **(A) certain** – …itching dermatitis to at least one fine perfume or aftershave and also to other scented products;
- **(B) probable** – …one or more scented products…perfume has not been identified …;
- **(C) questionable** – …various cosmetics with or without fragrances, …other materials than fragrance constituents …
- **(D) none** – has never reacted to scented materials

	Pos to ox limonene (n=152) Certain or probable	Pos to ox linalool (n=200) Certain or probable
Overall	**23%**	**21%**
Range at different test centres	**0-58%**	**9-60%**

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Do the patients only allergic to ox limonene (no other fragrance) experience more problems with fragranced products than those negative all fragrances?

Certain or probable reaction to a fragranced product (question posed before testing)

	Pos to ox limonene (n=152)	Pos only ox limonene (no other fragrances) (n=86)	Neg to all fragrances (n=1737)
Certain or probable (A or B)	23%	**18%***	**8%***

→Significantly more patients experienced problems with fragrances in limonene-allergic group. Supports clinically relevant reactions

*P<0.01, Fisher´s exact test two tailed

Bråred Christensson et al. *Contact Dermatitis.* 2014; 71: 264-7

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Step 2: Is the allergic patient using products containing limonene/linalool on dermatitis areas?

- Asking patients about products used
- Patients bring in products
- Find culprit products

- Time consuming
- Big help for patients: discuss their allergy
- Efficient advice, explain where to look

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Relevant exposure found by clinician, past or present

	Patients pos to ox limonene--limonene	Patients pos to ox linalool--linalool
Overall	**36%**	**42%**
N=2900		
Bråred Christensson et al. *Contact Dermatitis.* 2014; 71: 264-7 Bråred Christensson et al. *Contact Dermatitis.* 2012; 67: 247-59		
Overall	**37%**	
N=4731		
Audrain et al *British Journal of Dermatology* 2014: 171: 292–297		

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Relevant exposure found by clinician, past or present

Relevant exposure found by clinician, past or present, multicentre study Bråred Christensson										Over-all
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
- Declaring ingredients differed EU/non-EU
- 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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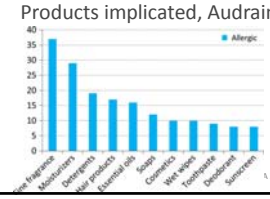
Which products were implicated?

- 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

Products implicated, Audrain et al



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

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Clinical relevance, step 3. Concomitant reactions to fragrance markers (FM I, FM II, HICC, Myroxylon pereirae, colophonium)

Strong statistical significance, p<0.000001

	≥1 fragramce marker a/o colophonium	
Positive ox linalool	79/ 200	40%
Doubtful ox linalool	51/271	19%
Negative ox linalool	230/2388	10%

Bråred Christensson et al. *Contact Dermatitis.* 2012; 67: 247-59

	≥1 fragrance marker a/o colophonium	
Positive ox limonene	64/152	42%
Doubtful ox limonene	40/204	20%
Negative ox limonene	267/2519	11%

Bråred Christensson et al. *Contact Dermatitis.* 2013;68:214-23

4. Assessing clinical relevance: Repeated open application test

ROAT in ox linalool-allergic patients: ox linalool in perfume- and cream base: eczematous reactions to 0.3% oxidized linalool (0.056% (560µg/g) linalool hydroperoxides)

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

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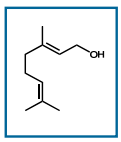
Oxidized linalyl acetate

- Presence of linalyl acetate in cosmetic products and essential oil is difficult to assess, as it is not labelled on products in the EU.
- When products have been analyzed, linalyl acetate has been found in 30-90% of the products.
- Linalyl acetate hydroperoxides were detected early in the autoxidation process and accumulated to a concentration of 37% at 42 weeks of air exposure
- The linalyl acetate hydroperoxides were classified as moderate sensitizers
- 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

Rastogi S C et al, *Contact Dermatitis* 2001: 45: 221-5.
Rastogi S C et al,. *Contact Dermatitis* 1998: 38: 29-35.
Sköld M et al. Contact Dermatitis 2008: 58: 9-14
Hagvall L, et al Contact Dermatitis. 2015 ;72: 216-23

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]



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

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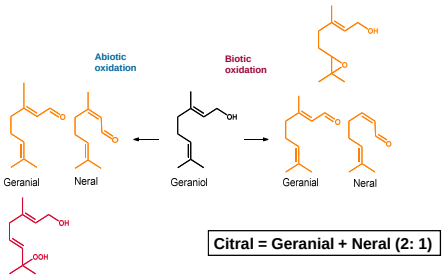
Geraniol



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

How are people exposed to fragrance terpenes?

