



UNIVERSITY OF GOTHENBURG

# Cross reactivity when patch testing with oxidized materials

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# Cross reactivity

## Definition:

The receptor of a memory cell for **antigen 1** cannot distinguish between **antigen 1** and an **antigen 2** created from another hapten and will thus react also to **antigen 2**.

# Cross reactivity

- **Haptens A** and **B** are chemically and structurally similar.
- **A** is metabolised to a compound similar to **B**.
- **B** is metabolised to a compound similar to **A**.
- **A** and **B** are metabolised to the same compound.

However,

- Small changes in structure and configuration could prevent from cross reactivity.



# Cross reactivity

- True cross reactivity studies must be performed experimentally under controlled exposure conditions.
- So far the most reliable are guinea pig studies
  - Live animals
  - Both induction and elicitation
  - No concomitant exposure
- Clinical studies can only give indications since **no control of the exposure**



# Cross reactivity

Prehaptens – the haptens are formed outside skin

Prohaptens – the haptens are formed in the skin

In both cases identification and testing with the real haptens will help in detecting contact allergy.



# Cross reactivity

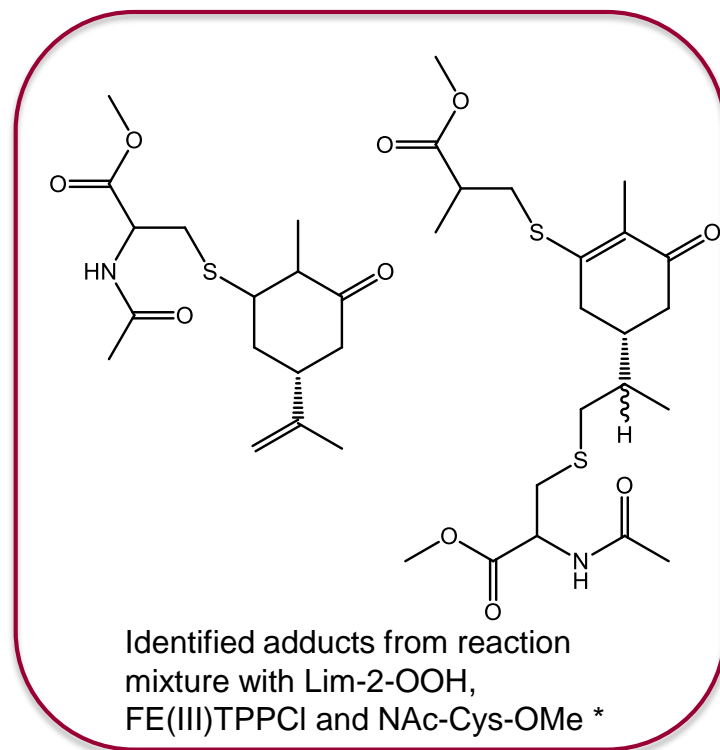
Prehaptens and the haptens formed outside skin by abiotic oxidation:

- The primary oxidation products – the hydroperoxides

# Specific immunogens formed

## Formation of immunogenic complexes

- ✓ Involvement of carbon centered, alkoxy, and peroxy radicals has been demonstrated with radical trappers
- ✓ Investigations with peptides and nucleophiles show specific complex formation with limonene-2-OOH
- ✓ Presence of iron complexes necessary (Fe(II)/Fe(III))
- ✓ Radical mechanism involved
- ✓ Formation of non-specific peptide oligomers





# Cross reactivity

- Specific cross-reactivity pattern demonstrated in guinea-pigs according to the Freund's complete adjuvant test method\*

\*Klecak G. *Curr Probl Dermatol* 1985; 14: 152–171  
Hoffman La Roche, Basel

- One of the accepted methods in the original OECD Guideline 406

## Pragmatic and regulatory reasons:

- OECD Guideline 406 reduced the methods to two: GPMT (with FCA) and Buehler test (without FCA)





# Freund's complete adjuvant test method

- Klecek G. "The Freund's complete adjuvant test and the open epicutaneous test. A complementary test procedure for realistic assessment of allergenic potential." *Curr Probl Dermatol* 1985; 14: 152–171.
- Especially developed for natural substances in small amounts.
- Good agreement between FCAT and GPMT when testing colophony allergens (mainly hydroperoxides)



# Positive reaction at challenge testing in guinea pigs



# Cross reactivity pattern- for different monoterpenes and one diterpene

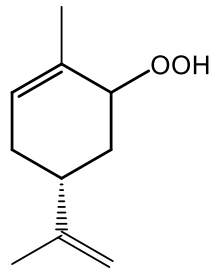
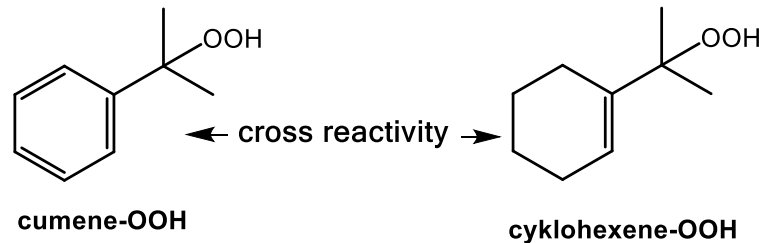
Induction compounds:	Challenge compound 1	Challenge compound 2	Challenge compound 3	Challenge compound 4
	Cumene-OOH	Limonene-2-OOH	Cyclohexene-OOH	15-Hydroperoxy dehydroabietic acid
<u>Group A</u> Cumene-OOH	pos	neg	pos	neg
<u>Group B</u> Limonene-2-OOH	neg	pos	neg	NT

