Minimization of pre-hapten conversion (focus on oxidation) to haptens by improved formulation, storage and packaging

17.6.2015

Givaudan

engage your senses

Hydroperoxide formation in products and formulation parameters

1. **Background what we know and what we do not know**

2. **Constraints for oxidation:**
   1. Availability of Oxygen
   2. Presence of antioxidants
   3. Competing ingredients form product base

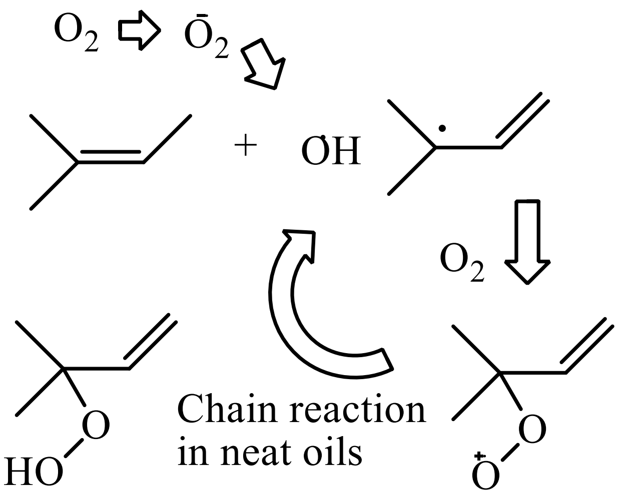
3. **Can we learn from oil oxidation in food and cosmetic products?**

4. **What do we know on terpene hydroperoxide formation?**
   1. Experience from neat products and essential oils
   2. Experience from formulated products

Background

- Fact is: Many fragrance components derived from terpenes can undergo oxidation if
  - Saturated with air/oxygen by repeated strong stirring in presence of air
  - If stored under these conditions as neat oils, not diluted in a product matrix

- Very limited information is available
  - What happens if the ingredients are diluted into a product matrix
  - What happens under typical oxygen availability of a formulated product



Constraints for oxidation: Oxygen availability in neat products - theory

- Oxygen availability depends on packaging
- **Pressurized sprays / aerosols:** very limited /no oxygen
- **Pump sprays**: Headspace, increases during product use
- **Creams and body lotions**: headspace in partly used products

- **Stochiometry – theory pump spray:**
- 100 ml bottle – 50 ml used, 50 ml headspace
  - 0.44 mmol O_2
- 10% perfume, 20% with oxidizable double bonds
  - 6 mmol oxidizable product (1 g)
- 7 % oxidation **theoretically** possible with one renewal of headspace.
- **Availability of oxygen in partly used products appears not a limiting factor**

Constraints for oxidation: Presence of antioxidants

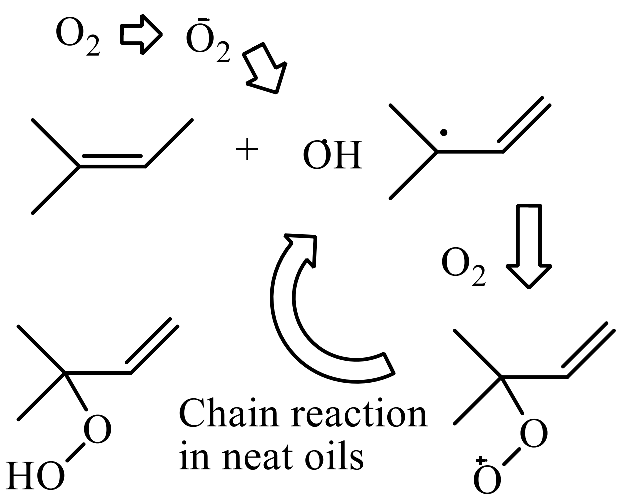
- Antioxidants are routinely added to cosmetic products
- A) **Enhance stability of product – perfume and oils contained in base**
  - Use of antioxidants in cosmetic products for stability is mainly based on empirical grounds
  - Mainly decided upon by **sensorial attributes** (avoid color change, avoid off-odors, odor change)
  - Normally not based on any analytical measurement of oxidation in final product
- B) **Cosmetic benefits**
  - Many cosmetics contain Vitamine E, C and other antioxidants
  - Main reason to improve skin health and appearance
  - These antioxidants also may enhance product stability as 'side effect'

  - **I am not aware of detailed chemical / analytical knowledge of antioxidant effect on hydroperoxide formation in final cosmetic products**

Constraints for oxidation: Effect of competing products in product base

- In concentrated (essential) oils or pure chemicals – each formed radical can again abstract electron from double bond in other parent molecules
- Therefore chain reaction leads to progressive consumption (often exponential)
- In product formulation, base ingredients may consume radicals - shorter lived radicals may not 'find' new parent molecule for propagation

- **Difficult to predict in complex bases – the only thing we know is that oxidation will be dramatically different once molecule enters formulation**.



