IDEA workshop,
Bruxelles 27-28
August 2013

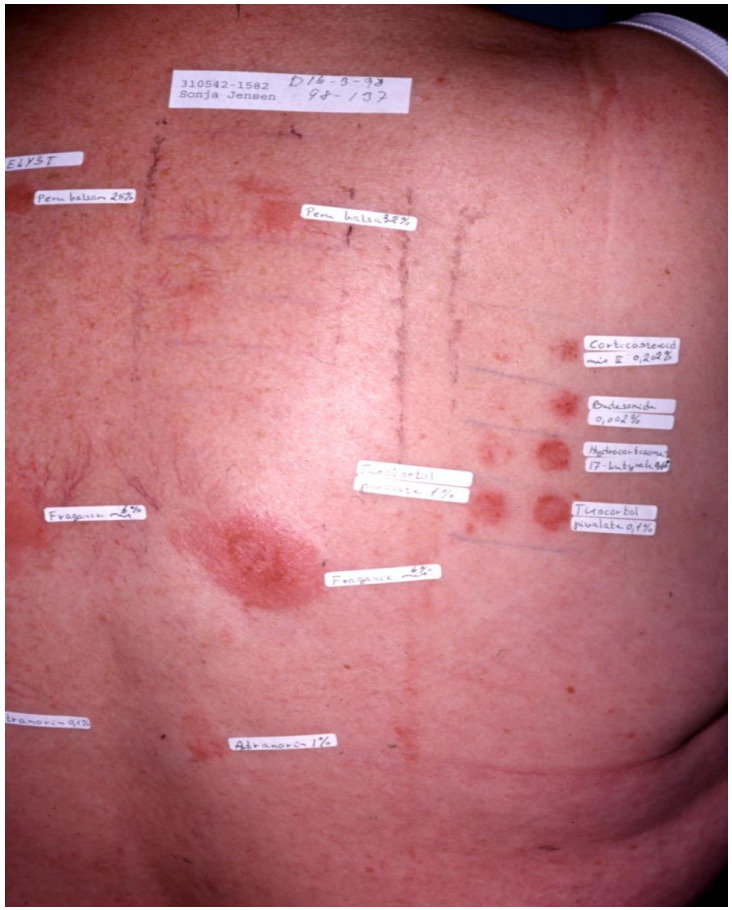
# Available data from diagnostic patch test and clinical studies (HRIPT, ROAT, etc.)

Klaus Ejner Andersen

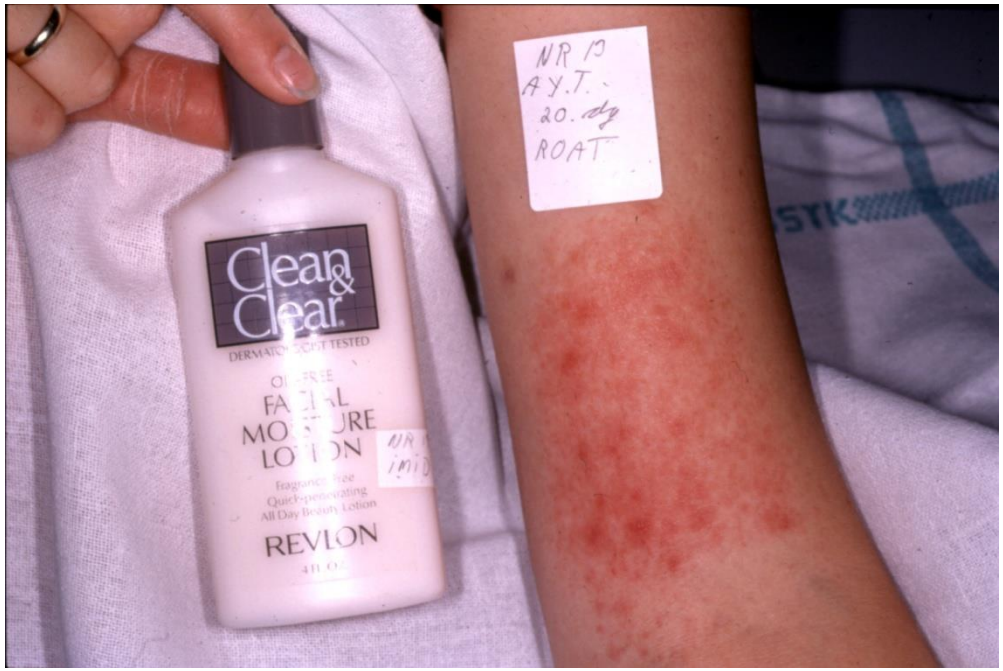
Department of Dermatology and Allergy Centre, Odense University Hospital, University of Southern Denmark

# Elicitation tests for contact allergy/allergic contact dermatitis

- Diagnostic patch test




- Repeated Open Application Test (ROAT)



# How common is perfume allergy?

- 949 consecutive eczema patients answered a questionnaire on perfume allergy prior to patch testing:
- The responses were categorised as ”certain”, ”probable”, ”possible” or ”none”

- 7.4% had a ”certain” parfume allergy defined by a history that they had developed an itching dermatitis from exposure to one denominated fine perfume or aftershave, and they had reacted to other perfumed products

Schollhammer et al *Contact Dermatitis*, **66**, 340–355 2012

# Patch test studies

# Fragrance mix I patch test reactions in 5006 consecutive dermatitis patients tested simultaneously with TRUE Test® and Trolab® test material

CHARLOTTE GOTTHARD MORTZ AND KLAUS EJNER ANDERSEN

*Contact Dermatitis 2010: 63: 248–253*

- Retrospective study - 5006 patients
- The patches were read on D3 and D5- D7.
- If a patient had a positive reaction to one of the FM I tests at the first reading, usually at D3, the individual constituents of FM I (Trolab®) each in 1% concentration in petrolatum were tested whenever possible and read once at the second reading.
- All patients with a +, ++ or +++ to one or both FM I test materials were regarded as positive and included in the analysis.
- The clinical relevance was recorded as present or past.

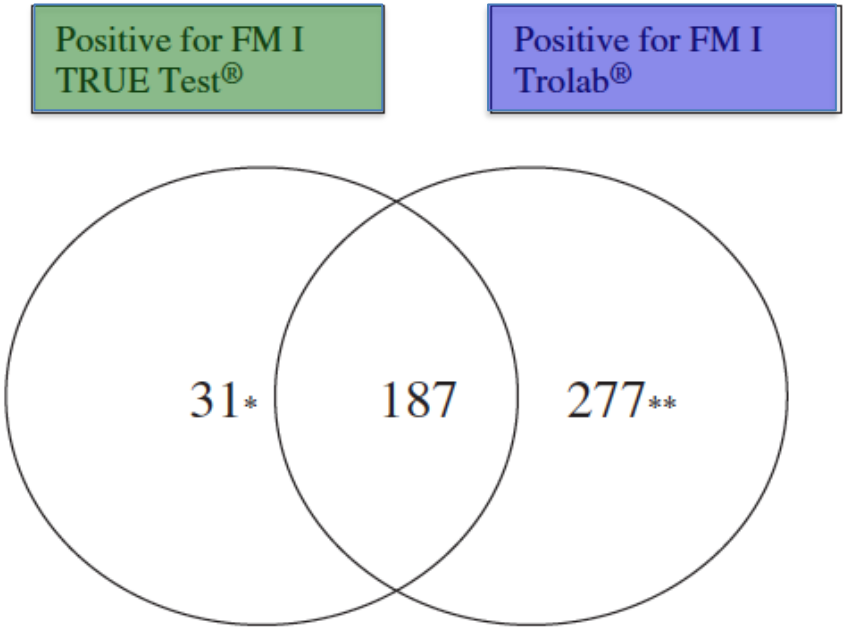
Results,

- 9.9% had a positive reaction to one of the FM I
- 4.4% had a positive reaction to FM I (TRUETest®)
- 9,3% had a positive reaction to FM I (Trolab®)
- 3.7% had a positive reaction to both.

Significant difference between the 2 tests in frequency of positive reactions ($p<0.0001$).

Patients with stronger reaction to FM I TRUETest® (++/+++) reacted almost all to FM I Trolab® (resp. 98% and 100%).