\*Bråred Christensson et al. Contact Dermatitis 2006: 55: 230–237

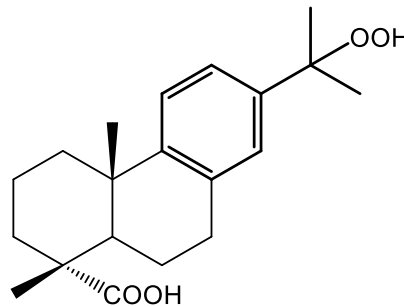
# Cross reactivity pattern

No general cross reactivity found

Cross reactivity when overall structural similarity



limonene-OOH

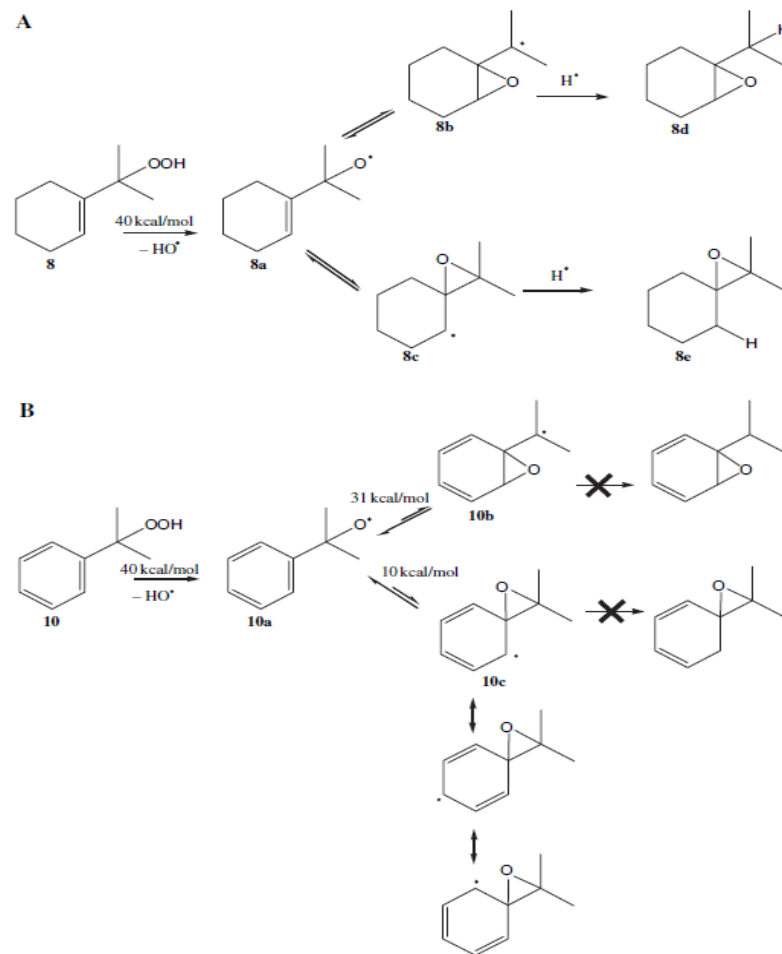


15-hydroperoxydehydroabiatic acid

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

# Cross reactivity pattern

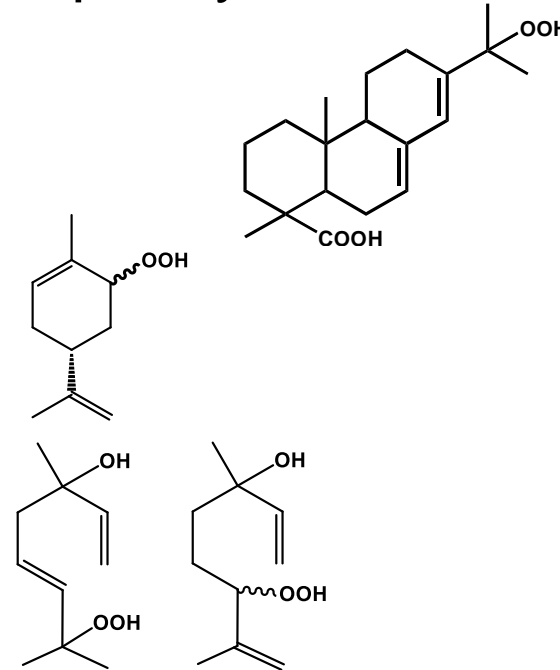
Supported by determination of the formation energies of the intermediary radicals.



# Cross reactivity pattern

29 individuals sensitised to colophony  
tested with:

- ✓ 15-Hydroperoxyabietic acid  
(important hapten in colophony)
- ✓ Limonene-2-hydroperoxide
- ✓ Linaloolhydroperoxides



**Only 1/29 reacted to more than one hydroperoxide**

1/29 reacted to colophony to 15-HPA **and** to limonene-2-OOH



# Cross reactivity pattern

28/ 29 individuals reacted to colophony at retesting

13/29 (36%) reacted to: 15-hydroperoxyabiatic acid (15HPA)

1/29 reacted only to linalool-OOH and colophony

1/29 reacted only to limonene-2-OOH and colophony

1/29 reacted to limonene-2-OOH, 15HPA and colophony

**Thus, no over all unspecific reactivity**

# Simultaneous reactions to ox. limonene and its hydroperoxide fraction in 2273 patients in a European multicenter study

**63 patients** reacted either to ox.limonene or to the hydroperoxide fraction or to both = **63/2273 = 2.8%**.

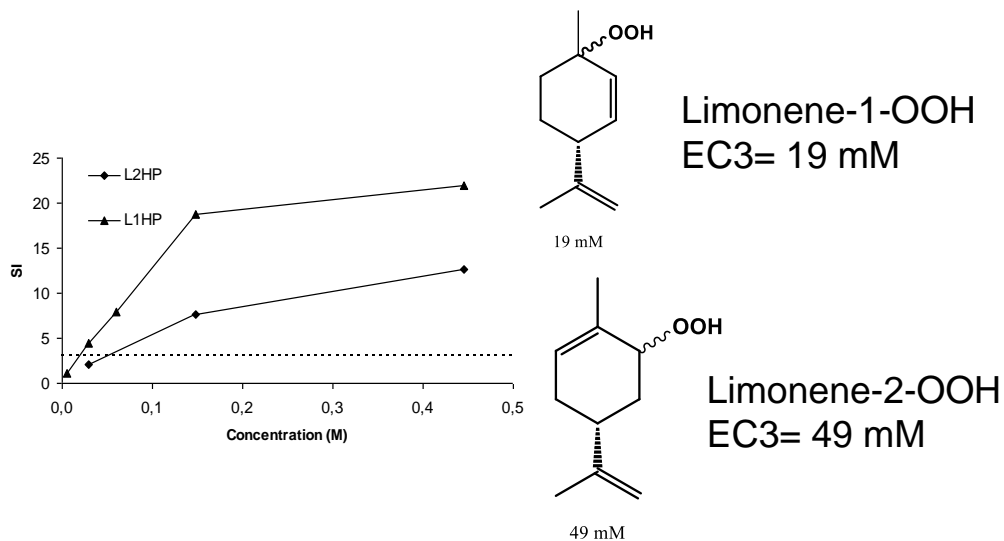
	<i>R</i> -Limonene-	hydroperoxide	fraction
	Positive	Negative	Total
Total ox. <i>R</i> -limonene mixture			
Positive	27/63 (43%)	26/63 (41%)	53/63 (84%)
Negative	10/63 (16%)	2210	2220
Total	37/63 (59%)	2236	2273

Matura M et al. Contact Dermatitis 2003: 49: 15-21

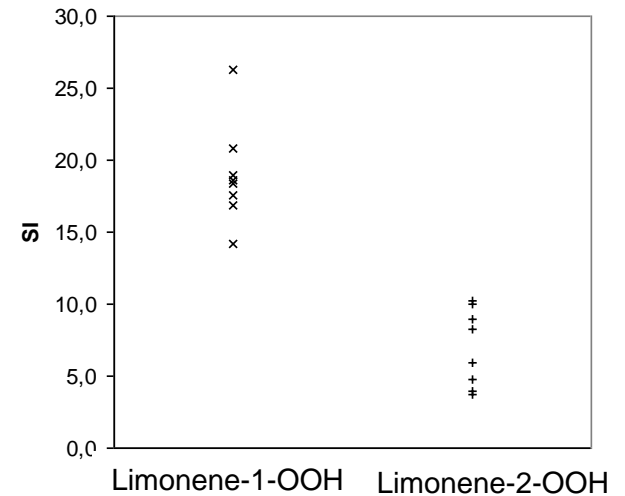


# Limonene-1-OOH and Limonene -2-OOH

The limonene hydroperoxides differ in sensitizing potency in LLNA



Statistically significant





# Limonene-1-OOH and Limonene -2-OOH

## Small clinical study:

7 patients allergic to ox. limonene patch tested with Lim-1-OOH and Lim-2-OOH

### Results:

- 7/7 reacted to Lim-1-OOH
- 3/7 reacted to Lim-2-OOH

- Lim-1-OOH stronger allergen
  - in modified LLNA
  - more positive patch test reactions in limonene-allergic patients

Bråred Christensson et al. Contact Dermatitis: 2008;59:344-52.