Can we learn from oil oxidation in food and cosmetic products?

- Fact is: Oils containing PUFA in complex food matrices are prone to oxidation
  - This is widely studied
- Fact is: Experimental emulsion and liposomes from PUFA containing oils are widely used as experimental model to study antioxidant effects
  - In most of these studies, oxidation is initiated, e.g. by radical forming organic compounds, metals, photosensitizers
  - These studies clearly show that in case of PUFA, oxidation does happen in experimental emulsions
  - Although some of these experimental emulsions are a good model for a cosmetic product, these studies do not directly teach us whether oxidation happens in real cosmetics
- **BUT: I found no published analytical data on hydroperoxide formation from PUFA / other unsaturated fatty acids in formulated cosmetic products**
  - Cosmetic companies may have internal data
  - If available, They were not publsihed / not found

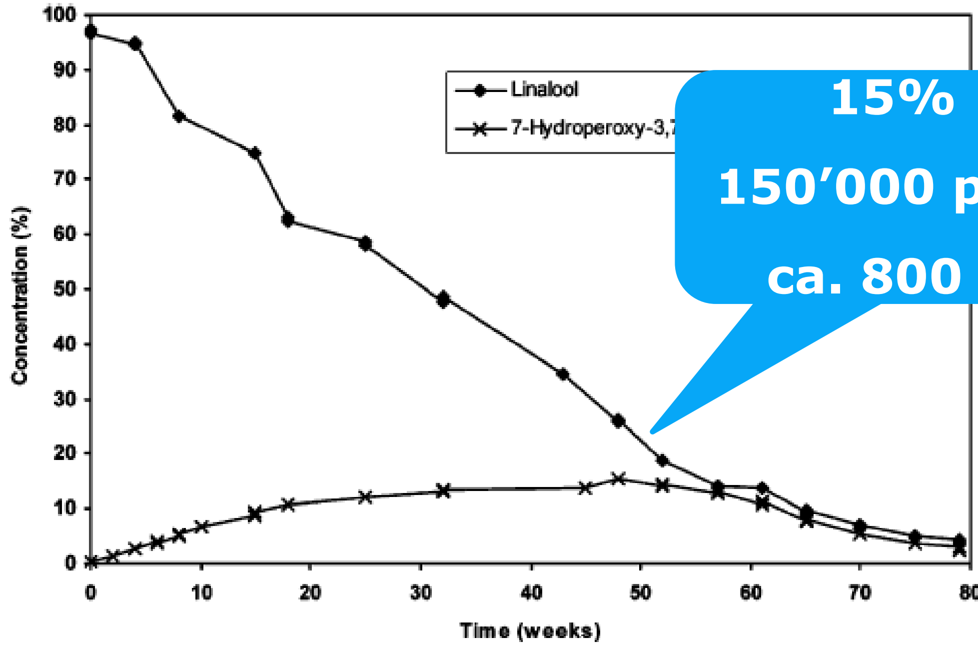
What do we know on terpene hydroperoxide formation in products ?

- **VERY LITTLE**

- **But not nothing.....**

Experience from neat products and essential oils

- Under air saturation, complete degradation of Linalool, with formation of primary and secondary oxidation products
- Similar effects for citronellol, geraniol, linalyl acetate, lavender oil.
- This oxidation protocol initially developped in 1991 to' mimic industrial handling of limonene (as a solvent)', originally not related to fragrance industry



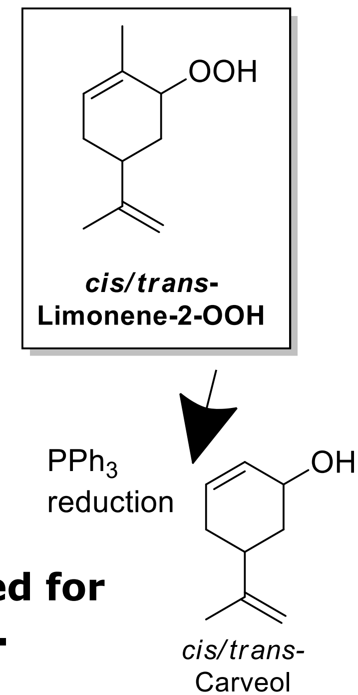
15% = 150'000 ppm = ca. 800 mM

Figure 2. Concentrations of linalool and the major hydroperoxide 7-hydroperoxy-3,7-dimethylocta-1,5-diene-3-ol **4** in air-exposed linalool, over time. Quantification of linalool was performed with GC using the on-column technique. For the hydroperoxide, HPLC was used.