Conversely, only 58% with a ++ and 86% with a +++ reaction to FM I Trolab® had concomitant positive reaction to FM I TRUETest®.




| | FM I Trolab® | | |
|---|---|---|---|
| **FM I TRUE Test®** | **Negative** | **Positive** | **Total** |
| **Negative** | 4511 | 277 | **4788** |
| **Positive** | 31 | 187 | **218** |
| **Total** | **4542** | **464** | **5006** |

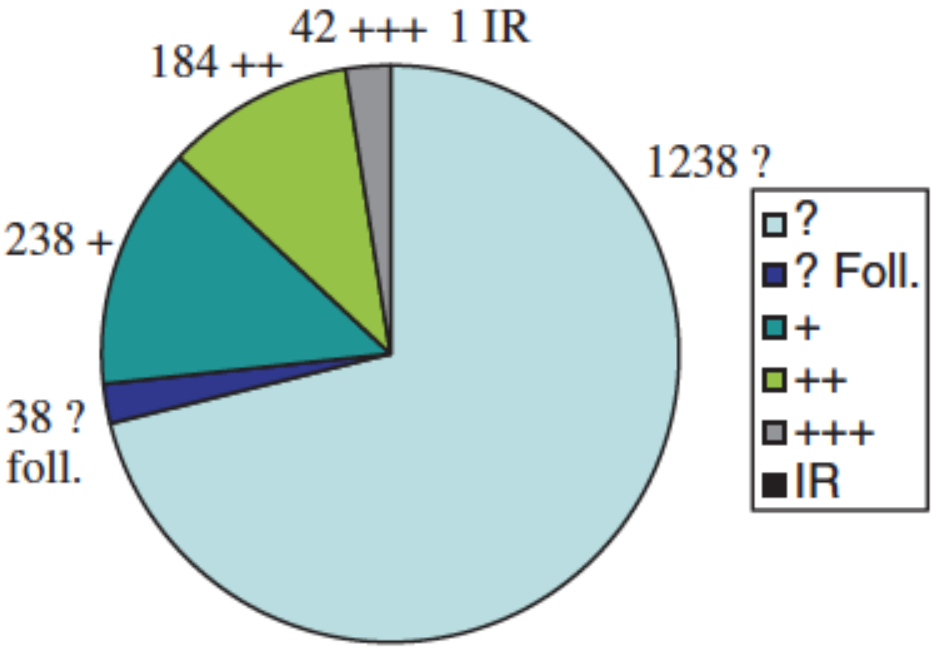
*Fig. 1.* Patch test results in 5006 patients tested with fragrance mix (FM) I both in TRUE Test® and in pet. (Trolab®).
*Of the 31 (29+ and 2++) patients positive only in FM I TRUE Test, 19 had a ? reaction to FM I from Trolab®, and 12 had no reaction.
**Of the 277 (193+, 78++, and 6+++) patients positive only in FM I Trolab, 103 had a ? reaction to FM I TRUE Test, 12 had a follicular reaction, and 162 had no reaction.

*Table 1.* Relationship between the strength of reaction to fragrance mix (FM) I TRUE Test® and the number of reactions to FM I Trolab®

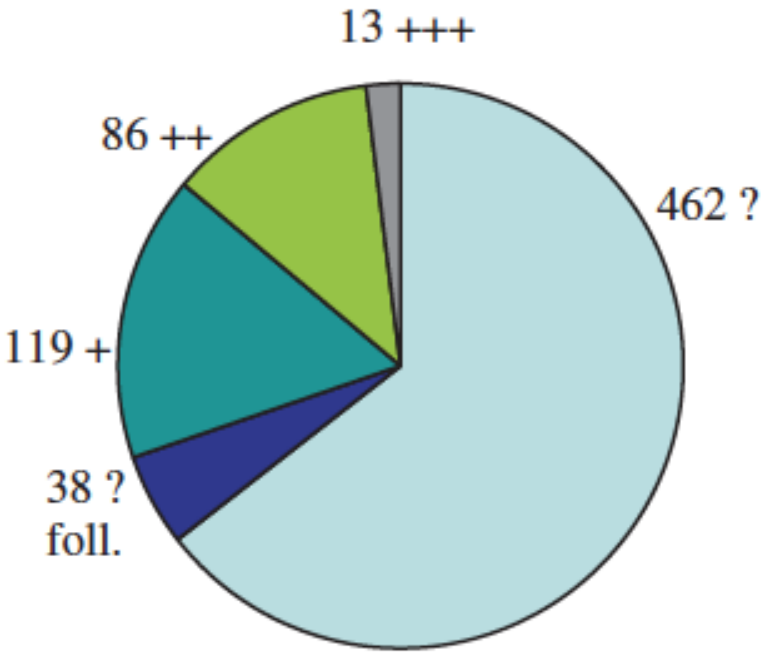
| Strength of TRUE Test® FM I test reaction | Number of patients | Number (%) of patients with concomitant reaction to FM I Trolab® |
|---|---|---|
| + | 119 | 90 (75.6) |
| ++ | 86 | 84 (97.7) |
| +++ | 13 | 13 (100.0) |
| All | 218 | 187 (85.8) |

*Table 2.* Relationship between the strength of reaction to fragrance mix (FM) I in pet. (Trolab®) and the number of reactions to FM I TRUE Test®

| Strength of Trolab® FM I test reaction | Number of patients | Number of patients (%) with concomitant reaction to FM I TRUE Test® |
|---|---|---|
| + | 238 | 45 (18.9) |
| ++ | 184 | 106 (57.6) |
| +++ | 42 | 36 (85.7) |
| All | 464 | 187 (40.3) |




FM I reactions to Trolab®, $n = 1741$




FM I reactions to TRUE Test®, $n = 718$

*Fig. 2.* Number of doubtful, irritant and graded positive reactions to fragrance mix (FM) I from TRUE Test and Trolab.

Same distribution of scoring with the 2 test materials

# Reproducibility

236 patients were tested more than once

FM I TRUETest® gave reproducible test results in 229/236 patients (97.0%).

FM I Trolab® gave reproducible test results in 216/236 patients (91.5%).

*Table 4.* The relationship between strengths of reaction in the two tests and associated clinical relevance

| Strength of FM I patch test | % (number) of patients with clinical relevance of FM I TRUE Test® | % (number) of patients with clinical relevance of FM I Trolab® |
|---|---|---|
| + | 64.1 (59/92) | 52.7 (87/165) |
| ++ | 82.2 (60/73) | 70.6 (101/143) |
| +++ | 84.6 (11/13) | 91.9 (34/37) |

Clinical relevance determined from the available information in the patient's record

Clinical relevance of a positive patch test:

FM I TRUETest® : 73.0% (130/178)

FM I Trolab® : 64.3% (222/345)

*Table 5.* The relationship between strengths of reaction in the two tests and positive reactions to the constituents of fragrance mix (FM) I

| Strength of FM I patch test reaction | % (number) of patients with a positive reaction to FM I TRUE Test® and with reaction to constituents of the mix | % (number) of patients with a positive reaction to FM I Trolab® and with reaction to constituents of the mix |
|---|---|---|
| + | 54.3 (50/92) | 29.2 (50/171) |
| ++ | 83.3 (60/72) | 74.8 (110/147) |
| +++ | 100 (7/7) | 96.7 (29/30) |

## Conclusion

• The analysis cannot conclude which of the 2 FM I test preparations is the best for diagnostic purposes.

•We included both FM I tests in the baseline series to obtain a graded degree of FM I allergy for the individual patient.

• With investigator-loaded Finn Chamber the major problem may be false-positive reactions

• With TRUETest® the major problem is false negative reactions.

• Weak positive reactions to FM I (Trolab®) should be evaluated carefully for clinical relevance.

•*Limitations*: the study is retrospective and supplementary testing with constituents of FM I was performed in a selected group of patients. Determination of clinical relevance may be biased.

*Contact Dermatitis*, **66**, 131–136 2012

# Patch test concentrations (doses in mg/cm$^2$) for the 12 non-mix fragrance substances regulated by European legislation

**Magnus Bruze[1], Cecilia Svedman[1], Klaus Ejner Andersen[2], Derk Bruynzeel[3], An Goossens[4], Jeanne Duus Johansen[5], Mihaly Matura[6], David Orton[7] and Martine Vigan[8], on behalf of the ESCD**

**Results.** The predetermined maximum patch test concentrations/doses could be tested for all 12 fragrance substances, with no observable adverse reactions being noted.

Comment:
FMI constituents are tested at 1% pet – this may be too low

| | CAS | Maximum concentration (%) to be tested |
|---|---|---|
| Amyl cinnamyl alcohol | 101-85-9 | 15.0 |
| Anise alcohol | 105-13-5 | 25.0 |
| Benzyl alcohol | 100-51-6 | 30.0 |
| Benzyl benzoate | 120-51-4 | 30.0 |
| Benzyl cinnamate | 103-41-3 | 30.0 |
| Benzyl salicylate | 118-58-1 | 30.0 |
| Butylphenyl methylpropional | 80-54-6 | 22.5 |
| *Evernia furfuracea* | 90028-67-4 | 3.0 |
| $\alpha$-Isomethyl ionone | 127-51-5 | 30.0 |
| D-Limonene | 5989-27-5 | 30.0 |
| Linalool | 78-70-6 | 30.0 |
| Methyl 2-octynoate | 111-12-6 | 0.5 |

*Contact Dermatitis*, **64**, 96–103 2010

# Screening for Compositae sensitization with pure allergens: implications of molecular structure, strength of reaction, and time of testing

**Evy Paulsen and Klaus E. Andersen**

**Table 4.** Association between time of application and number of positive reactions to constituents of the sesquiterpine lactone (SL) mix in persons with different degrees of SL sensitivity

| SL mix reaction | Day of application of constituents | 0 positive | 1 positive | 2 positive | 3 positive (% with 2 or 3 reactions) |
|---|---|---|---|---|---|
| 1+ ($n = 15$) | D1/D2 | — | 2 | 0 | 0 (0) |
| | D3–5 | 2 | 4 | 1 | 0 (14) |
| | Later | 1 | 1 | 3 | 2 (71) |
| 2+ ($n = 73$) | D1/D2 | — | 7 | 16 | 7 (76)* |
| | D3–5 | 4 | 15 | 9 | 5 (42)* |
| | Later | — | 1 | 4 | 7 (92) |
| 3+ ($n = 32$) | D1/D2 | 1 | — | 5 | 12 (94) |
| | D3–5 | — | 1 | 5 | 3 (88) |
| | Later | — | 1 | 2 | 3 (83) |

*$p = 0.0099$.

