A Risk Assessor's Perspective on Pre- and Pro-Haptens

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Risk Assessment considering pre- & pro-haptens

Toxicological questions:

- Are Terpene hydroperoxides (such as oxidized linalool or limonene) relevant for allergic contact dermatitis through use of cosmetic products?

Pre-clinical data
see also next slide:

Table 6: LLNA studies based on different analytical grades of pure linalool

Test material	Concentration of the test material (pure linalool)	SI	EC3
Linalool (commercial)	25%	2.5	30%
	50%	4.8	
	100%	8.3	
Linalool (purified, redistilled)	25%	2.1	55%
	50%	2.9	
	100%	4.9	

RAC 2015

Exposure:

- different markets across EU have identified the concentration of linalool in consumer products to between approximately 0.001% and 0.35%)
- Linalool together with limonene, has been identified as the most ubiquitous fragrance in cosmetics among the 26 fragrance substances to be labelled in the EU (SCCS, 2012)

Clinical data:

- Testing of oxidized limonene/linalool revealed 281 of 2900 consecutive patients reacted to either oxidized R-limonene (3.0% containing limonene hydroperoxides at 0.33%) and oxidized linalool (6% containing linalool hydroperoxides at 1%) Brared-Christensson et al., 2016
- Specificity of elicitation reactions? Defined hydroperoxides instead of oxidation mixture?

Pre-Clinical effects of Linalool, oxidized Linalool and Linalool hydroperoxide

LLNA

Sköld et al, 2004

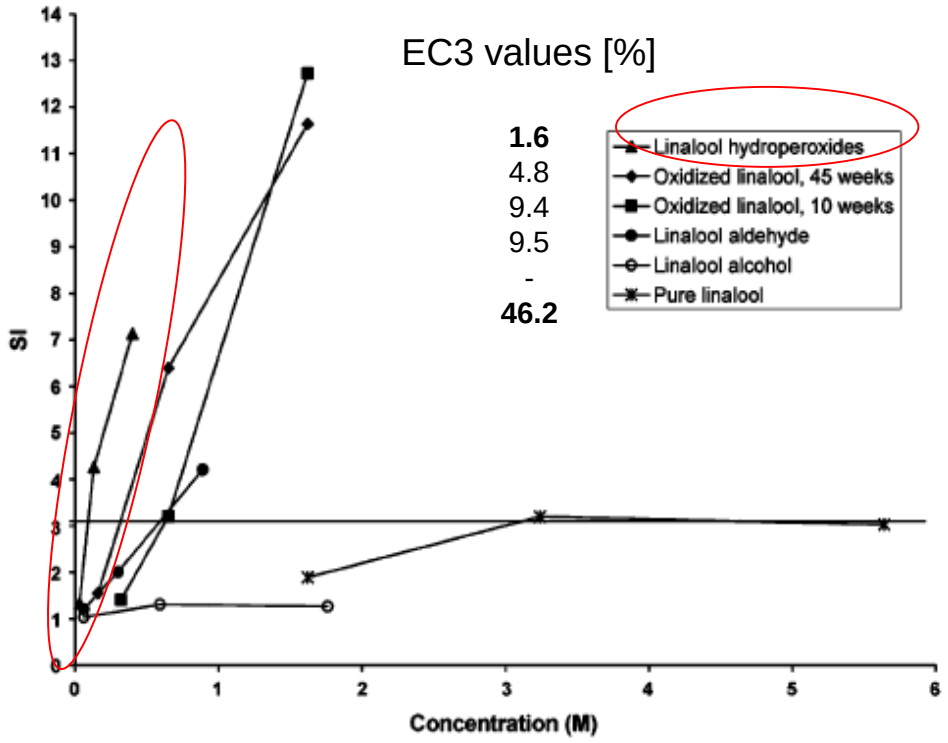

Figure 6. Dose response curves for the compounds tested in the local lymph node assay (LLNA). The concentrations are given in M. The molar concentrations of oxidized linalool are calculated using the molecular weight of linalool.

Consecutive patients

Brared Christensson et al., 2012

	Oxidized linalool 6.0% (Lin-OOHs 1%) pet.			
Test centre (9)	Total no. tested	No. of positive patch test reactions (%)	No. of doubtful patch test reactions (%)	No. of irritant patch test reactions (%)
Total	2900	200 (6.9)	266 (9.2)	37 (1.3)

Linalool Assessment by ECHA-RAC

RAC opinion

- average concentration of linalool oxides on aged (at least two years) commercial products not above 1.8%, average 0.6% Kern et al. (2014),
- test material for pre-clinical studies with oxidised linalool studies up to 19%.
- Likelihood of stabilized/non-stabilised linalool to reach high concentration of oxidation products is very low

Risk Assessment perspective

Abiotic transformation

- unlikely
  e.g. ECHA-RAC, Natsch et al 2019 in Food Chem Toxicol

Biotic transformation

- Can biotic transformation be a relevant cause of allergic contact dermatitis through the use of cosmetic products?