# Limonene-1-OOH and Limonene -2-OOH

## Enlarged clinical study:

Testing in 763 consecutive patients

Reactions to	Also reactions to Ox. R-lim.	Also reactions to Lim-1-OOH	Also reactions to Lim-2-OOH	No pos reactions to other limonene markers
Ox. R-lim. (3%)*	9 (total)	6	4	2
Lim-1-OOH (0.5%)	6	18 (total)	8	7
Lim-2-OOH (0.5%)	4	8	13 (total)	4

\*Content of Lim-1-OOH 0.0003% and of Lim-2-OOH 0.002%

# Limonene-1-OOH and Limonene -2-OOH

Concomitant reactions to **other** fragrance markers

Patch test material	Also reactions to FM 1	Also reactions to FM 2	Also reactions to Myroxylon Pereirae	Also reactions to Colophony
Ox. R-lim. (3%)	45%	0	36%	2%
Lim-1-OOH (0.5%)	28%	0	39%	17%
Lim-2-OOH (0.5%)	38%	0	61%	23%

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

# Thorough presentation of all 25 cases

**Table 3.** Clinical data from individual patients showing maximum positive patch test readings to oxidized R-limonene, limonene-1-hydroperoxide, and limonene-2-hydroperoxide; the table also shows concomitant positive patch test reactions to fragrance markers and/or colophonium in the baseline series, as well as to oxidized linalool 6.0% pet.

	Age (years)	Male (M) female (F)	Oxidized limonene 3.0%	Limonene-1-hydroperoxide 0.5%	Limonene-2-hydroperoxide 0.5%	Other fragrance markers and oxidized linalool					Location of eczema	Relevant exposure <sup>a</sup>
						Fragrance Mix I	Fragrance Mix II	Myroxylon perexiae	Colophonium	Oxidized linalool		
2007	64	F	?	++	++	+	-	+	-	+	Hands	Cleaner
	49	M	-	+	+	-	-	-	-	+	Legs	-
	36	F	-	+	?	-	-	-	-	-	Around mouth	-
	36	M	++	++	++	+	-	+	-	++	Foot	-
	33	F	-	+	-	-	-	-	-	-	Axillae	Deodorant
2008	33	F	-	-	+	-	-	+	-	-	Dry skin, eczema on hands	Shower gel
	81	F	+++	+	+	++	?	+	+	++	Torso	-
	74	F	+	-	-	-	-	+	-	+	Axillae, torso	Positive reaction to deodorant
	66	F	+	+	-	-	-	-	-	-	Hands	Works in cookie factory
	72	F	-	++	+	-	-	-	-	-	Around ears	-
	55	F	?	+	+	?	-	++	++	+	Face	Many perfumed products
	47	F	-	-	+	-	-	-	-	-	Arms, hands	Works with paints
	33	F	+	+	+	++	-	++	++	+	Hands	-
	21	M	?	?	+	++	-	++	-	+	Around mouth	-
	49	F	-	+	-	-	-	-	-	-	Face	-
	21	F	-	+	-	-	-	-	-	-	Scalp	-
	14	F	-	+	-	-	-	-	-	-	Face, arms	Shampoo
2009	54	F	+	+	-	-	-	+	-	-	Face, around mouth	-
	32	M	+	+	?	+	-	?	-	-	Hands, feet, arms	Baker, pizza maker
	32	F	++	-	++	-	-	-	-	-	Genital area	-
	24	F	+	?	-	++	-	-	-	+	Hands	-
	57	F	-	+	+	-	-	++	-	-	Hands	-
	56	F	-	+	?	-	-	-	-	+	Hands, torso	Works in biscuit factory
	14	M	-	+	-	-	-	-	-	-	Face	-
38	F	-	-	++	-	-	-	-	-	Hands	Sunscreen	

<sup>a</sup>When positive readings for any of the test materials were obtained, questions regarding exposure to limonene-containing products were asked. A positive patch test reaction was assessed as relevant if the patient used products in which limonene was declared among the contents at the site of eczema or if the patient came into contact with products expected to contain limonene (i.e. citrus flavouring, citrus-based degreasers, or citrus-based paint thinners) at the site of eczema.



**All** other clinical studies are on oxidized limonene or oxidized linalool (oxidation mixtures)

- ✓ **Mixtures of:**
- ✓ Non-oxidized parent compounds
- ✓ Primary oxidation products
- ✓ Secondary oxidation products
- ✓ Dimers?



# Concomitant reactions to other fragrance markers in old studies

	Positive reactions in the baseline series		
	Fragrance mix 1	MP	Colophony
<b>91-95.</b> Pos. to oxidized limonene (49/2800 pat.)	<b>41%</b>	<b>24%</b>	<b>24%</b>
<b>97-99.</b> Pos. to oxidized limonene (63/2273 pat.)	<b>37%</b>	<b>21%</b>	<b>22%</b>
<b>2001.</b> Pos. to oxidized limonene (63/2411 pat.)	<b>33%</b>	<b>22%</b>	<b>29%</b>
<b>2002.</b> Pos. to oxidized linalool (25/1511 pat.)	<b>40%</b>	<b>20%</b>	<b>32%</b>



## Concomitant reactions to other fragrance markers in dose response study with ox. linalool

Concomitant reactions	Pos to ox. linalool			Neg to ox. linalool (n=929)
	Conc. 4% (n=30)	Conc. 6% (n=55)	Conc. 11% (n=72)	
FM I, FM II, MP, Colophonium	<b>47%</b>	<b>42%</b>	<b>39%</b>	<b>12%</b>

