Analysis of hydroperoxides in consumer products

Progress report on the work under the International Dialogue on the Evaluation of Allergens (IDEA)

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The question:

- High frequency of positive patch test reactions to oxidized terpenes are reported.
- No known consumer exposure source for sensitizing doses of hydroperoxides (HP) from ox. terpenes.
- Fragrance products are under suspicion – *is this assumption correct?*
- This question needs to be resolved by analytical methods

IDEA analytical taskforce: Mission

1. Provide methods to determine hydroperoxides formed from Linalool and Limonene
2. Validate methods for analysis of consumer products
3. Perform market surveillance to test **consumer/ patient exposure** from fragranced products

Key research steps:

- **Method evaluation**: methods from 6 laboratoires
- Two blind-coded ring trials for pre-selection and evaluation of methods
- **Method validation** by 2 **blinded ring trials**
- **Market surveillance** with validated method on **consumer products and products from patients**

1. Toolbox of methods

- GC-MS reduction method: Reduces HP to alcohols; alcohols are reliably measured in different bases
    - Widely available, robust
    - May lead to some overestimation
- Three LC-based methods: Directly detect parent HP, can be used for confirmatory analysis

2. Validation of methods by blinded ring-trials

- Two studies: a) fine fragrances and b) creams and lotions
- Blind-coded samples
- 4 laboratories used GC-MS reduction method in parallel
- 3 laboratories made confirmatory analysis by different LC-MS methods

Table 1: Detection of Linalool-OOH in Eau de Toilette (EdT) by **Toolbox of methods**

(data in µg/ml)	EdT No Spike	EdT Low Spike	EdT High Spike
LC-Q-TOF MS	0.0	90.0	279.0
HPLC-CL	0.0	79.5	310.7
LC-orbitrap-MS	0.2	95.7	398.7
Red-GC-MS	6.1	88.6	325.4
spike level added	0.0	92.0	322.0

Figure 1: Reduction **method validation In blind-coded ring trial**
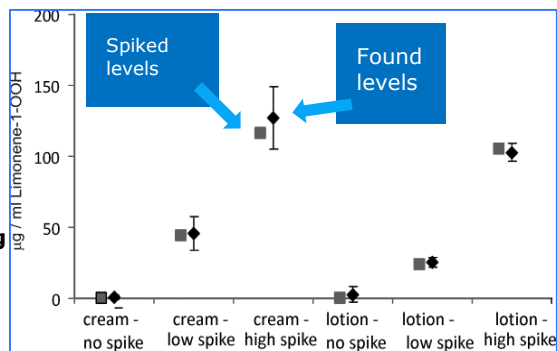
3. Market Surveillance study: Key findings

- 104 products analyzed by independent third party CRO
- Only one product with confirmed HP level > 50 µg/ml (i.e. 90 µg/ml limonene-1-OOH)
- **No HP detected in products from (patch test positive) patients**
- **No evidence for HP accumulation in aged samples**
- All results validated by standard addition

Market Sample: Dose-per area calculation:

- For the single sample with highest level (91 µg/g), exposure per area calculated
- Compared with reporting limit and with toxicological data and patch test dose

Example of a patient product

Sample and history of donating patient	Analytical methods	Limonene-1-OOH	Limonene-2-OOH	Linalool-7-OOH	Linalool-6-OOH	
O12, Body cream, Positive some fragrances, Positive Limonene ox	GC-MS red. (µg/ml)	<22	<22	<22	<22	⇐ Reduction method
	GC-MS red. (% recovery)	69%	70%	59%	84%	⇐ Spike recovery
	LC-Orbitrap-MS (µg/ml)	NF	nr	NF	NF	⇐ LC-MS method 1
	LC-Q-ToF-MS (µg/ml)	<5	<5	<5	11	⇐ LC-MS method 2
	LC-CL (µg/ml)	NF	NF	NF	NF	⇐ Chemilum. method

Dose per area calculations for limonene-1-OOH

	Dose of hydroperoxide in test preparation	Dose per area
LLNA Dose inducing sensitisation (EC3)	3300 µg/g (0.33%)	82.5 µg/cm²
Patch test limonene-HP *, **routine diagnostic level**	3300 µg/g (0.33%)	156 µg/cm²
Patch test limonene-1-OOH **, diagnostic level	5000 µg/g (0.5%)	228 µg/cm²
Defined reporting limit	50 µg/g	0.1 – 0.5*** µg/cm²
Analytical data market surveillance: (Max. value of n = 104)	90 µg/g (0.009%)	0.2 µg/cm² ****

* Mixture of isomers, not specifically 1-OOH-isomer
** Dose used in study on specific Limonene-1-OOH isomer by Christensson, Contact Dermatitis 2015
*** Different dose depending on product type (Cream 10 mg/cm² higher than fine fragrance, 2.2 mg/cm²)
**** Based on the typical application dose of fine fragrance per area

Key conclusions:

- Toolbox of methods established
- GC-MS method validated in blinded ring study
- Market surveillance found very low incidence of positive samples
- Low dose in single confirmed positive sample
- Neither detected level nor incidence of positive samples can explain patch test frequency
- **Exposure source for potential sensitization remains elusive**