View on further needs:

- Assess possible routes of bioactivation of linalool/limonene in skin - confirm that activation is unlikely in healthy skin – consider 'danger' skin conditions (e.g. inflammation)
  - evaluate if under any conditions results support or question clinical findings

Activation in the skin possible?

Cysteine Peptide Depletion of **Geraniol** based on horseradish *peroxidase* (HRP)

Depletion	- HRP	+ HRP
%	2.4	9.3

Gerberick et al., 2009

- *protein reactivity is key step in skin sensitization development*
- *peptide reactivity provides indication of a sensitizing potential*

Is geraniol activation relevant for linalool?

SUMMARY/OUTLOOK

Data generation is recommended to close gaps regarding
- bio-activation possible/relevant?
- relation to inflammatory skin conditions?

Risk assessment goal: pre-clinical data and clinical data are in accordance

EXAMPLE

Pre-Clincial data (Goebel et al 2012, 2014)

Hair dye precursor	**Consumer exposure** hair dyeing event (MEL; µg/cm²)	**Allergy induction threshold** (NESIL; µg/cm²)	**Comparison**	**Associated with Contact Dermatitis among hair dye users**
PPD	16.1	27.5	Same order of magnitude	**yes**
PTD	22.7	41.6	Same order of magnitude	**yes**
Resorcinol	3.1	350	Consumer exposure << Induction threshold	**no** relevant hair dye allergen

Clincial data (Sosted et al 2013)

Table 2. Results from patch testing of 27 hair dye ingredients in 2939 consecutive patients

All centres	Substances	Chemical cluster (A)	No. tested	No. positive +	++	+++	Total (%)	No. doubtful (%)	No. irritant (%)
	m-Aminophenol	5	2939	12	13	4	29 (1)	13 (0.4)	1 (0.03)
	p-Aminophenol	5	2939	17	23	13	53 (1.8)	121 (4.1)	3 (0.1)
	Resorcinol	6	2939	0	2	1	3 (0.1)	16 (0.5)	0
	PTD	8	2939	25	33	25	83 (2.8)	103 (3.5)	11 (0.4)
	PPD	8	2939	47	49	37	133 (4.5)	119 (4.0)	0

Back up slides

Bio Activation?

Cysteine Peptide Depletion of **Geraniol** based on horseradish *peroxidase* (HRP)

Depletion	- HRP	+ HRP
%	2.4	9.3

Gerberick et al., 2009

- *protein reactivity is key step in skin sensitization development*
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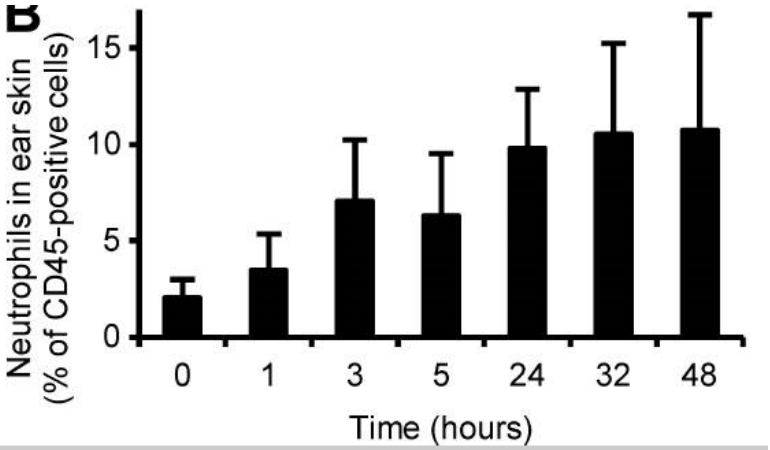
Is geraniol activation relevant for linalool?