Air Exposure Procedure. Linalool (Lancaster) was air-exposed in an Erlenmeyer flask, covered with aluminum foil to prevent contamination. It was stirred for 1 h, four times a day for 80 weeks, as previously described (*13*). Samples were taken

Excurs – analysis of hydroperoxides

- **Iodometric titration**: IFRA quality control method –– measures oxidation of iodide.
  - Standard method used in fat oxidation studies
  - Sensitive – detects all peroxides.
  - Further validation is now possible based on synthetic references
  - Not selective – detects different (hydro)peroxides, and potentially other oxidants
- **HR-LC-MS of the hydroperoxide directly**
  - Selective and sensitive.
  - Feasabilty shown in complex products for linalool-OOH
  - Difficult / not useful for Limonene-OOH in complex products
- **Reduction to alcohol – followed by GC-MS**
  - Selective and sensitive
  - May give overestimation due to alcohol already in product
  - **I learned on Monday's meeting from a food chemist that it is used for quantitative analysis of PUFA-OOH – se we reinvented the wheel.**

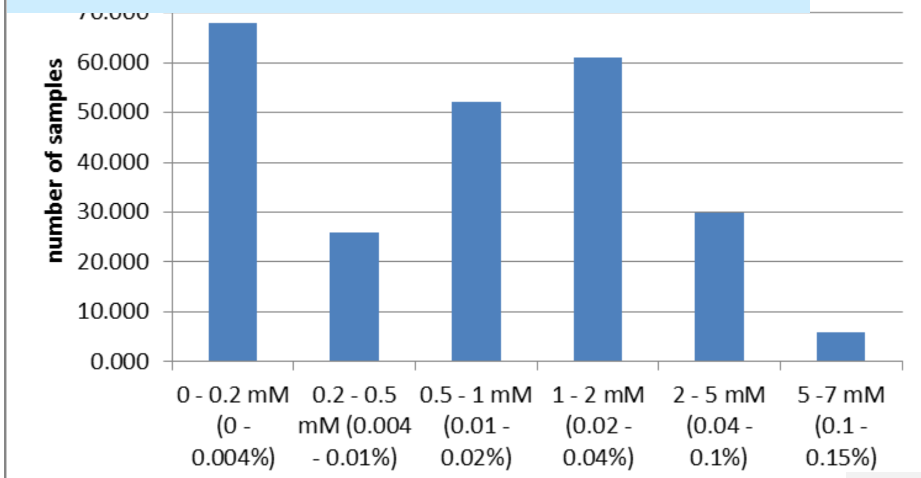


Peroxide levels along the fragrance value chain: Raw materials

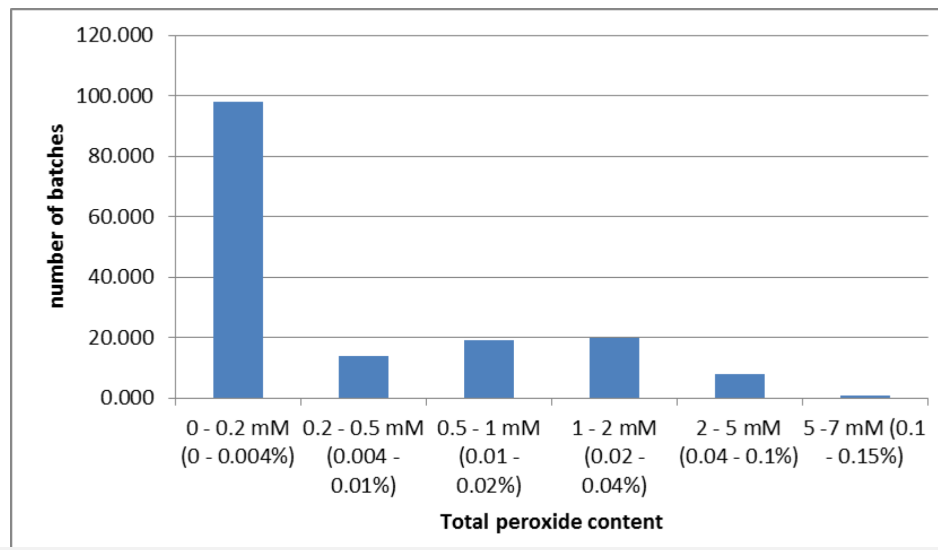
- Raw materials are screened to comply with IFRA standards before added to fragrance compound / fragrance oil