Comment:
A routine procedure where mix constituents are applied at D3 after seeing a positive reaction to the mix – may give false negative reactions to the mix constituents!

*Contact Dermatitis*, **66**, 340–355 2012

# The diagnostic value of patch tests with two fragrance mix I preparations for detection of clinically relevant perfume allergy

**Liv Schollhammer, Klaus Ejner Andersen and Charlotte Gotthard Mortz**

949 consecutive eczema patients – prior to patch testing – answered questionnaire:

(1) Certain: has reacted with an itching dermatitis to at least one denominated fine perfume or aftershave, and has reacted to other perfumed products;
(2) Probable: has reacted to one or more perfumed products (e.g. deodorant), but a certain perfume has not been identified as the cause of a clinical reaction;
(3) Possible: has reacted to various cosmetic products with and without perfume; materials other than fragrance constituents may be the cause of the reaction; and
(4) None: has never reacted to a perfumed material.

In order to compare the diagnostic value of the fragrance mixes in this study, a gold standard for the diagnosis of fragrance contact allergy was defined on the basis of the questionnaire responses as a certain history of adverse reaction to fragrances. Thus, patients with a probable or possible history were excluded from further analysis.

**Table 1.** Distribution of results for patch testing with FM1 TRUE Test® and FM1 Trolab® in the study population after exclusion of patients with a probable or possible history; results of calculations of sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and *p*-values

| | History | | | Sensitivity (95% CI) | Specificity (95% CI) | PPV (95% CI) | NPV (95% CI) | *p*-value |
|---|---|---|---|---|---|---|---|---|
| FM I Trolab® | Certain | No | Total | 38.6% (35.1–42.1%) | 96% (94.6–97.4%) | 49% (45.4–52.6%) | 94% (92.3–95.7%) | <0.001 |
| Positive reaction | 27 | 28 | 55 | | | | | |
| Negative reaction | 43 | 637 | 680 | | | | | |
| Total | 70 | 665 | 735 | | | | | |
| | History | | | Sensitivity (95% CI) | Specificity (95% CI) | PPV (95% CI) | NPV (95% CI) | *p*-value |
| FM I TRUE Test® | Certain | No | Total | 28.6% (25.5–31.7%) | 98% (96.9–99%) | 63% (59.5–66.5%) | 93% (91.1–94.8%) | <0.001 |
| Positive reaction | 20 | 12 | 32 | | | | | |
| Negative reaction | 50 | 653 | 703 | | | | | |
| Total | 70 | 665 | 735 | | | | | |

Comment:
The prevalence of ”certain” perfume allergy was 7.4%
The poor performance of FM I underlines the importance of testing with other fragrance allergens - even with FM II many perfume reactions may be missed

*Contact Dermatitis 2010: 63: 270–276*

# Fragrance mix II in the baseline series contributes significantly to detection of fragrance allergy

Maria V Heisterberg[1], Klaus E Andersen[2], Christian Avnstorp[3], Berit Kristensen[4], Ove Kristensen[4], Knud Kaaber[5], Grete Laurberg[6], Torkil Menné[1], Niels Henrik Nielsen[7], Mette Sommerlund[8], Jens Thormann[9], Niels K Veien[6], Susanne Vissing[10] and Jeanne D Johansen[1]

*Method:* Retrospective study of 12 302 patients consecutively patch tested with FM II by members of the Danish Contact Dermatitis Group 2005–2008.

*Results:* FM II gave a positive patch test in 553 patients (4.5%), and in 72.2% of these patients the reaction was judged to be clinically relevant. FM II ranked second in detecting fragrance allergy, after FM I. If FM II had not been included as a screening marker in the baseline series, 15.6% ($n = 202$) of individuals with fragrance allergy would not have been identified by the other fragrance screening markers (FM I, *M. pereirae* or HICC).

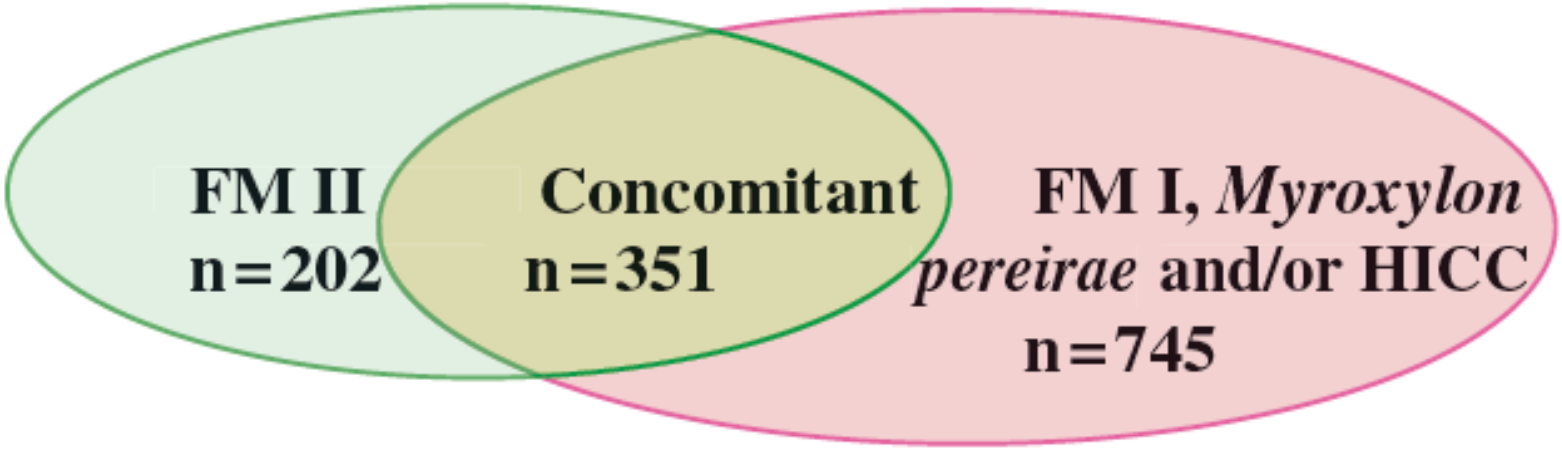



*Fig. 3.* Illustration of concomitant reactions to fragrance mix II (FM II) and subjects allergic to fragrance mix I (FM I), *Myroxylon pereirae*, and/or hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC). Subjects with fragrance allergy identified with FM II, FM I, *Myroxylon pereirae* and HICC ($n = 1298$). FM II additionally identified 202 (15.6%) allergic subjects.