Bråred Christensson J et al. Contact Dermatitis 2010;62:32-41

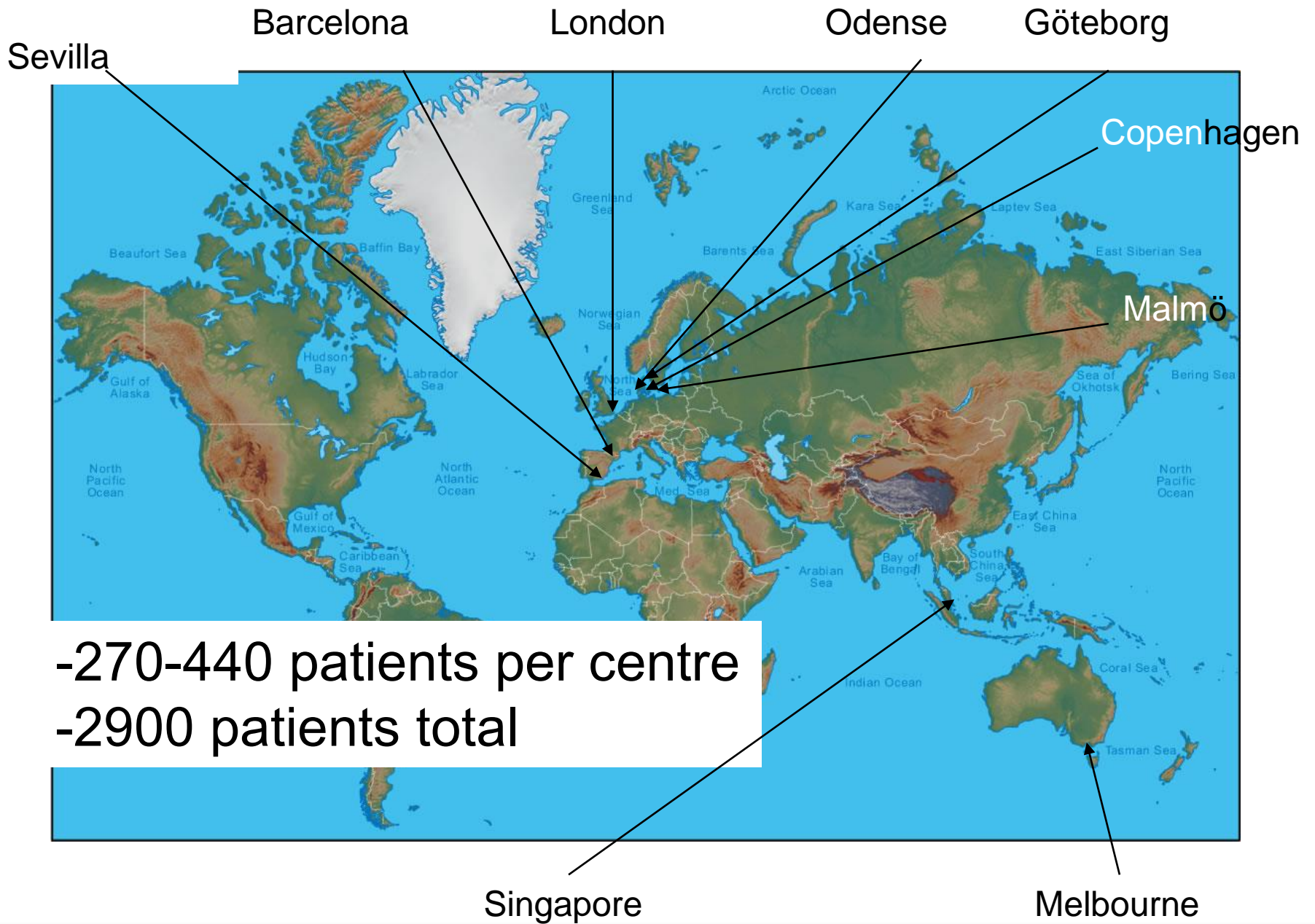


# International multicentre study oxidized linalool

- *Contact Dermatitis* 2012; 67: 247-259.
- Klaus E Andersen, Magnus Bruze, Jeanne Duus Johansen, Begoña Garcia-Bravo, Ana Gimenez Arnau, CL Goh, Rosemary Nixon, Ian R White, Johanna Bråred Christensson
- Ann-Therese Karlberg
- Charlotte Siwmark, Bo Niklasson



Bråred Christensson J et al. *Contact Dermatitis* 2012;**67**: 247–259



## Results ox linalool 6% containing Lin-OOHs 1% pet.

Tested	Positive	Doubtful	Irritation
2900	200	266	39
%	6.9%	9.2%	1.3%
range	(3-14%)	(0-36%)	(0-5%)

40% of patients with positive reactions reacted to other fragrance marker and/or colophonium



# International multicentre study oxidized *R*- limonene

- *Contact Dermatitis* 2013: 68:214-223
- Klaus E Andersen, Magnus Bruze, Jeanne Duus Johansen, Begoña Garcia-Bravo, Ana Gimenez Arnau, CL Goh, Rosemary Nixon, Ian R White, Johanna Bråred Christensson
- Ann-Therese Karlberg
- Charlotte Siwmark, Bo Niklasson



Bråred Christensson J et al. *Contact Dermatitis* 2013;**68**: 214–223

Results ox limonene 3% containing Lim-OOHs 0.33% pet

Tested	Positive	Doubtful	Irritation
2900	152	204	25
%	5.2%	7.0%	0.9%
range	(2-12%)	(0-25%)	(0-4%)

42% of patients with positive reactions had reaction to other fragrance marker and/or colophonium



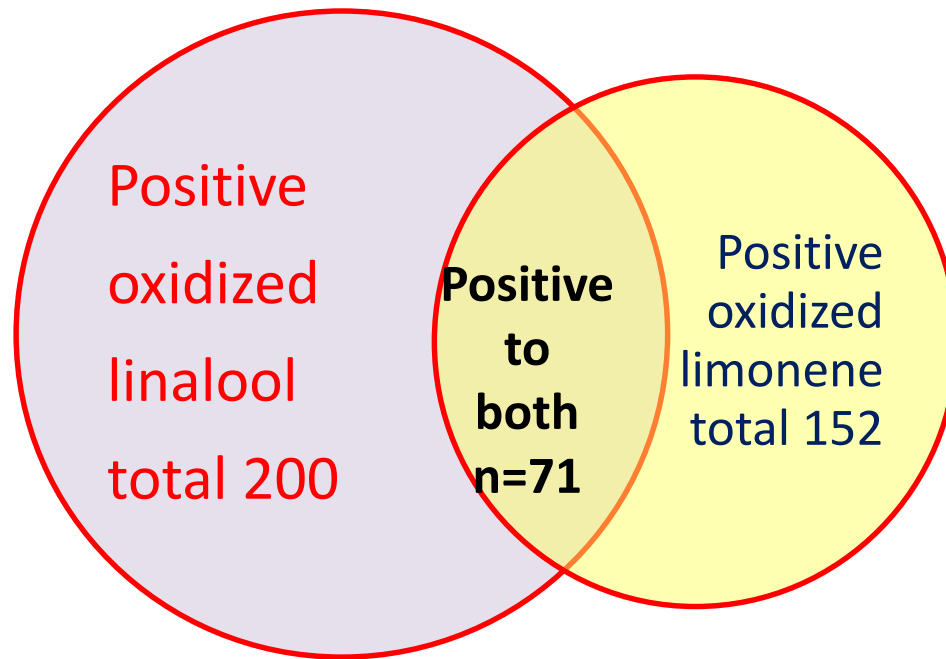
# Non-specific reactivity between ox. Limonene and ox. Linalool?

- A total of **281** patients reacted (+, ++, +++) to either oxidized *R*-limonene or oxidized linalool.
- **75%** of the patients reacted **only to one** of the oxidation mixtures, thus supporting the specificity of the reactions.
- **2619** patients did **not** react

Bråred Christensson J et al. Manuscript in preparation



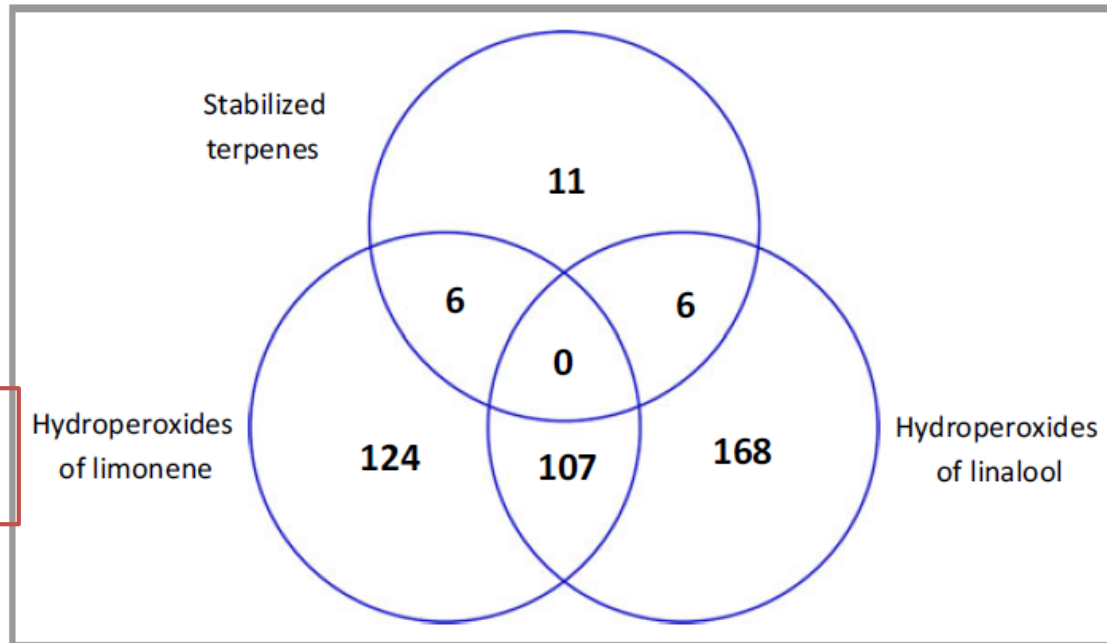
25% reacted to both - 75% reacted only to one