1.1 mM = 180 ppm
Top quartile: 0.6% limonene in product (EDT)
Top quartile: 2.8 mM in raw materil
Gives **2.8 ppm in final eau de toilette**
i.e. Level of Lim-OOH typically added to commercial product

	Linalool Synth.	Orange terp.
average	**0.46 mM**	**1.10 mM**
median	**0.00 mM**	**0.80 mM**
n	160	243



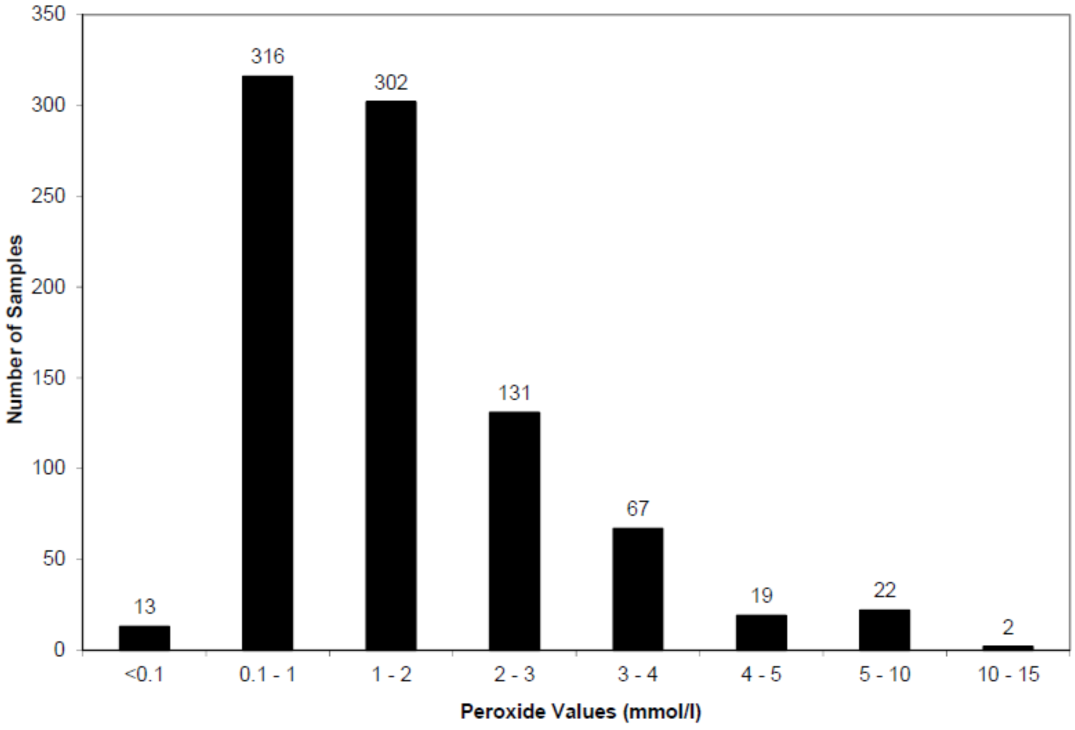
- Synthetic linalool (main source of commercial linalool)



Source: Givaudan raw material quality control, 2013

Peroxide levels along the fragrance value chain: Fragrance oils

- In fragrance oils, peroxides from all possible oxidized raw materials and also from other materials which may give a signal in the iodometric titration are summed up
- With this method, similar low levels of peroxides as in the raw material quality assessment are found-



	fragrance oils
Mean	**1.66 mM**
Median	**1.31 mM**
n	875

Source: Takasago / RIFM study on peroxide formation along the value chain

Peroxide levels along the fragrance value chain: Hydroalcoholics

- Hydroalcoholics = fine fragrances as used by consumers
  - In final fragrance, all peroxides from fragrance oil and potential base ingredients are summed up
  - This level cannot be related to the level of a single ingredient
- Still this first study with the titration method indicated only a slight and non-significant (p=0.2) increase from moment when samples are sold to typical levels in old products used by consumers.
  - Caveat: Not tested on same sample – two unrelated random samples

	Freshly bought	Retrieved from consumers
Average	**1.01 mM**	**1.39 mM**
Median	**0.86 mM**	**0.82 mM**
n	34	80