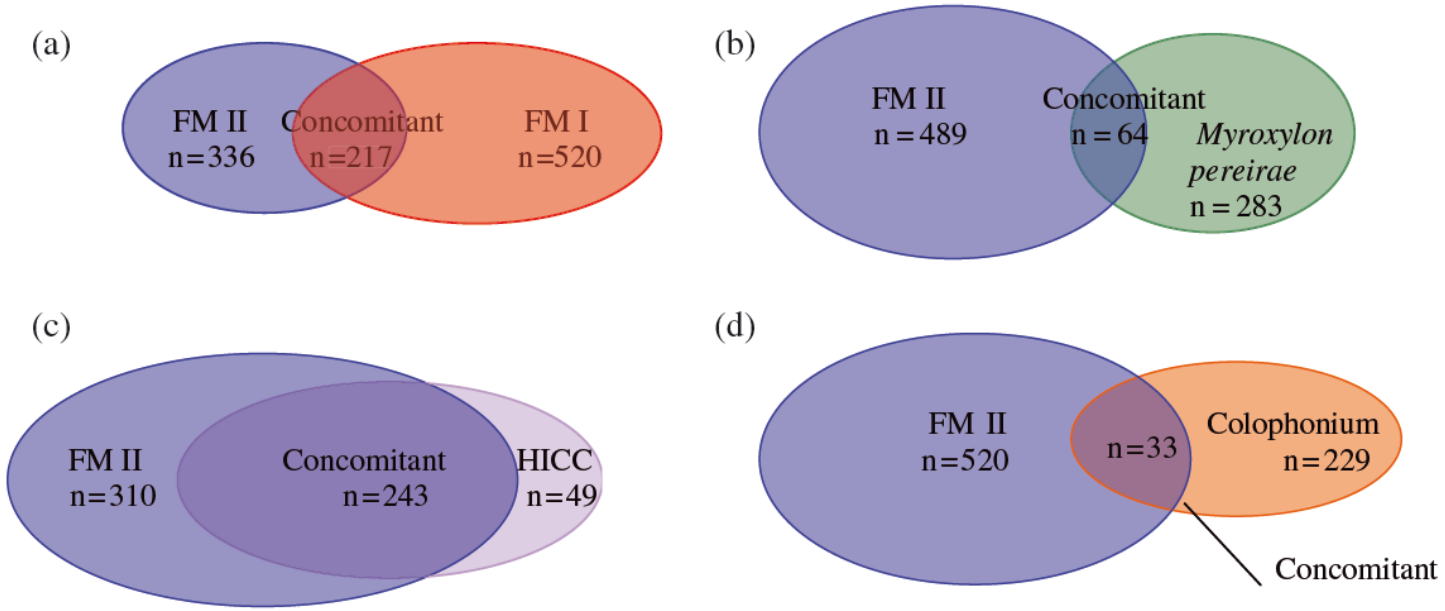



*Fig. 2.* (a) Illustration of concomitant reactions to fragrance mix II (FM II) and fragrance mix I (FM I). Subjects with fragrance allergy identified by FM II and FM I = 1073. A significant statistical association was observed between the two groups, $\chi^2$, $P < 0.0000001$. OR = 14.0 (CI 11.5–16.9). (b) Illustration of concomitant reactions to fragrance mix II (FM II) and *Myroxylon pereirae*. Subjects with fragrance allergy identified by FM II and *Myroxylon pereirae* = 836. A significant statistical association between the two groups was observed, $\chi^2$, $P < 0.0000001$. OR = 5.3 (CI 4.0–7.1). (c) Illustration of concomitant reactions to fragrance mix II (FM II) and hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC). Subjects with fragrance allergy identified by FM II and HICC = 602. A significant statistical association was observed between the two groups, $\chi^2$, $P < 0.000001$. OR = 187.2 (CI 135.3–259.6). (d) Illustration of concomitant reactions to fragrance mix II (FM II) and colophonium. Subjects with fragrance allergy identified by FM II and colophonium = 782. A significant statistical association was observed among the two groups, $\chi^2$, $P < 0.0000001$. OR = 3.2 (CI 2.2–4.7).

*British Journal of Dermatology* 1999; **141:** 1076–1083.

# Lyral® is an important sensitizer in patients sensitive to fragrances

P.J.FROSCH, J.D.JOHANSEN,* T.MENNÉ,* S.C.RASTOGI,† M.BRUZE,‡ K.E.ANDERSEN,§ J.P.LEPOITTEVIN,¶ E.GIMÉNEZ ARNAU,¶ C.PIRKER, A.GOOSSENS** AND I.R.WHITE††

**Table 1.** Results of patch testing with 8% fragrance mix (FM) and 5% Lyral® in a total of 1855 patients from six centres. Positive and doubtful/irritant reactions (second reading)

| | | Positive reactions (+, + +, + + +) | | Doubtful reactions | |
|---|---|---|---|---|---|
| Centre | No. patients tested | FM | Lyral® | FM | Lyral® |
| Dortmund | 281 | 32 (11·4%) | 4 (1·4%) | 15 (5·3%) | 1 (0·3%) |
| Malmö | 395 | 55 (13·9%) | 12 (3·0%) | 18 (4·0%) | 2 (0·5%) |
| Odense | 331 | 33 (9·9%) | 8 (2·4%) | 42 (12·6%) | 7 (2·1%) |
| Copenhagen | 303 | 25 (8·2%) | 8 (2·6%) | 30 (9·9%) | 8 (2·6%) |
| Leuven | 70 | 16 (23·0%) | 12 (17·0%) | 4 (5·7%) | 0 (0·0%) |
| London | 475 | 49 (10·3%) | 6 (1·2%) | 7 (1·5%) | 2 (0·4%) |
| Total | 1855 | 210 (11·3%) | 50 (2·7%) | 116 (6·2%) | 20 (1·0%) |

# An international multicentre study on the allergenic activity of air-oxidized *R*-limonene


*Contact Dermatitis*, **68**, 214–223

**Johanna Bråred Christensson[1,2], Klaus E. Andersen[3], Magnus Bruze[4], Jeanne D. Johansen[5], Begoña Garcia-Bravo[6], Ana Giménez-Arnau[7], Chee-Leok Goh[8], Rosemary Nixon[9] and Ian R. White[10]**

**Table 1.** Total number of patients from each test centre and the number and percentage of positive reactions, doubtful reactions and irritant reactions to oxidized *R*-limonene 3.0% [limonene hydroperoxides (Lim-OOHs) 0.33%] pet.

| | Oxidized *R*-limonene 3.0% (Lim-OOHs 0.33%) pet. | | | |
|---|---|---|---|---|
| Test centre | Total no. tested | No. (%) positive | No. (%) doubtful | No. (%) irritant |
| Barcelona | 299 | 13 (4.3) | 0 (0) | 4 (1.4) |
| Copenhagen | 440 | 16 (3.6) | 53 (12.0) | 17 (3.9) |
| Gothenburg | 397 | 10 (2.5) | 1 (0.2) | 0 (0) |
| London | 271 | 8 (2.9) | 9 (3.3) | 0 (0) |
| Malmö | 300 | 7 (2.3) | 11 (3.7) | 0 (0) |
| Melbourne | 289 | 18 (6.2) | 11 (3.8) | 0 (0) |
| Odense | 298 | 23 (7.7) | 73 (24.5) | 3 (1.0) |
| Seville | 300 | 20 (6.7) | 6 (2.0) | 0 (0) |
| Singapore | 306 | 37 (12.1) | 40 (13.1) | 1 (0.3) |
| Total | 2900 | 152 (5.2) | 204 (7.0) | 25 (0.9) |

# Air-oxidized linalool – a frequent cause of fragrance contact allergy

Johanna Bråred Christensson[1,2], Klaus E. Andersen[3], Magnus Bruze[4], Jeanne D. Johansen[5], Begoña Garcia-Bravo[6], Ana Giménez Arnau[7], Chee-Leok Goh[8], Rosemary Nixon[9] and Ian R. White[10]



*Contact Dermatitis*, **67**, 247–259

**Table 2.** Total number of patients from each respective test centre and the number and percentage of positive reactions, doubtful reactions and irritant reactions to oxidized linalool 6.0% [linalool hydroperoxides (Lin-OOHs) 1%] pet.

| | Oxidized linalool 6.0% (Lin-OOHs 1%) pet. | | | |
|---|---|---|---|---|
| Test centre | Total no. tested | No. of positive patch test reactions (%) | No. of doubtful patch test reactions (%) | No. of irritant patch test reactions (%) |
| Barcelona | 299 | 11 (3.7) | 0 (0) | 0 (0) |
| Copenhagen | 440 | 21 (4.8) | 68 (15.4) | 24 (5.4) |
| Göteborg | 397 | 15 (3.8) | 11 (2.8) | 1 (0.25) |
| London | 271 | 14 (5.2) | 9 (3.3) | 0 (0) |
| Malmö | 300 | 10 (3.3) | 13 (4.3) | 0 (0) |
| Melbourne | 289 | 24 (8.3) | 7 (2.4) | 0 (0) |
| Odense | 298 | 24 (8.0) | 108 (36.2) | 7 (2.3) |
| Seville | 300 | 43 (14.3) | 6 (2.0) | 3 (1.0) |
| Singapore | 306 | 38 (12.4) | 44 (14.4) | 2 (0.65) |
| Total | 2900 | 200 (6.9) | 266 (9.2) | 37 (1.3) |

# Use tests

## Use-tests in contact dermatitis

A ”Use-test” is a general term denoting clinical observations of outcome following usage of a product in selected individuals/patients

- Observation of possible skin changes following intended usage
- Observation following exaggerated usage
- Repeated Open Application Test (ROAT) – a standardized clinical exposure test

*Contact Dermatitis* 1986: **14:** 221–227

# The repeated open application test (ROAT)

MATTI HANNUKSELA AND HEIKKI SALO

dermatitis on the test site appeared. About 0.1 ml of test material was applied twice daily to the flexor aspect of the forearm near the cubital fossa, to an area approximately 5 × 5 cm.