"Tandem exposure"

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

26% reacted to both -74% reacted only to one



Ox. limonene 3%  
containing  
Lim-OOHs 0.33% pet

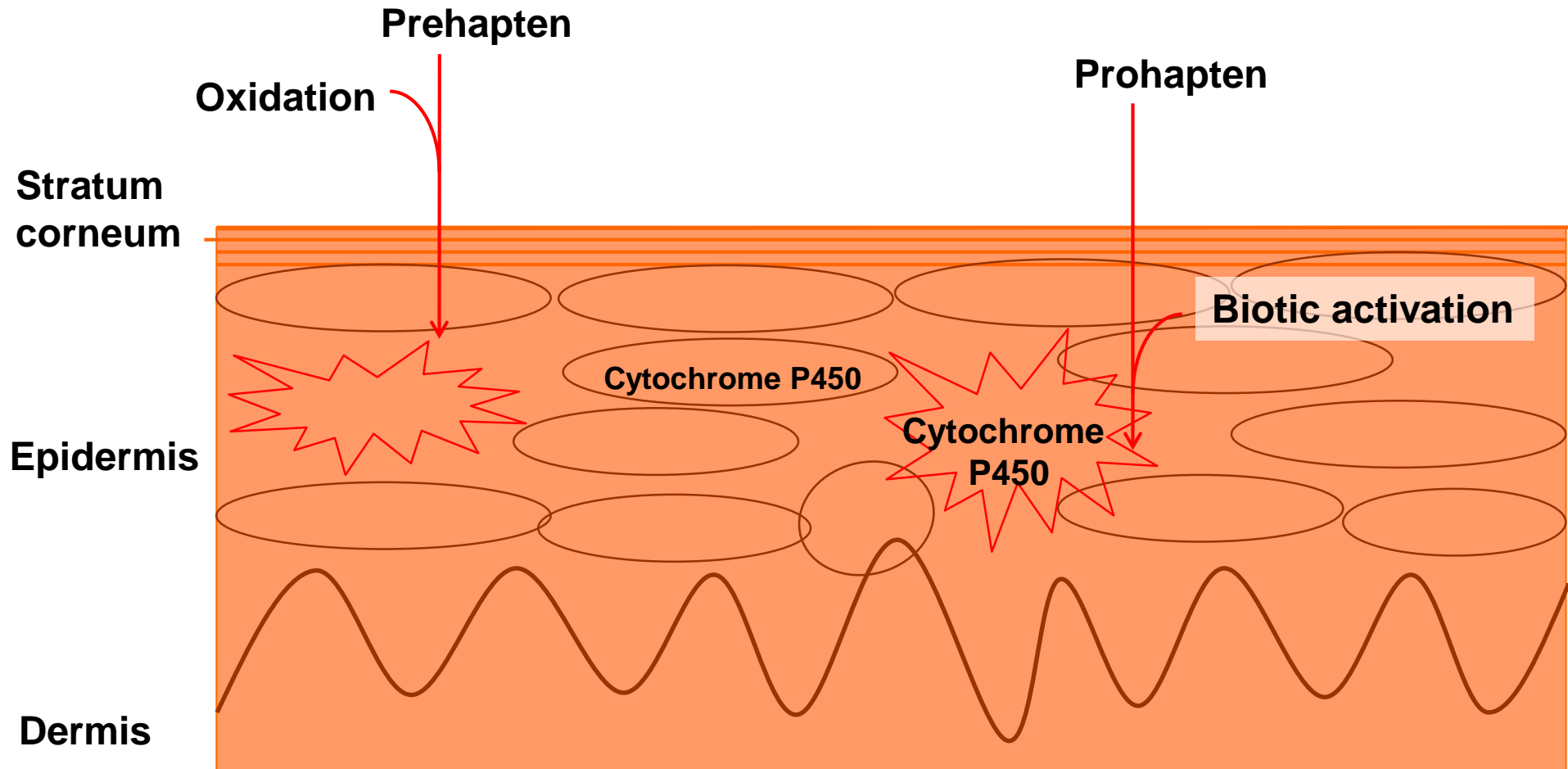
Ox. linalool 6%  
containing Lin-OOHs 1%

Fig 1. Venn diagram showing the relationship between allergic reactions to the stabilized terpenes and hydroperoxides of linalool and limonene in 4731 patients tested (422 positive reactions in total).

Audrian et al. British Journal of Dermatology 2014; 171: 292–297



# Activation of pre- and prohaptens to sensitizers - Cross Reactivity





# High frequency of concomitant reactions to citral and geraniol

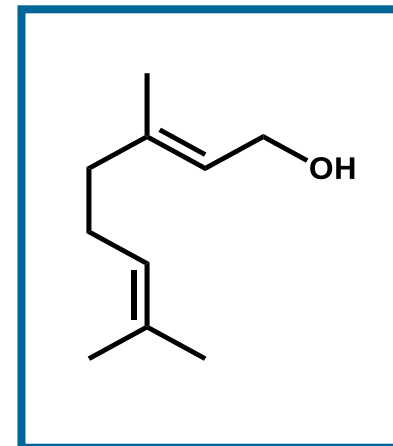
- German multicentre study<sup>1</sup>
- 2021 patients patch tested with both geraniol and citral
- 83.3% of citral-allergic patients reacted also to geraniol

<sup>1</sup>Schnuch et al. Contact Dermatitis 2007;57;1-10



Are citral and geraniol activated to the same haptens?