Source: Takasago / RIFM study on peroxide formation along the value chain

Effects on formulation parameters: controled study

- Again little information available: Mainly from our studies on stability in fine fragrance
- Different parameters screened: Temperature, antioxidants, headspace air, bottle opening
- **No effect for these parameters – high stability in all conditions**

Table 2 Stability of pure linalool formulated as a hydroalcoholic fragrance in a 2-month standardized stability test

Linalool type	Storage temperature (°C)	Stabilizers	Half full	Half full/opened	Linalool (μg/g)[a]	Linalool7-hydroperoxide (μg/g)[b]	*cis*/*trans*-Linalool oxide (μg/g)	7-Hydroxylinalool (μg/g)
Synthetic	45	+			105,091±33	<LOD	3.6±0.2	<LOQ
Synthetic	45	+	+		105,978±7,708	<LOD	3.4±0.1	3.1±0.9
Synthetic	45	+	+	+	97,330±1,666	<LOD	3.7±0.2	3.6±0.4
Synthetic	5	+			100,003±1,405	<LOD	<LOD	<LOQ
Synthetic	45	-			100,008±2,032	<LOD	<LOQ	4.2±0.8
Synthetic	45	-	+		98,656±646	<LOD	<LOQ	3.7±0.8
Synthetic	45	-	+	+	104,931±2,552	<LOD	<LOQ	4.6±0.5
Synthetic	5	-			106,885±5,275	<LOD	<LOD	3.8±0.4

Formulation parameters – naturally derived linalool

- Naturally derived linalool contains higher hydroperoxide levels
- This quality also contains higher secondary oxidation products
- This is a niche product, less than 1% of industrially used linalool
- **Again not affected by any of the studied formulation parameters**

Table 2 Stability of pure linalool formulated as a hydroalcoholic fragrance in a 2-month standardized stability test

Linalool type	Storage temperature (°C)	Stabilizers	Half full	Half full/opened	Linalool (μg/g)[a]	Linalool 7-hydroperoxide (μg/g)[b]	*cis*/*trans*-Linalool oxide (μg/g)	7-Hydroxylinalool (μg/g)
Natural grade	45	+			100,344±2,587	63±0	332±32	36±4
Natural grade	45	+	+		102,854±4,314	64±5	352±14	43±0.2
Natural grade	45	+	+	+	105,429±7,797	64±3	355±15	44±2
Natural grade	5	+			102,966±1,067	60±3	347±0.2	41±5
Natural grade	45	-			93,930±1,309	60±5	339±0.6	38±4
Natural grade	45	-	+		105,421±1,589	70±5	364±0.7	40±1
Natural grade	45	-	+	+	110,298±545	74±1	391±17	39±2
Natural grade	5	-			98,059±10,779	70±9	287±2	33±5

Kern, S., et al.. Anal. Bioanal. Chem., 2014. 406(25): p. 6165-78.

Prolonged storage

- Samples with highest risk – repeatedly opened
- Study prolonged to 9 months
- More sensitive LC-MS method for hydroperoxide detection developped
  - Hydroperoxide detected in synthetic linalool
- **No effect of storage temperature or antioxidants**

Table 3 Detailed analytical results after 9 months' storage for linalool formulated as a hydroalcoholic fragrance

	Storage temperature (°C)[b]	Linalool (µg/g)[a,c]	Linalool hydroperoxide (sum of isomers) (µg/g)[d]	*trans*-Linalool oxide (µg/g)[c]	*cis*-Linalool oxide (µg/g)[c]
Synthetic linalool plus stabilizers	45	110,553±2,499	18±0.4	10±1.3	<LOD
Synthetic linalool	45	113,100±5,102	15±0.2	<LOQ	<LOD
Synthetic linalool plus stabilizers	5	103,531±1,152	14±0.2	<LOD	<LOD
Synthetic linalool	5	117,980±664	14±0	<LOD	<LOD

Kern, S., et al.. Anal. Bioanal. Chem., 2014. 406(25): p. 6165-78.