# The repeated open application test: suggestions for a scale of evaluation

J. D. JOHANSEN[1], M. BRUZE[2], K. E. ANDERSEN[3], P. J. FROSCH[4], B. DREIER[4], I. R. WHITE[5], S. RASTOGI[6], J. P. LEPOITTEVIN[7] AND T. MENNÉ[1]

*Contact Dermatitis* 1997: **39**: 95

Toxicology and Applied Pharmacology 170, 166–171 (2001)
doi:10.1006/taap.2000.9095, available online at http://www.idealibrary.com on IDEAL®

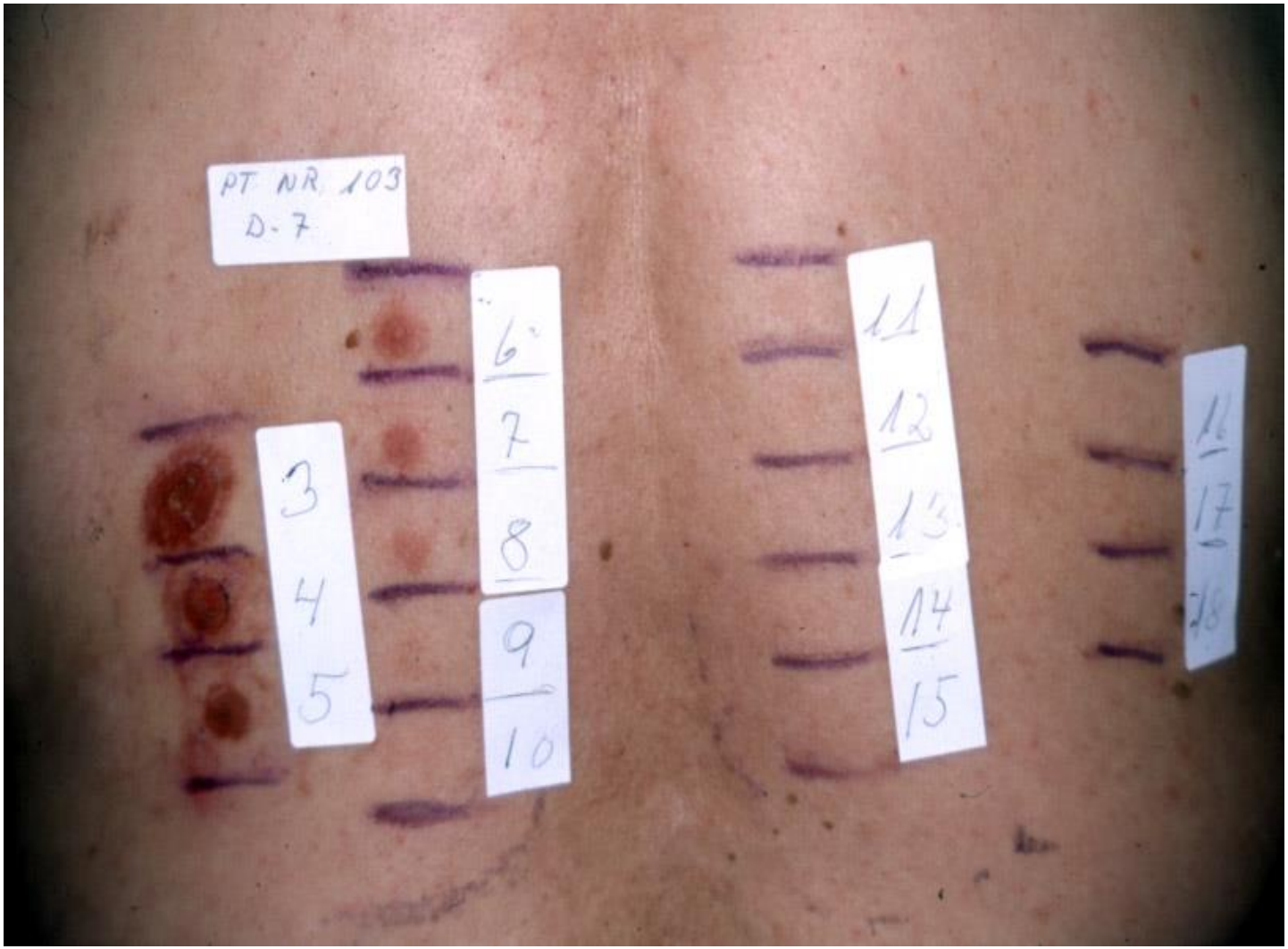
# The Time–Dose–Response Relationship for Elicitation of Contact Dermatitis in Isoeugenol Allergic Individuals

Klaus E. Andersen,* Jeanne D. Johansen,† Magnus Bruze,‡ Peter J. Frosch,§ An Goossens,¶ Jean-Pierre Lepoittevin,‖ Suresh Rastogi,** Ian White,†† and Torkil Menné†

- 27 isoeugenol sensitive patients
- Serial dilution patch test with isoeugenol eth. concentrations: 2.0% - 0.00006%
- Reading D3 and D7
- Treshold concentration established
- Double blind ROAT for up to 28 days
- Endpoints: days to pos. ROAT and amount of isoeugenol solution used until pos. ROAT or max. 28 days

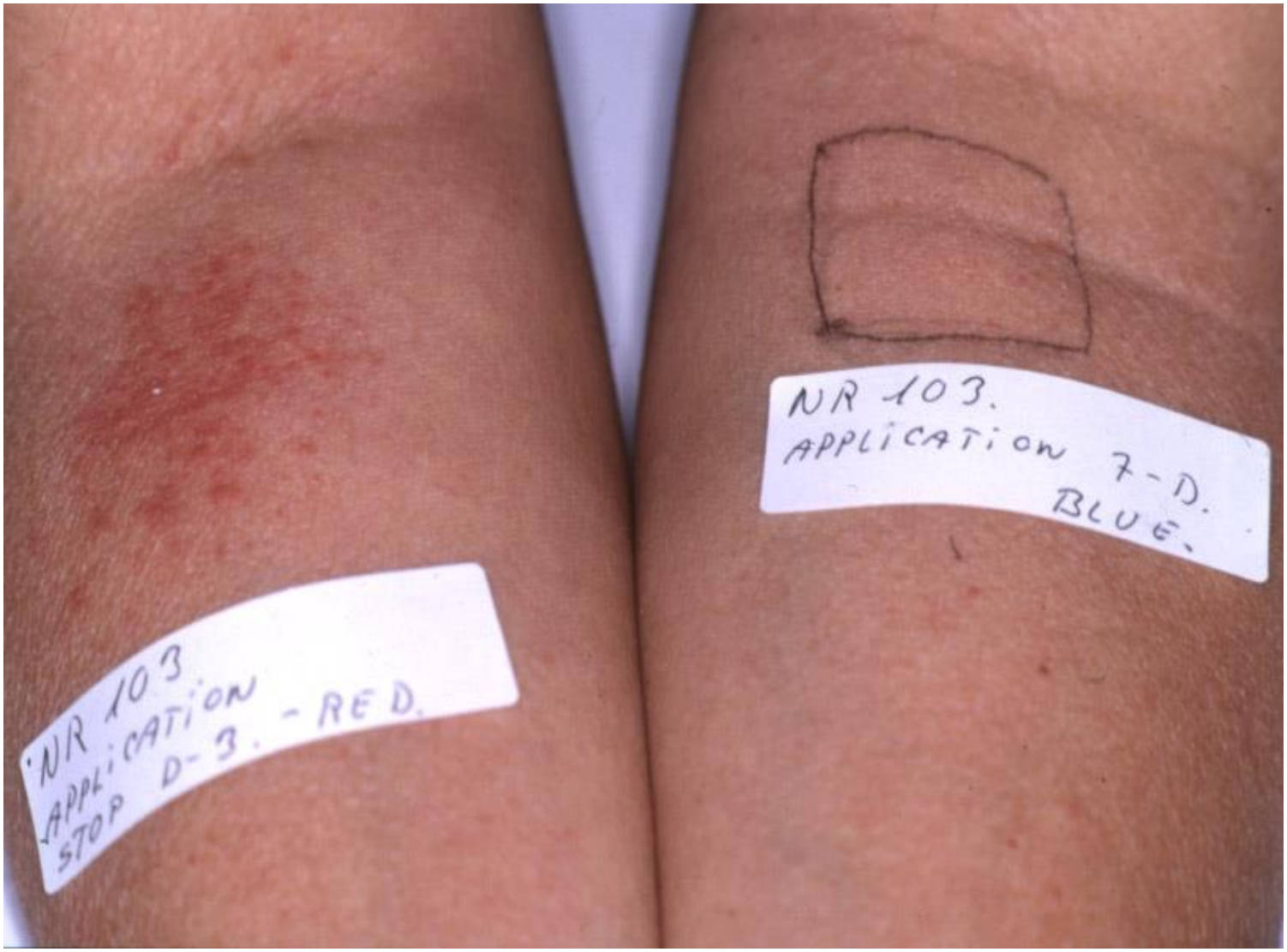
Patch tests with a dilution series of isoeugenol in eth.