- Fragrances in many products
- 15-30% of volume in perfumes, 0.1-2% of detergents, soaps, shampoos
- Diverse products: big industries, small companies, home made products.
- Plant oils in ecological/nature based products

Hydroperoxides in commercial products:

- Very difficult to identify hydroperoxides in complex matrices
- Labile compounds, have not been able to be detected by analytic methods until today

Autoxidation occurs in essential oils

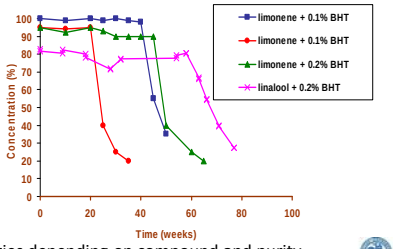
- **Petitgrain oil**: linalyl acetate hydroperoxides, linalool hydroperoxides[1]
- **Sweet orange oil:** limonene hydroperoxides[1]
- **Lavender oil**: linalyl acetate hydroperoxides, linalool hydroperoxides[2]

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

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

Effect of antioxidant on the air oxidation of limonene and linalool

BHT (butylated hydroxy-toluene): antioxidant, used as preservative



The effect varies depending on compound and purity.

Karlberg A-T, et al. Ann Occup Hyg 1994: 38: 199-207

Sensitization may be enhanced by formulations

Sensitization and elicitation responses enhanced in mixtures of fragrances compared to single fragrance (in mice)

Menné Bonefeld et al. *Contact Dermatitis* 2011: **65**: 336-42.

Formulation with ethosomes increases sensitization (LLNA) and elicitation in ROAT

Madsen et al. *Contact Dermatitis* 2010: **63**: 209-14

Reactivity to fragrances enhanced in combination with SLS

Heydorn et al. *Contact Dermatitis* 2003: 49: 133-139

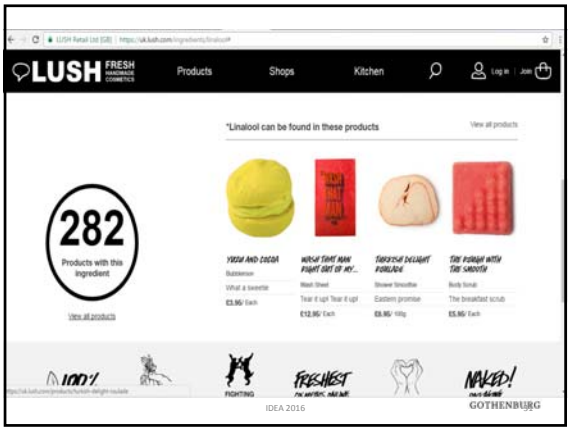
Hydroperoxides in commercial products

- 40 recalled used perfumes:

Linalool hydroperoxides detected. In 1 of 40 (2.5%) perfumes - amount of linalool hydroperoxides (132 ug/g) only 5 times below lowest reaction in ox linalool ROAT (560 ug/g)

- Kern et al. *Anal. Bioanal.Chem* 2014: 406: 6165-6178

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



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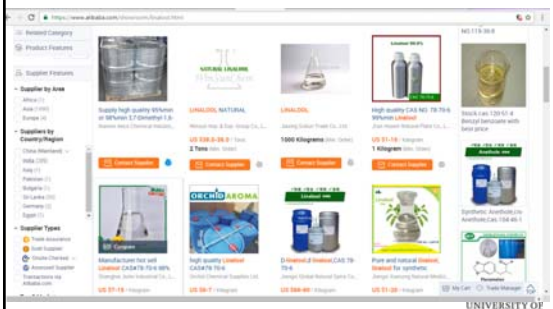
Who is buying linalool: ask Sigma Aldrich, …?
→ Alibaba: 45 internet pages of suppliers of linalool….



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Oxidation products in commercial products?

- Mixing oils at spa centre Gothenburg, used for massage
- (Photo from spa)

- Room scents
- Natural ointments
- Home made remedies

Recipe fron Swedish television: hand cream with lavender oil

"A paraffinbased cream with eterical lavender oil.
Suitable for hairy areas.
1 litre"

Household products?

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Many fragrance terpenes are altered by air oxidation or metabolism

	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
Alpha-terpinene	Yes	Yes
Citronellol	Yes*	Yes**

Karlberg A-T et al. *Contact Dermatitis.* 2013; 69: 323-34
- Rudbäck J et al. *Contact Dermatitis* 2014; 70: 329–339

** Not enough to cause sensitization. Delaine T et al. Chem. Res. Toxicol. 2014; 27: 1860−1870

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In near future

- Planned study with University of Copenhagen, ROAT study oxidized limonene and oxidized linalool
- Discussion of studies of clinical relevance

Future outlook?

- More oxidized fragrance terpenes to be studied experimentally and clinically
- Continuing work to develop practical analytical methods
- Studies on exposure, (ROATs) repeated open application tests of oxidized fragrances, use tests of products and model products
- Work towards a scientific understanding
- Safer products, better protection for consumer

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