Prolonged storage – natural linalool

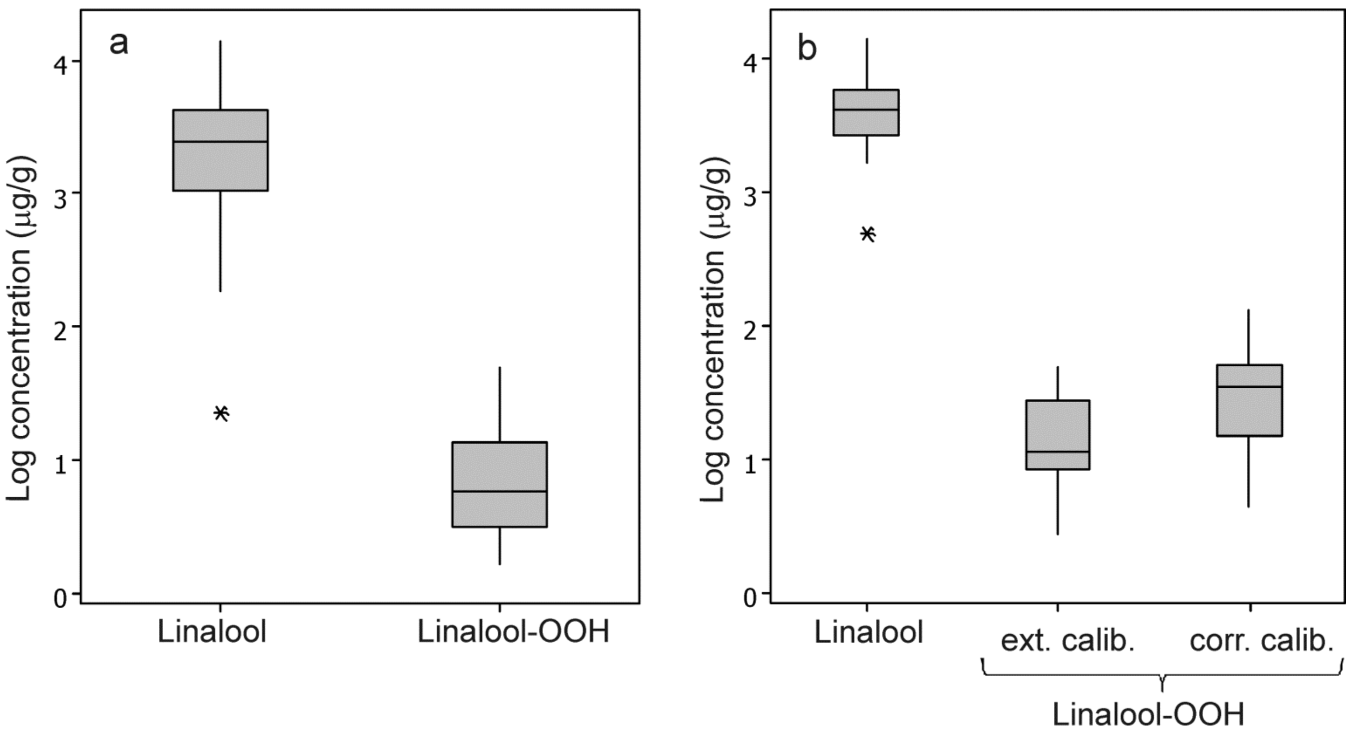
- Again higher levels in natural linalool
- No effect of temperature or antioxidants

Table 3 Detailed analytical results after 9 months' storage for linalool formulated as a hydroalcoholic fragrance

	Storage temperature (°C)[b]	Linalool (μg/g)[a,c]	Linalool hydroperoxide (sum of isomers) (μg/g)[d]	*trans*-Linalool oxide (μg/g)[c]	*cis*-Linalool oxide (μg/g)[c]
Natural linalool plus stabilizers	45	105,780±9,042	83±4	46±4	115±11
Natural linalool	45	107,732±5,033	83±4	49±4	29±5
Natural linalool plus stabilizers	5	108,424±2,403	97±0.1	20±2	75±0.1
Natural linalool	5	100,600±2,499	92±0.2	17±2	68±3

Linalool in fragrances aged 2 – 10 years in consumer homes

- Linalool hydroperoxide detectable in 33 of 39 fragrances
- Geometric mean **14 ppm** in positive samples, including matric effect (= 0.66% of linalool content)
- Maximal level in one sample 130 µg/g
- We do not know how much is formed in product



A: all 30 samples

B: 18 samples reanalized with spiking experiments

Kern, S., et al.. Anal. Bioanal. Chem., 2014. 406(25): p. 6165-78.

Limonene in 9 months stability study

- Partly filled, repeatedly opened bottles
- Parent limonene levels remains constant over 9 months stability study

	Storage Temp. [1)]	Theoretical limonene level (µg/g)	Detected limonene level (µg/g)
Fragrance B5	45°C	475 ± 47	428 ± 4
Fragrance B20	45°C	1900 ± 190	1976 ± 15
Fragrance B50	45°C	4750 ± 470	4935 ± 117
Commercial Fragrance D	45°C	990	840 ± 26
Fragrance B50	5°C	4750 ± 470	5037 ± 76
Commercial Fragrance D	5°C	990	922 ± 40

Kern, S., et al. Flavour Fragrance J., 2014. 29(5): p. 277-286.