Threshold patch test conc. is 0.016% eth



# ROAT with isoeugenol 0.2% R and 0.05% L

Comment:
Note follicular pattern of response

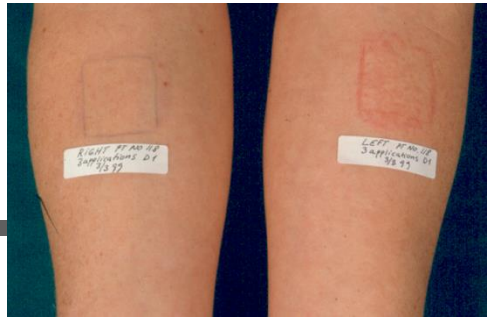


## TABLE 1

## Results of ROAT with 0.2 and 0.05% Isoeugenol in Ethanol in 24 Patients with a Positive Patch Test to Isoeugenol

| | ROAT concentration | |
|---|---|---|
| | 0.2% | 0.05% |
| Patients with positive ROAT | 16/24 | 10/24 |
| No. of days to positive ROAT (median) | 7 | 15 |
| Range (days) | 2–26 | 3–28 |
| Mean ± SD (days) | 8.4 ± 6.2 | 15.2 ± 8.8 |

# Exposure dose - accumulated

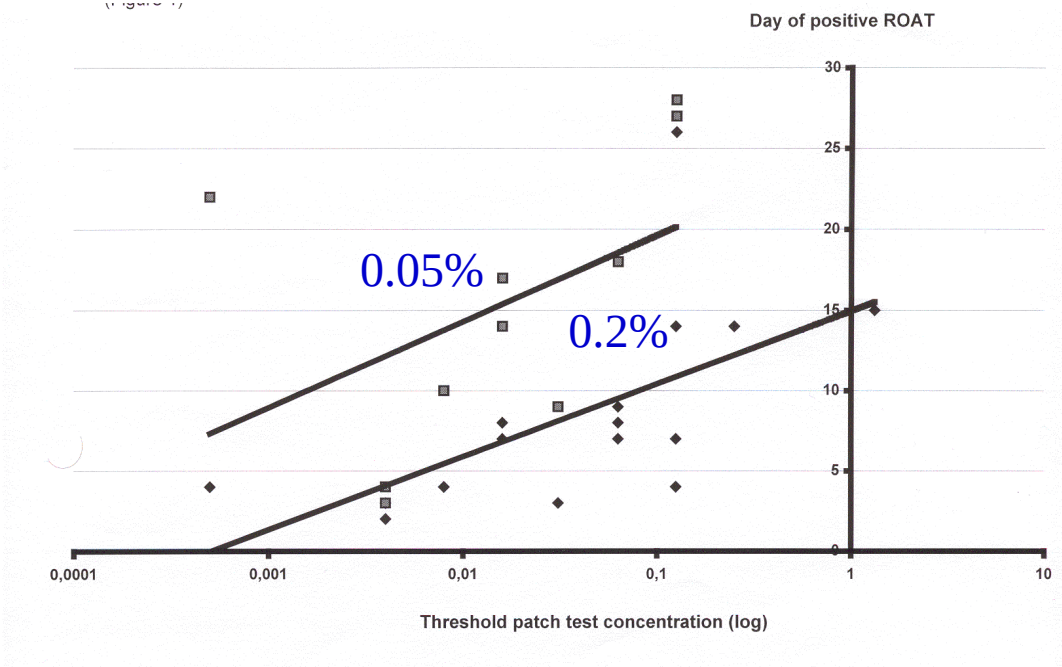


Number of days (exposures) until elicitation depends on exposure concentration:

- 0.2%: 7 days of exposure (median)
- 0.05%: 15 days of exposure (median)

-And the individual level of sensitivity

ROAT med isoeugenol 0.05% og 0.2%

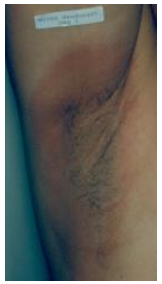


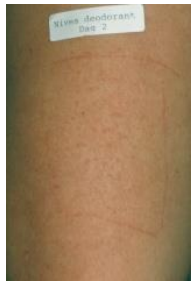

Andersen KE et al. Toxicol Appl Pharmacol 2001:170:166-171

# Region and previous eczema

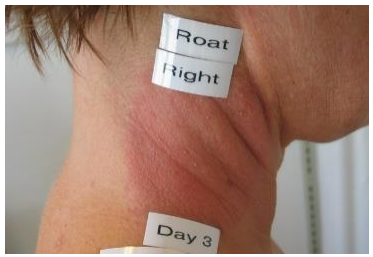
## Sensitivity depends on region

Axilla > arm





Face=neck> arm




Outer aspect of upper arm

Upper back > lower back

Zachariae C. CD 2006; 54: 21-24

## Previous allergic eczema

Experimental nickel contact dermatitis

*Challenge later - after*
- 8 months
- 4 months
- 1 months

Significantly higher reactivity at previous allergic eczema sites

Hindsén M et al. CD 1997;37: 101-106

*Contact Dermatitis 2009: 61: 201–208*

# The dose–response relationship between the patch test and ROAT and the potential use for regulatory purposes

Louise Arup Fischer[1], Aage Voelund[2], Klaus Ejner Andersen[3], Torkil Menné[1] and Jeanne Duus Johansen[1]

*Objectives:* The aim was to develop an equation that could predict the response to an allergen in a ROAT based on the dose–response curve derived by patch testing.

*Table 2.* Spearman's rank correlation between the threshold concentrations in the patch test and repeated open applications test (ROAT) for the nickel study (13), the methyldibromo glutaronitrile (MDBGN) study (14) and for the hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) study (15)

| Allergen | Number of patients | Correlation coefficient | *P*-value |
|---|---|---|---|
| Nickel | 18 | 0.45 | 0.033 |
| MDBGN | 15 | 0.76 | 0.0021 |
| HICC | 16 | 0.59 | 0.011 |

Therefore, based on the above analysis, it was concluded that all three conditions are fulfilled, and an equation to convert the patch test dose–response data into the ROAT dose–response data was possible by means of the following equation:

$$ED_{xx}(\text{ROAT}) = 0.0296 ED_{xx}(\text{patch test}) \quad (4)$$

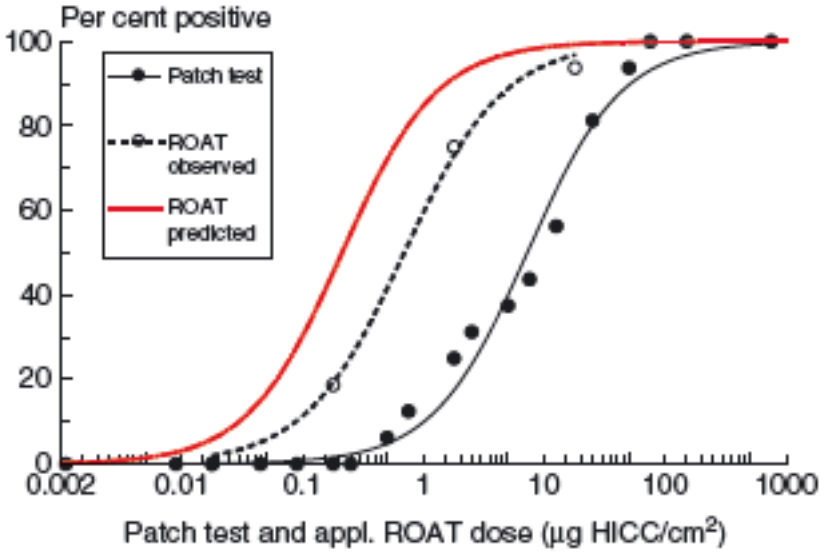



*Fig. 3.* Comparison of predicted and observed (black broken line) dose–response relations for repeated open applications test (ROAT) of hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) (15). The predicted dose–response curve (red line) is obtained from the fitted patch test curve by multiplication of the patch test doses by a factor 0.0296, i.e. $ED_{xx}$(ROAT) = 0.0296 $ED_{xx}$(patch test).