# Geraniol

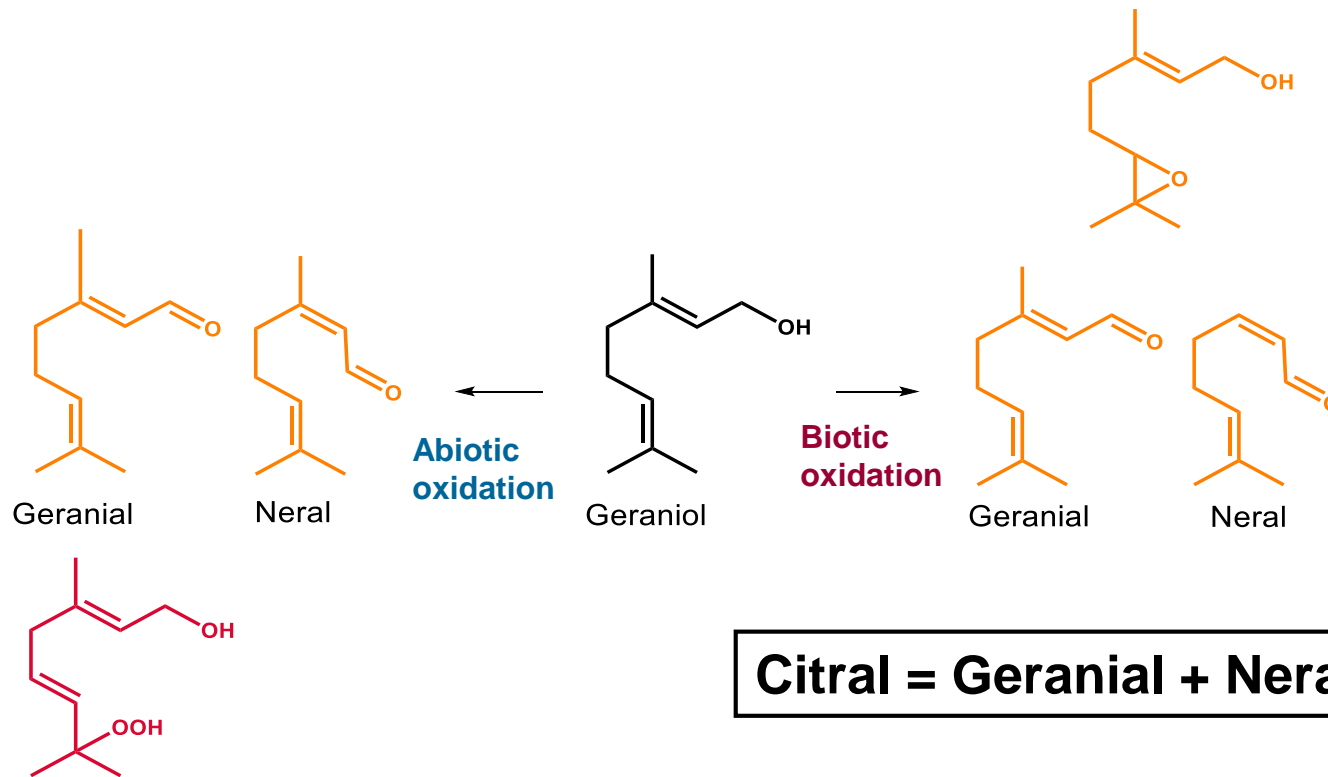


- Geraniol is both a pre- and a prohaptan
- A hydroperoxide and sensitizing aldehydes are formed in autoxidation of geraniol<sup>1</sup>
- Sensitizing aldehydes and epoxides are formed in metabolic activation of geraniol in the skin<sup>2</sup>



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

# Sensitizers formed in the activation of geraniol



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 Air Exposed Geraniol

## Clinical Observations and Report of 14 Cases

Patient number		1	2	3	4	5	6	7	8	9	10	11	12	13	14
Age		20	15	40	44	51	12	76	54	49	54	31	23	55	52
Sex		F	M	F	M	F	M	F	F	F	F	F	M	M	F
Geraniol	2.0%	-	++	-	++	-	+	-	-	-	-	-	-	-	-
Ox. Geraniol	2.0%	++	++	-	++	nt	+	++	+	++	-	+	++	++	+
Citral	1.5%	-	++	-	++	+	+	++	-	++	+	nt	-	nt	nt
Geranial	1.0%	-	++	+	++	+	+	++	-	nt	nt	nt	nt	nt	nt
Geranial	1.5%	nt	nt	nt	nt	-	nt	nt	nt	++	++	+	-	++	+
Neral	1.0%	-	++	-	++	+	?	++	-	nt	nt	nt	nt	nt	nt
Neral	1.5 %	nt	nt	nt	nt	nt	nt	nt	nt	-	-	+	nt	nt	nt
FM	8.0%	+	+++	-	++	+	+	+++	+	++	++	+	++	+	-
FM II	14%	nt	nt	-	++	nt	-	-	+	++	++	+	++	+	+
Dermatitis location		legs, face	axillae, perioral	axillae, face	hands	face	general	legs	hands	hands, face	hands	hands, feet	neck, arms	general	hands

Hagvall L. et al. Contact Dermatitis 2012;**67**: 20–27



Patient number		1	2	3	4	5	6	7
Age		20	15	40	44	51	12	76
Sex		F	M	F	M	F	M	F
Geraniol	2.0%	-	++	-	++	-	+	-
Ox. Geraniol	2.0%	++	++	-	++	nt	+	++
Citral	1.5%	-	++	-	++	+	+	++
Geranial	1.0%	-	++	+	++	+	+	++
Geranial	1.5%	nt	nt	nt	nt	-	nt	nt
Neral	1.0%	-	++	-	++	+	?	++
Neral	1.5 %	nt	nt	nt	nt	nt	nt	nt
FM	8.0%	+	+++	-	++	+	+	+++
FM II	14%	nt	nt	-	++	nt	-	-
Dermatitis location		legs, face	axillae, perioral	axillae, face	hands	face	general	legs

Hagvall L. et al. Contact Dermatitis 2012;**67**: 20–27



Patient number		8	9	10	11	12	13	14
Age		54	49	54	31	23	55	52
Sex		F	F	F	F	M	M	F
Geraniol	2.0%	-	-	-	-	-	-	-
Ox. Geraniol	2.0%	+	++	-	+	++	++	+
Citral	1.5%	-	++	+	nt	-	nt	nt
Geranial	1.0%	-	nt	nt	nt	nt	nt	nt
Geranial	1.5%	nt	++	++	+	-	++	+
Neral	1.0%	-	nt	nt	nt	nt	nt	nt
Neral	1.5 %	nt	-	-	+	nt	nt	nt
FM	8.0%	+	++	++	+	++	+	-
FM II	14%	+	++	++	+	++	+	+
Dermatitis location		hands	hands, face	hands	hands, feet	neck, arms	general	hands

Hagvall L. et al. Contact Dermatitis 2012;**67**: 20–27



# Contact Allergy to Air Exposed Geraniol

## Clinical Observations and Report of 14 Cases

Patient number		1	2	3	4	5	6	7	8	9	10	11	12	13	14
Age		20	15	40	44	51	12	76	54	49	54	31	23	55	52
Sex		F	M	F	M	F	M	F	F	F	F	F	M	M	F
Geraniol	2.0%	-	++	-	++	-	+	-	-	-	-	-	-	-	-
Ox. Geraniol	2.0%	++	++	-	++	nt	+	++	+	++	-	+	++	++	+
Citral	1.5%	-	++	-	++	+	+	++	-	++	+	nt	-	nt	nt
Geranial	1.0%	-	++	+	++	+	+	++	-	nt	nt	nt	nt	nt	nt
Geranial	1.5%	nt	nt	nt	nt	-	nt	nt	nt	++	++	+	-	++	+
Neral	1.0%	-	++	-	++	+	?	++	-	nt	nt	nt	nt	nt	nt
Neral	1.5 %	nt	nt	nt	nt	nt	nt	nt	nt	-	-	+	nt	nt	nt
FM	8.0%	+	+++	-	++	+	+	+++	+	++	++	+	++	+	-
FM II	14%	nt	nt	-	++	nt	-	-	+	++	++	+	++	+	+
Dermatitis location		legs, face	axillae, perioral	axillae, face	hands	face	general	legs	hands	hands, face	hands	hands, feet	neck, arms	general	hands