Limonene-hydroperoxide in 9 months stability study

- No hydroperoxide found after 9 months stability study of limonene-containing fragrance
- No effect of storage parameters

Analyte	***trans*-carveol (µg/g)** [1)]		
Reduction	**No** PPh_3	**With** PPh_3 **reduction**	
Spiking agent	**none**	**none**	**115 µg/g *trans*-limonene-2-OOH**
Fragrance B5, 45°C	<LOD	<LOD	122 ± 5 [2)]
Fragrance B20, 45°C	<LOD	<LOD	125 ± 9
Fragrance B50, 45°C	<LOD	<LOD	122 ± 17
Commercial Fragrance D, 45°C	<LOD	<LOD	96 ± 1
Fragrance B50, 5°C	<LOD	<LOD	103 ± 11
Commercial Fragrance D, 5°C	<LOD	<LOD	112 ± 22

Carveol below limit of detection after PPh_3 reduction

Quantitative Carveol detection in spiked samples

All samples analyzed

⇒ **Method / negative result validated**

Kern, S., et al. Flavour Fragrance J., 2014. 29(5): p. 277-286.

Limonene in aged consumer fragrances

- 39 fragrances tested
- Limonene-OOH detected by reduction
- Only trace levels found (< 10 ppm)

Shown are the 10 samples with highest limonene content

Carveol detected in 9 of them

Successfull detection proven by spiking results

Analyte	Limonene (µg/g)	*trans*-carveol (µg/g) [2]		
Reduction		No PPh_3	With PPh_3 reduction	
Spiking agent		none	none	115 µg/g *trans*-limonene-2-OOH
Sample 31 (5) [1]	9343	2.8	4.9	123
Sample 26 (5)	8301	<LOD	<LOQ	130
Sample 24 (5)	7407	<LOD	<LOQ	124
Sample 7 (5)	6821	3.0	3.2	135
Sample 27 (5)	6748	1.7	3.9	112
Sample 37 (3)	6384	<LOQ	4.4	134
Sample 17 (2-3)	5941	<LOQ	2.8	134
Sample 30 (5)	5559	<LOD	<LOD	141
Sample 35 (7)	5152	1.9	1.7	138
Sample 33 (7)	5008	2.4	2.7	116

Terpene hydroperoxides in products – conclusion based on **the current state of the art**

- Conclusions below strongly affected by experience from hydroalcoholic products, and partly, antiperspirants / deodorants
  - i.e. products giving highest local fragrance exposure
- Currently we have no indication that oxidation takes place in final product
- Oxidation mainly takes place in essential oils and neat products
  - Low /trace levels of hydroperoxides may then come into products by formulation
  - These levels are quite stable
  - Levels tend to be higher when natural ingredients are being used
- Storage / product parameters have surprisingly little effect
- BUT: Of course proper formulation with clean raw materials is needed
- So far we cannot derive any need for additional antioxidants, fixed shelf-life and expiry date, etc.
- Question is whether other product types show a different picture

Key conclusion:

- A source of exposition of the general population to hydroperoxides derived from those prehaptens at toxicologically relevant concentrations is currently not kown