*Table 5.* Main findings

| Main findings | Relevance |
|---|---|
| The dose (µg/cm²) per application required to elicit a reaction in a repeated open applications test (ROAT) is lower than the dose required to elicit a reaction in a patch test. | When patch test data are used to determine safe levels of allergen exposure, a negative dose in the patch test is not a safe dose. This is also relevant for the clinical situation. |
| For non-volatile compounds, the outcome of a ROAT can be expressed by: $ED_{xx}$(ROAT) = 0.0296 $ED_{xx}$(patch test). | After validation, regulatory intervention should be undertaken based on patch test dilution series. Safe levels of allergens that will prevent elicitation can be calculated and contact dermatitis be prevented in a majority of cases. |

# Elicitation study on oak moss absolute

**Funded by IFRA members**

Klaus Ejner Andersen, Flemming Andersen, Kristian F Mose and Kirsten Hammond Andersen

**AIM**

To determine the elicitation frequency of ACD of a new quality of Oakmoss absolute (with lowered atranol and chloroatranol content) compared to the "classic" quality of Oakmoss absolute (containing higher levels of atranol and chloroatranol) using ROAT and a patch test serial dilution test in 30 OM allergic volunteers and 30 controls with negative patch tests to fragrance alergens

A randomized and double blind placebo controlled design was applied – adapted from the "old" isoeugenol study published in 2001. The ROAT was performed over a 21-day period and after a 4-week rest period the volunteers had serial dilution patch tests with COM and NOM.

# Elicitation study on oak moss absolute

Classic oakmoss:
Chloratranol 15000 ppm, atranol 27000 ppm

New oakmoss
Chloratranol 37 ppm, atranol 48 ppm

# Participants

**30 controls**

25 females, 5 males, age ≥ 18: with no prior history of hypersensitivity to perfume, fragrance mix, or/nor balsam of Peru

**30 oakmoss allergics**

19 females, 11 males, age ≥ 18, with privious positive patch tests to oak moss.

All with no active dermatitis nor in immunomodulary treatment

Dermatological Investigations Scandinavia™

OUH Odense Universitetshospital Svendborg Sygehus

# Solutions for ROAT

Classic Oak moss (COM) 0.1% dilution in 2:98 DEP:EtOH

Vehicle control: 2:98 DEP:EtOH

New Oak moss (NOM) 0.1% dilution in 2:98 DEP:EtOH

Vehicle control:2:98 DEP:EtOH

Dermatological Investigations Scandinavia™

OUH Odense Universitetshospital Svendborg Sygehus

# Solutions for Patch Tests

12 dilutions of the “classic” Oakmoss and “new” Oakmoss in 2:98 DEP:EtOH (v/v), respectively:

2.0, 1.32, 0,67, 0.22, 0.074, 0.025, 0.0082, 0.0027, 0.00091, 0.0003, 0.0001 and 0.00003% (w/v)

Three control patches with 2:98 DEP:EtOH, EtOH, and 100% DEP

Dermatological Investigations Scandinavia™
OUH Odense Universitetshospital Svendborg Sygehus

# Repeated Open Application Test

4 test areas on lower volar forearm, 2 on each arm

Double blind and randomized test design

Solutions (A,B,C,D) applied twice daily for 21 days or until positive reaction:
0.1% “classic” Oakmoss
0.1% “new” Oakmoss
two samples of the vehicle control; 2:98 DEP:EtOH.

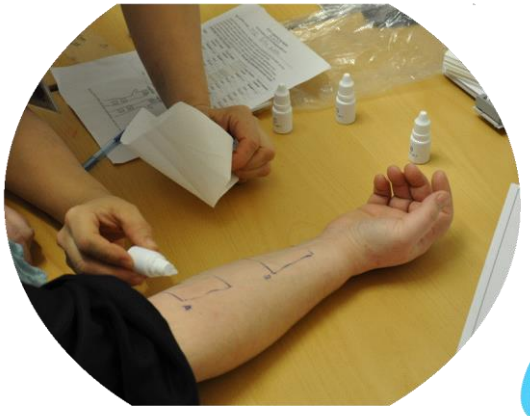
Volunteers documented treatments in a log. Bottles weighed before and after study

Areas were evaluated once a week and

when volunteers reported a positive reaction

Procedure and scoring adapted from

Johansen, J.D., et al (1997).



Dermatological Investigations Scandinavia™
OUH Odense Universitetshospital Svendborg Sygehus

# Patch test procedure

8 mm Finn Chambers® on Scanpor® tape + filter paper

15 µl (5.9 mg/cm2) of test solution applied to the chambers by a micropipette

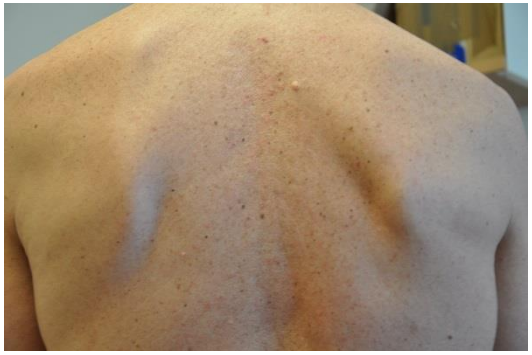
12 dilutions of COM and NOM + 3 vehicles – on each side of the upper back

Patches filled immediately before application

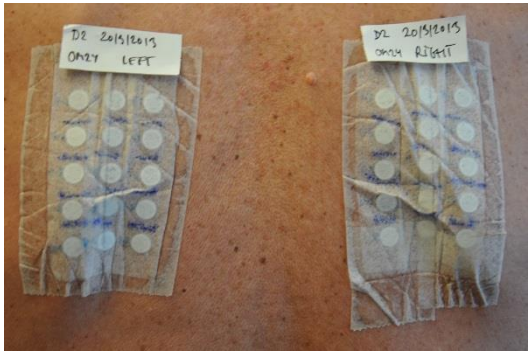
Test sites marked at time of application

Visual readings at D 3 and 7

Application and reading of the reactions were done in a double-blind fashion



Upper left and upper right



Pre-removal of 48-h patches

Dermatological Investigations Scandinavia™

OUH Odense Universitetshospital Svendborg Sygehus

# Results – ROAT

•All controls had negative ROAT to COM, NOM and vehicles

**•22/30 allergic volunteers had positive ROAT to COM, and only 6 reacted to NOM** (Fisher's Exact Test P < 0.0001).

•

No volunteer reacted to NOM only.

•

Removal of three volunteers with positive reactions to DEP in the patch test did not alter the outcome (Fisher's Exact Test P < 0.0001)

•

**The mean number of applications to elicit a positive ROAT were 14 for COM and 24 for NOM.**

• (Wilcoxon matched pairs test for 6 individuals reacting to both formulations (P = 0.013))

Dermatological Investigations Scandinavia™

OUH Odense Universitetshospital Svendborg Sygehus

# Results – ROAT

- No difference in amount of COM and NOM applied at each application (Friedmann Test with Dunn's Multiple Comparisons Test, P = 0,3647)

- Significant less COM was applied than both NOM and vehicle as expected

- No difference between the total amount of NOM and the amount of vehicle applied (Friedmann Test with Dunn's Multiple Comparisons Test, P < 0.0001)

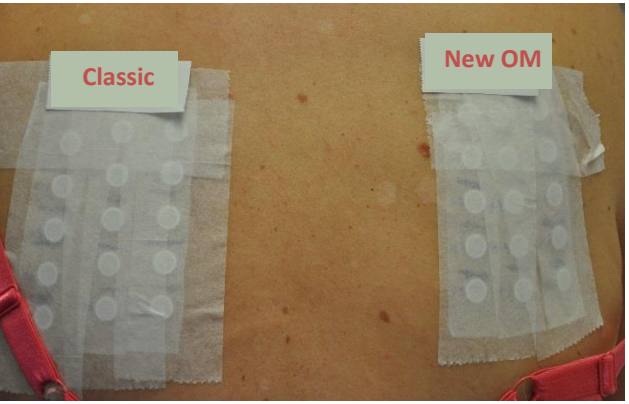
- Using McNemar's Test with the continuity correction it was demonstrated that COM caused significantly more reactions than NOM (p = 0.0002)

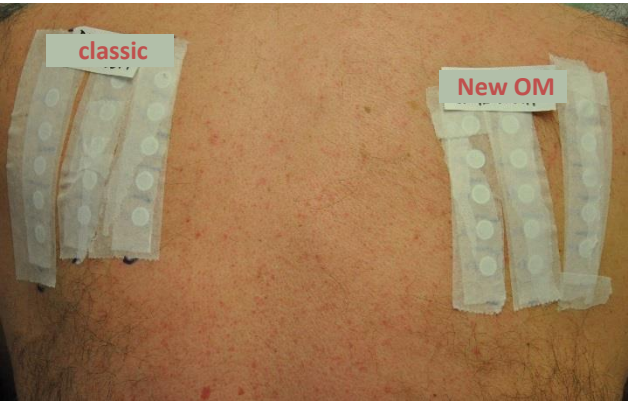
Dermatological Investigations Scandinavia™