Hagvall L. et al. Contact Dermatitis 2012:67: 20–27

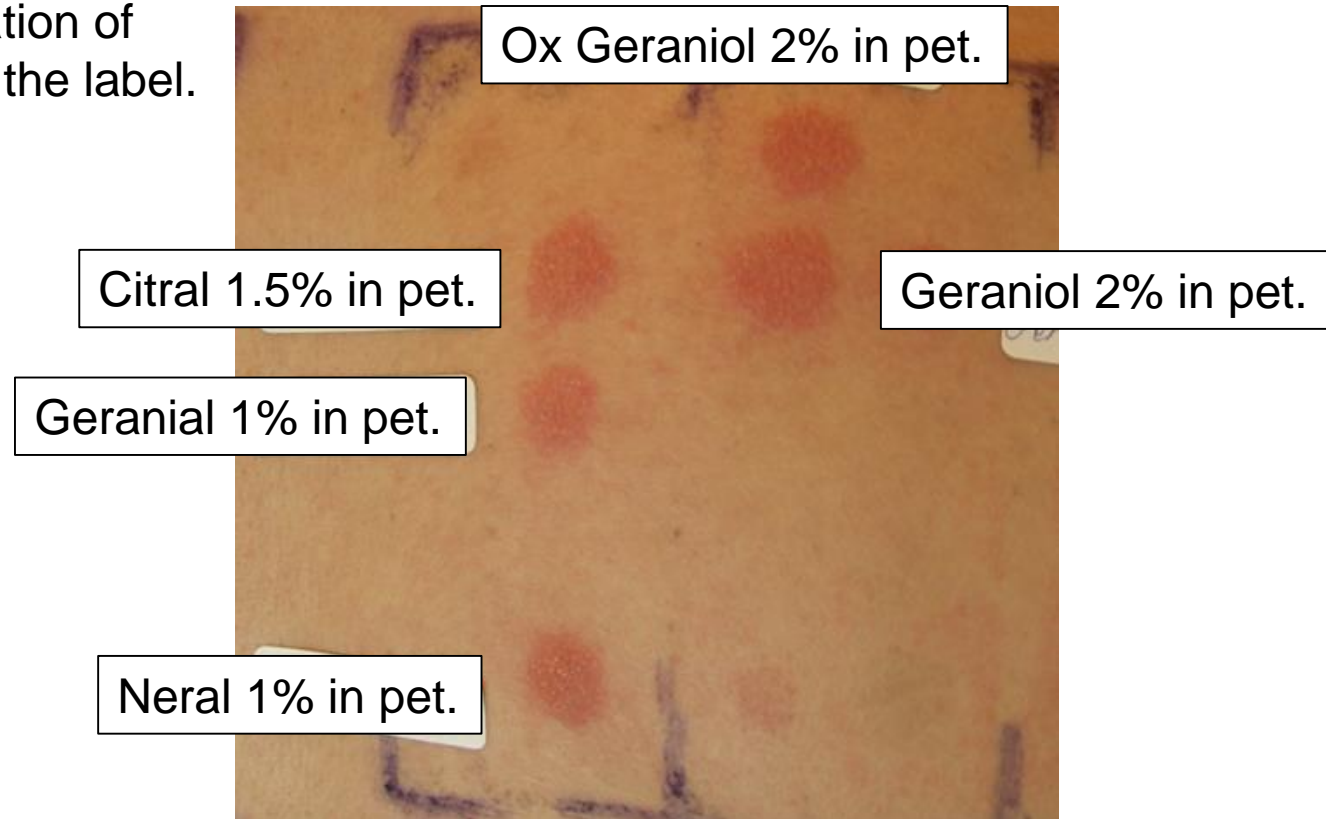
## Patient No. 2

20-year-old man, eczema of the axillae and the perioral region.

Pos. reactions to aftershave and deodorant.

Deodorant: citral and geraniol according to manufacturer.

Aftershave: no declaration of specific fragrances on the label.



# Cross-reactivity between citral and geraniol – can it be attributed to oxidized geraniol?

Lina Hagvall and Johanna Bråred Christensson

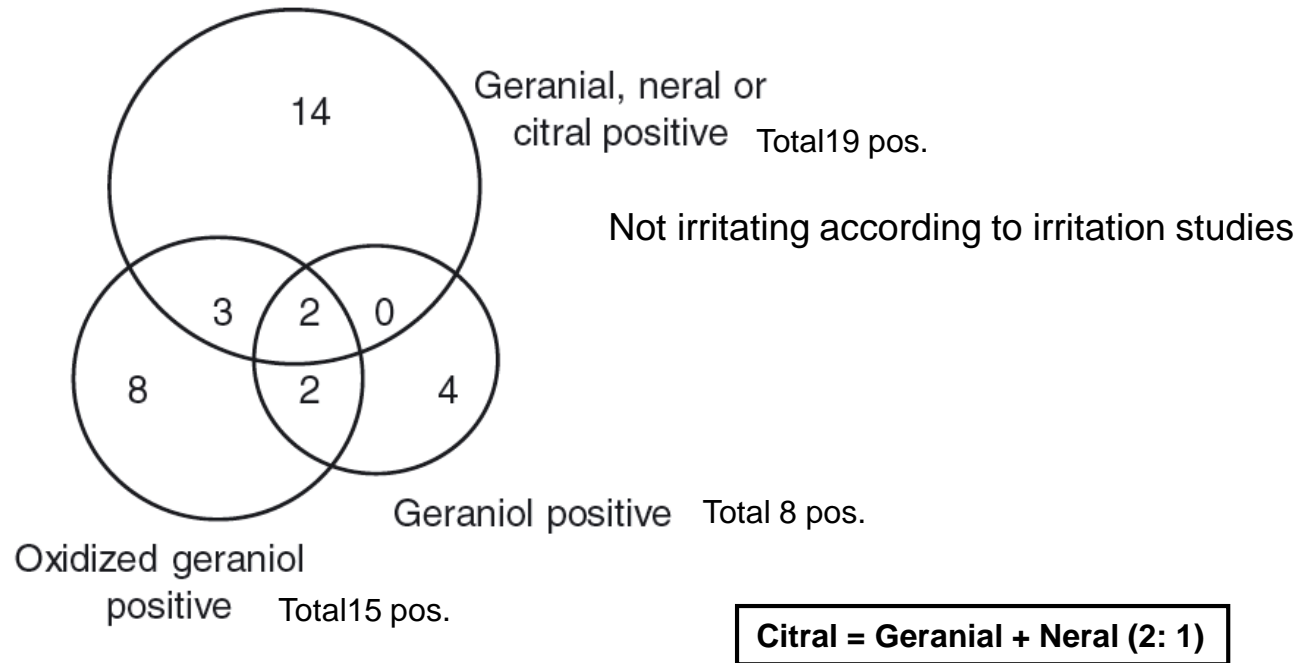
Test concentrations:

Geraniol 1.5% pet.

Neral 1.5% pet.

Citral 1.5% pet.

Pure Geraniol and Ox. Geraniol:  
4.0%, 6.0%, 11.0% pet.  
concomitantly



**Fig. 4.** Venn diagram representing the pattern of concomitant reactions to oxidized geraniol, geraniol and citral or its components among the 33 patients showing positive reactions to any of the test materials of the study.