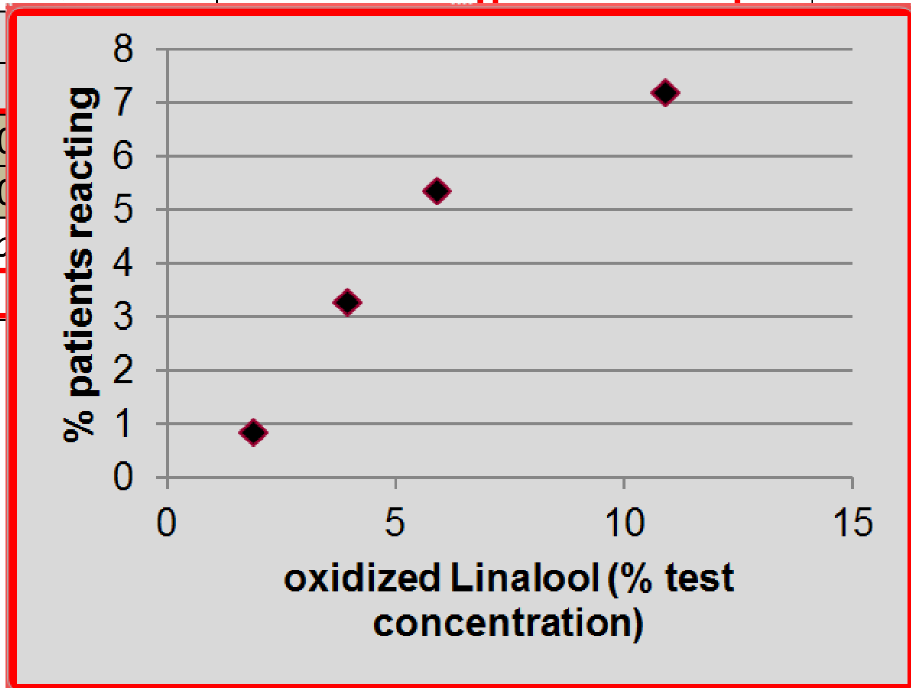
Thank you

Contact
Andreas Natsch
Givaudan Schweiz AG
andreas.natsch(at)givaudan.com

Studies in dermatological patients

Table 1. Literature review of positive and doubtful reactions to the terpene hydroperoxides

Study reference	N patients	Target hydroperoxide	Hydroperoxide level in the patch test preparation	% of positive /allergy skin reactions	% of doubtful / irritants
[1]	1693	Linalool-OOH	0.38%	0.83	1.9
[1]	2075	Linalool-OOH	0.76%	3.2	5.1
[1]	1725	Linalool-OOH	1.14%	5.3	6.4
[1]	1004	Linalool-OOH	2.1%	7.2	7.3
[2]	4731	Linalool-OOH	[illegible]	[illegible]	[illegible]
[3]	2800	Linalool-OOH	[illegible]	[illegible]	[illegible]
[4]	2800	Limonene-OOH	[illegible]	[illegible]	[illegible]
[2]	4731	Limonene-OOH	[illegible]	[illegible]	[illegible]
		Data for comp[illegible]			
[6]	*37270*	Lyral	[illegible]	[illegible]	[illegible]



⇒ **Dermatologists test at high concentrations –**

⇒ **high test concentrations lead to high numbers of reactions**

What does it all mean?
Analytical and literature data calculated as dose-per area

	Dose of hydroperoxide in test preparation	**Dose per area**
LLNA [a] Dose inducing sensitisation (EC3)	16‘000 µg/g (1.6%)	400 $\mu g/cm^2$
Patch test 2% oxidized linalool (**0.83% response**)	3’800 µg/g (0.38%)	152 $\mu g/cm^2$
Patch test 6% oxidized linalool**, diagnostic level**	10’000 µg/g (1%)	456 $\mu g/cm^2$
Patch test 11% oxidized linalool (7.2% response)	20’900 µg/g (2.09%)	836 $\mu g/cm^2$
Analytical data fine fragrance: median	14 µg/g (0.0014%)	0.031 $\mu g/cm^2$
Analytical data fine fragrance: (Max. value of n=39)	132 µg/g (0.0132%)	0.29 $\mu g/cm^2$

Levels in perfumes are 3 – 4 orders of magnitude lower as compared to levels in dermatological tests

Table 6 Comparison of analytical results with doses in clinical and animal studies expressed as micrograms per square centimetre of a single-dose application

	Dose of hydroperoxide in test preparation	Application density	Dose per unit area
LLNA[a] dose inducing sensitization (EC3)	16,000 μg/g (1.6 %)	25 mg/cm^2	400 μg/cm^2
Patch test 2 % oxidized linalool (0.83 % response)[b]	3,800 μg/g (0.38 %)	40 mg/cm^2	152 μg/cm^2
Patch test 6 % oxidized linalool (diagnostic level, approximately 6 % response)[c]	10,000 μg/g (1 %)	40 mg/cm^2	456 μg/cm^2
Patch test 11 % oxidized linalool (7.2 % response)[b]	20,900 μg/g (2.09 %)	40 mg/cm^2	836 μg/cm^2
ROAT 0.3 % oxidized linalool: LOEL for elicitation[e–g]	564 μg/g (0.056 %)	10 mg/cm^2	5.64 μg/cm^2
ROAT 0.1 % oxidized linalool: NOEL for elicitation[e,f,h]	188 μg/g (0.019 %)	10 mg/cm^2	1.88 μg/cm^2
Fine fragrance: (median of positive samples; with median matrix correction factor)	14 μg/g (0.0014 %)	2.21 mg/cm^2[d]	0.031 μg/cm^2
Fine fragrance: (single sample of $n=39$ with highest content including matrix correction factor)	132 μg/g (0.0132 %)	2.21 mg/cm^2[d]	0.29 μg/cm^2