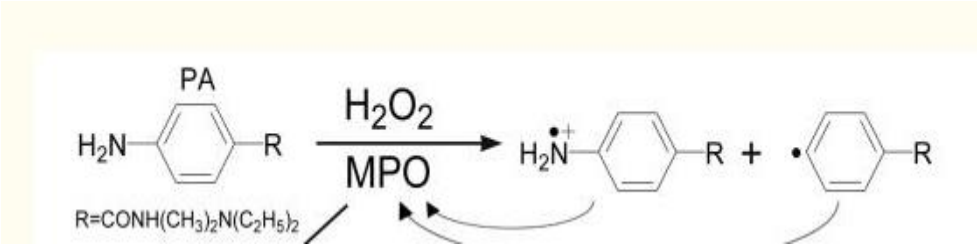
Role of neutrophils/MPO in activation of low molecular weight chemicals:

Infiltration of neutrophils to mouse ear after sensitization with TNCB



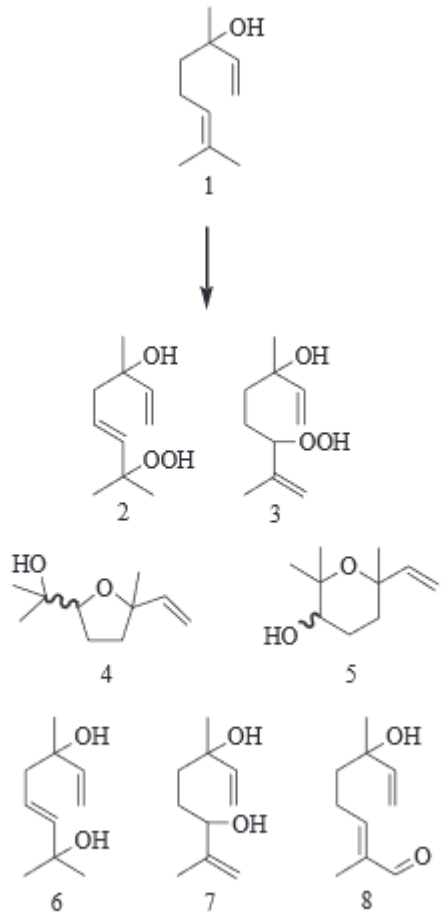
Weber et al., 2015

Activation of procainamide by MPO



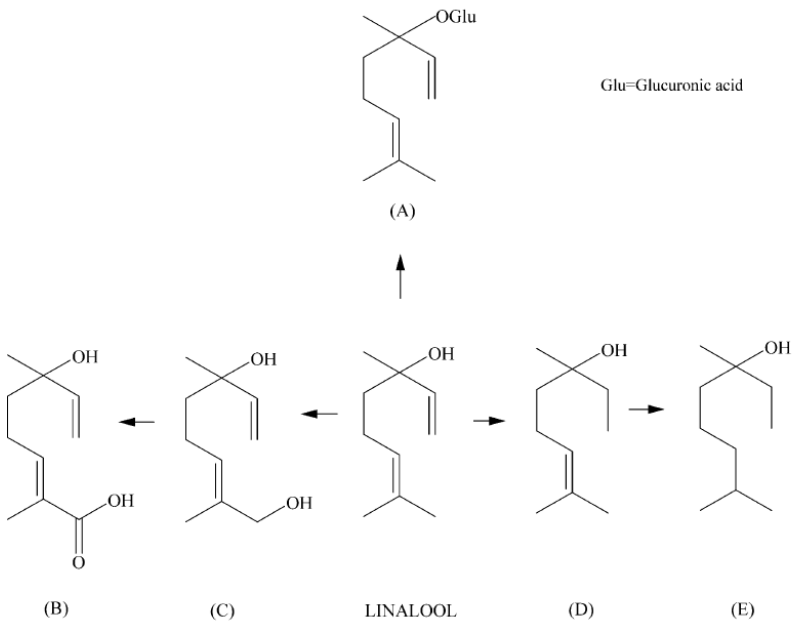
Siraki et al., 2008

Linalool autooxidation

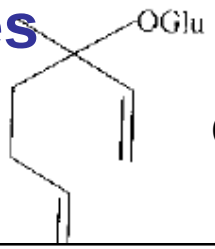


ig. 1. Identified oxidation products formed by autoxidation of

Linalool metabolism in rats: Urine metabolites



Linalool metabolism in rats: Urine metabolites



Glucuronidation (single dosing)