# Thorough presentation of all cases

**Table 4.** Concomitant reactions to citral, geranial, and neral, as well as to fragrance markers of the baseline series, in patients with positive reactions to any of the test materials of the study; exposure to citral-containing or geraniol-containing products at the site of the patients' dermatitis has been used as an approximation of exposure to the fragrance components, including oxidized geraniol

Patient	Sex	Age (years)	Citral 3.5%	Geranial 3.5%	Neral 3.5%	Geraniol <sup>a</sup> 6.0%	Geraniol <sup>a</sup> 11.0%	Oxidized geraniol <sup>a</sup> 6.0%	Relevance	FM II	FM I	MP	HICC	Colophonium
1	F	18	-	+	-	-	-	?	-	-	-	-	-	-
2	F	28	-	+	-	-	-	-	-	-	-	-	-	-
3	F	57	-	+	-	-	-	-	R	-	-	++	-	-
4	M	61	-	+	-	-	-	-	-	-	-	-	-	-
5	F	63	-	+	-	-	-	+	R	?	+	++	-	-
6	M	67	++	++	+	++	++	++	R	NT	NT	+	NT	-
7	F	28	+	++	-	-	-	++	R	+	++	-	-	-
8	F	24	-	+	-	NT	-	-	-	-	-	-	-	-
9	F	81	+	++	+	NT	+	++	R	+	++	-	-	-
10	F	47	-	+	-	-	-	++	R	-	-	-	-	-
11	F	23	-	+	-	-	-	?	-	-	-	-	-	-
12	F	50	-	+	+	-	-	-	-	-	-	-	-	-
13	F	59	-	+	-	-	-	-	-	-	++	++	-	-
14	F	64	-	-	+	-	-	-	-	-	-	-	-	-
15	F	25	-	-	++	-	-	-	-	-	-	-	-	-
16	F	49	-	-	+	-	-	-	-	-	-	-	-	-
17	M	33	+	-	-	-	-	-	-	-	-	-	-	-
18	M	69	+	-	-	-	-	?	R	-	+	+	-	-
19	F	50	+	-	-	-	-	-	-	-	-	-	-	-
20	F	78	-	-	-	-	+	-	-	-	-	-	-	-
21	F	51	-	-	-	-	++	-	R	-	++	-	-	-
22	F	34	-	-	-	-	-	+	R	-	-	-	-	-
23	F	54	-	-	-	-	-	+	R	-	-	-	-	-
24	F	63	-	-	-	+	-	-	-	-	-	-	-	-
25	F	32	-	-	-	-	+	-	-	-	-	-	-	-
26	F	27	-	-	-	-	-	+	R	-	-	-	-	-
27	F	63	-	-	-	+	+	+	-	-	-	-	-	+
28	M	66	-	?	-	-	-	++	-	-	-	-	-	-
29	F	67	-	-	-	-	-	+	-	-	++	+	?	-
30	F	38	-	-	-	-	-	+	-	-	-	-	-	-
31	F	63	-	-	-	-	++	++	-	-	-	-	-	-
32	F	22	-	-	-	-	-	+	R	-	-	-	-	++
33	F	69	-	-	-	-	-	+	-	-	-	-	-	-

F, female; FM, fragrance mix; M, male; MP, *Myroxylon pereirae* resin (balsam of Peru); HICC, hydroxyisoheptyl 3-cyclohexene carboxaldehyde; NT, not tested; R, probable relevance of the reaction.

<sup>a</sup>Data previously published (28).

Hagvall L. et al. Contact Dermatitis 2014;71: 280–288

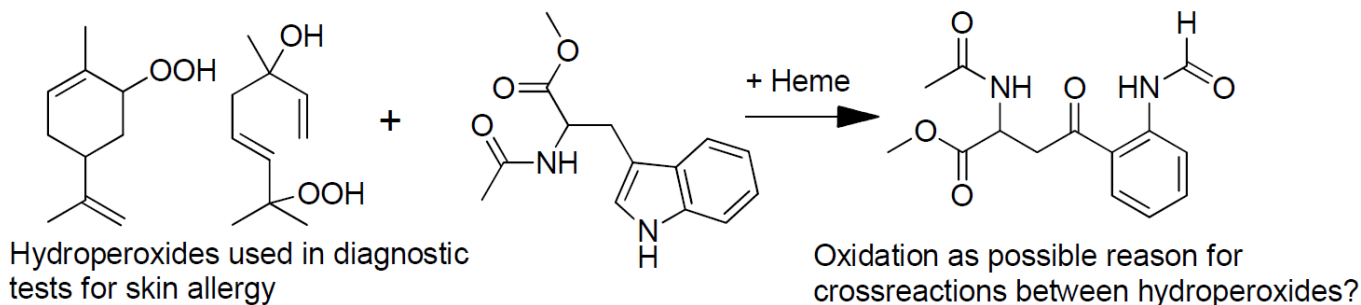
## Oxidative Tryptophan modification by terpene- and squalene-hydroperoxides and a possible link to cross-reactions in diagnostic tests

Andreas Natsch, Roger Emter, Remo Badertscher, Gerhard Brunner, Thierry Granier, Susanne Kern, and Graham Ellis

*Chem. Res. Toxicol.*, **Just Accepted Manuscript** • DOI: 10.1021/acs.chemrestox.5b00039 • Publication Date (Web): 05 May 2015

Hypothetically:

Table of content graphic





## Natsch et al.:

- "One possibility is that patch test to different terpene hydroperoxides do not only reveal a hapten-specific sensitization but rather a reactive state to other or multiples oxidizing agents."
- "It is important to be able to correctly interpret the results from diagnostic testig, since thousands of patients are currently being exposed to these hydroperoxides at high concentrations upon routine testing."
- "Indivual specific data are not made publically availabe."

No study with individual-specific data is discussed or referred to in this new publication from industry.



# Conclusions

- Cross reactions observed in clinic – only indicative since no control of exposure
- Animal study identified hydroperoxides as specific haptens
- Important to test with the real haptens formed from prehaptens or prohaptens (correct identity and dose)
- Clinical data show concomittant reactions to various markers for fragrance allergy – high simultaneous exposure
- One fragrance compound - oxidized to another common fragrance compound complicates the picture.
- Terpene hydroperoxides show specific reactions but are connected to reactions to other fragrance markers
- Only 25% of individuals reacting to ox. linalool or ox. limonene were positive to both.



# Gaps

- Are there more cross-reactivity studies in guinea pigs not shown?
- More experimental data on prohaptens and bioactivation needed
- How many of the fragrance compounds are pre-and prohaptens?
- Which compounds are changing into another known fragrance compound when activated abiotically and/or biotically?
- Investigations of fragrance mixtures - how much can the various fragrance compounds activate each other?
- What is happening on the skin?



# Guinea Pig Maximization Test

## Induction

## Test

Intradermal  
injections day 0

Epidermal  
application day 7

Patch testing  
day 22

Exposed animals

FCA + water  
Allergen in vehicle  
Allergen in vehicle + FCA

Allergen under  
occlusion for 48 h

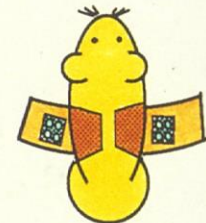
Epidermal exposure to  
the allergen for 24 h



Control animals

Same treatment but without allergen

Epidermal exposure to  
the allergen for 24 h



# Freunds Complete Adjuvance Test

Induction

Test

Intradermal injections days 0, 7, 14

Epidermal application day 7

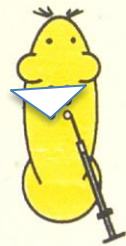
Patch testing day 21

Exposed animals

Allergen in FCA

Allergen under occlusion for 48 h

Epidermal exposure to the allergen for 24 h



Control animals

Same treatment but without allergen

Epidermal exposure to the allergen for 24 h