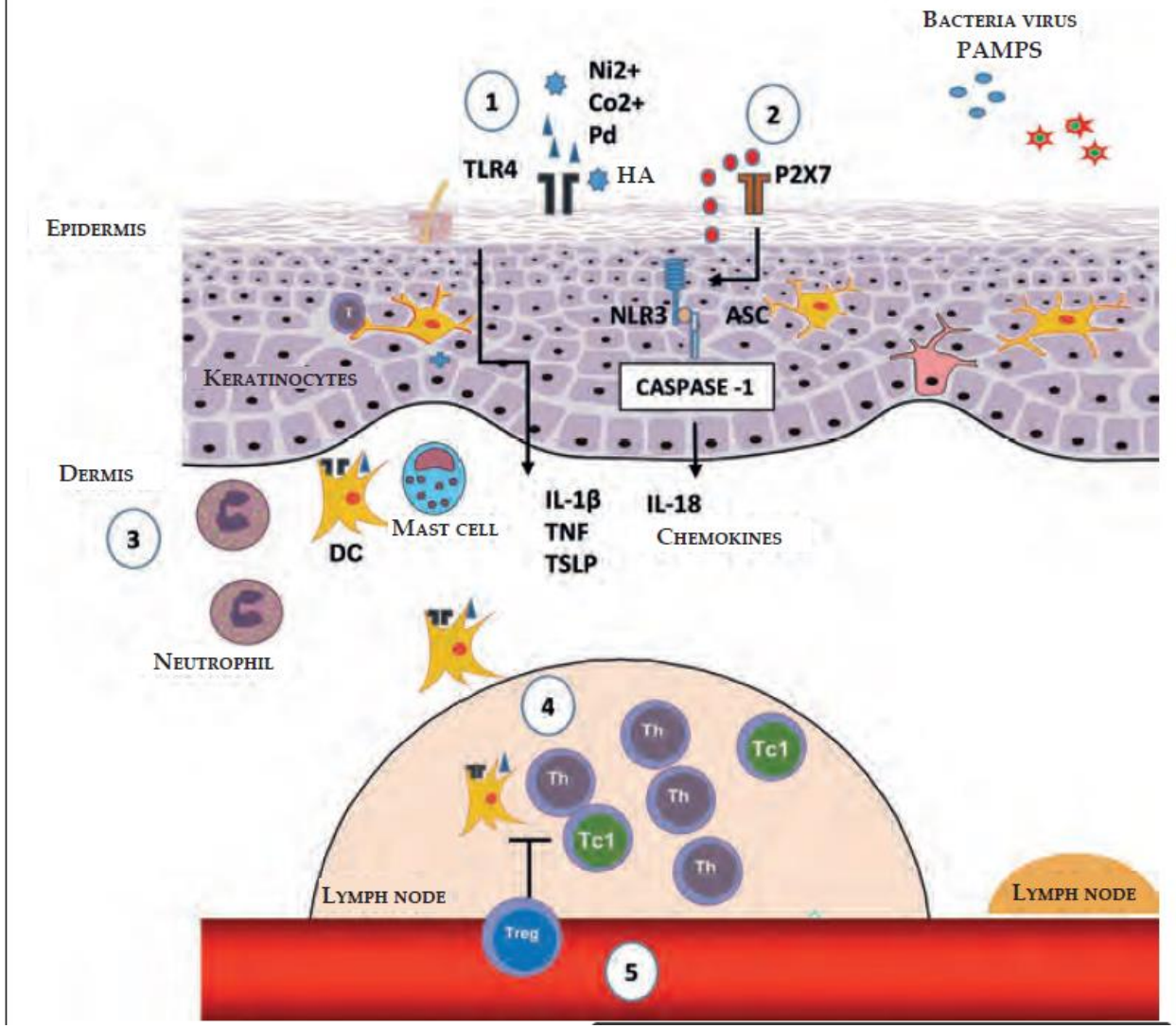
Conclusion on metabolism:

Linalool undergoes systemic glucuronidation/hydrolysis as well as hydroxylation

Skin?

(B) (C) LINALOOL (D) (E)

Fig. 2. Metabolism of linalool in mammals. These metabolites have been identified in the urine of rats after treatment with linalool by the oral route (JECFA, 1999). The major metabolite (A) was the glucuronic acid conjugate identified after a single dose. Metabolite B (8-carboxylinalool) and metabolite C (8-hydroxylinalool) were identified after treatment for 20 days. Metabolite D (dihydrolinalool) and metabolite E (tetrahydrolinalool) were identified after a single dose.


BACTERIA VIRUS
PAMPS
1
Ni2+
Co2+
Pd
TLR4
HA
2
P2X7
EPIDERMIS
NLR3
ASC
KERATINOCYTES
CASPASE -1
DERMIS
3
DC
MAST CELL
IL-1β
TNF
TSLP
IL-18
CHEMOKINES
NEUTROPHIL
4
Th
Tc1
Th
Th
Tc1
Th
LYMPH NODE
LYMPH NODE
Treg
5

Questions?

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