OUH Odense Universitetshospital Svendborg Sygehus

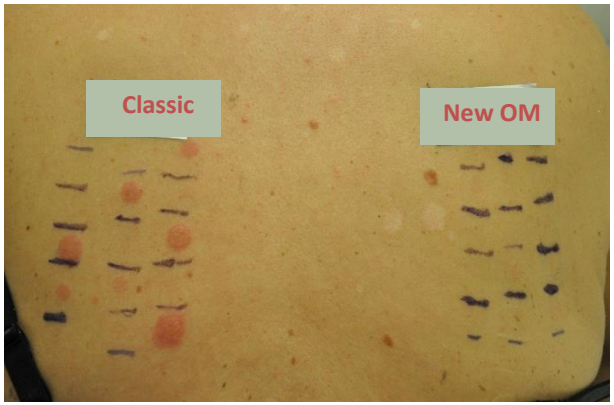
# Examples of patch test reactions

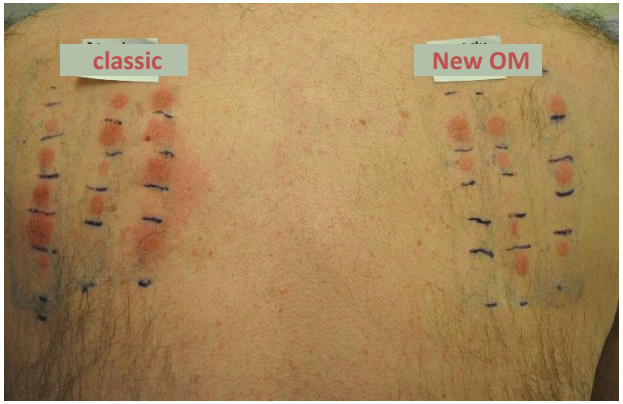
## Pre-removal of patches



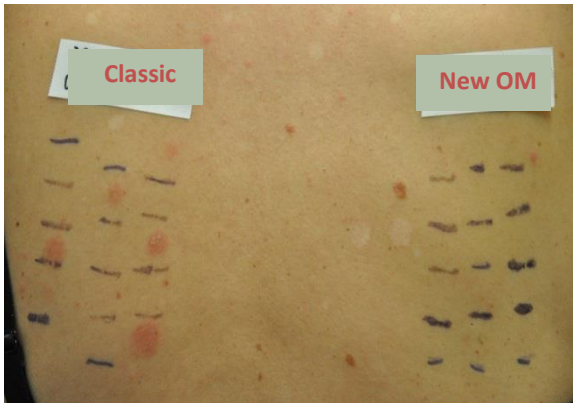



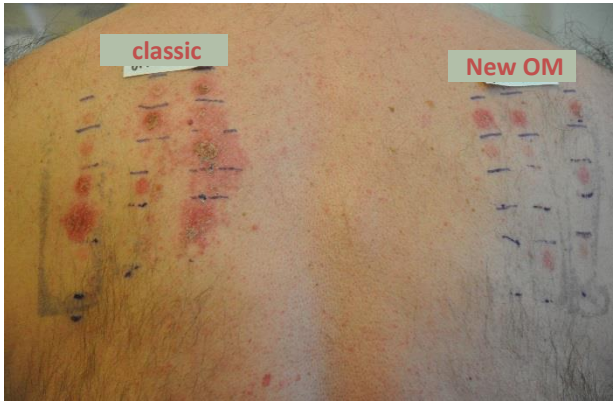

## D3-readings

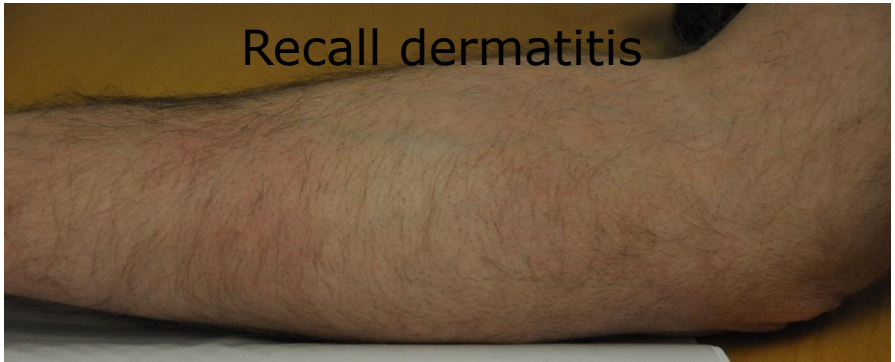



## D7-readings




Dermatological Investigations Scandinavia™
OUH Odense Universitetshospital Svendborg Sygehus

# Results – patch tests

- No controls had a positive reaction to COM, NOM and vehicles
- Three oak moss positives had positive reaction to 100% DEP
- 3/30 previous OM positive had negative patch tests
- Significantly more oak moss positive volunteers had positive reactions to COM than to NOM (Fisher's Exact Test P = 0.0005).
- Removal of three volunteers with positive reactions to DEP in the patch test did not alter the outcome (Fisher's Exact Test P = 0.0004).

Dermatological Investigations Scandinavia™
OUH Odense Universitetshospital Svendborg Sygehus

# Results – patch tests

3/60 had positive patch test to 100% DEP (diethylphthalate)

They may be irritant reactions??

DEP is a rare contact allergen!

Oliwiecki S  et al. Contact Dermatitis 1991;25: 264-265
Betts CJ et al. Contact Dermatitis 2007; 56: 70-75

Recommended patch test concentration: 2-10% pet
(De Groot AC. Patch testing, 3rd ed. 2008)

Dermatological Investigations Scandinavia™
OUH Odense Universitetshospital Svendborg Sygehus

# Controls

| Participant | | 1 | | 2 | | 6 | | 7 | | 8 | | 9 | | 10 | | 12 | | 13 | | 14 | | 16 | | 17 | | 18 | | 20 | | 23 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Original patch test | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | |
| Reaction | | COM | NOM | COM | NOM | COM | NOM | COM | NOM | COM | NOM | COM | NOM | COM | NOM | COM | NOM | COM | NOM | COM | NOM | COM | NOM | COM | NOM | COM | NOM | COM | NOM | COM | NOM |
| DEP | 100% | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| DEP/EtOH | 2/98% | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| EtOH | 100% | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ? |
| OM | 2,0% | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ? | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 1,32% | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ? | 0 |
| | 0,67% | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 0,22% | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 0,074% | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ? |
| | 0,025% | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 0,0082% | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ? |
| | 0,0027% | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 0,00091% | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ? |
| | 0,0003% | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 0,0001% | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 0,00003% | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ROAT | | No. of applications until positive reaction | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| COM | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | |
| NOM | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | |
| DEP/EtOH VEH1 | 2/98% | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | |
| DEP/EtOH VEH2 | 2/98% | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | |
| Amount used | | P.A. | Total | P.A. | Total | P.A. | Total | P.A. | Total | P.A. | Total | P.A. | Total | P.A. | Total | P.A. | Total | P.A. | Total | P.A. | Total | P.A. | Total | P.A. | Total | P.A. | Total | P.A. | Total | P.A. | Total |
| COM | | 0,0658 | 2,7620 | 0,1166 | 4,8980 | 0,0257 | 0,7190 | 0,1043 | 4,3800 | 0,093 | 3,92 | 0,0841 | 3,5330 | 0,0529 | 2,2230 | 0,0627 | 2,6340 | 0,2338 | 0,9350 | 0,0402 | 1,6870 | 0,2072 | 1,036 | 0,0659 | 2,7670 | 0,1178 | 0,5890 | 0,0318 | 1,3370 | 0,0331 | 1,3900 |
| NOM | | 0,0610 | 2,5610 | 0,1042 | 4,3770 | 0,0317 | 1,3320 | 0,1069 | 4,4910 | 0,0779 | 3,2700 | 0,0805 | 3,3800 | 0,0482 | 2,0240 | 0,0469 | 1,9700 | 0,0585 | 2,4570 | 0,0475 | 1,9960 | 0,0858 | 3,6030 | 0,0980 | 4,1150 | 0,0480 | 2,0180 | 0,0461 | 1,9350 | 0,0310 | 1,3030 |
| DEP/EtOH VEH1 | 2/98% | 0,0719 | 3,0200 | 0,072 | 3,02 | 0,0212 | 0,8920 | 0,1243 | 5,2190 | 0,0920 | 3,8660 | 0,0708 | 2,9740 | 0,0486 | 2,0410 | 0,0427 | 1,7930 | 0,0522 | 2,1920 | 0,0406 | 1,7040 | 0,0847 | 3,5570 | 0,0706 | 2,9670 | 0,0695 | 2,9170 | 0,0507 | 2,1310 | 0,0370 | 1,5540 |
| DEP/EtOH VEH2 | 2/98% | 0,0742 | 3,1170 | 0,0754 | 3,1670 | 0,0396 | 1,6650 | 0,0928 | 3,8960 | 0,0806 | 3,3870 | 0,0734 | 3,0840 | 0,0432 | 1,8150 | 0,0461 | 1,9380 | 0,0435 | 1,8290 | 0,0374 | 1,5720 | 0,0737 | 3,0970 | 0,0991 | 4,1640 | 0,0525 | 2,2070 | 0,0417 | 1,7530 | 0,0339 | 1,4220 |

| Participant | | 24 | | 25 | | 31 | | 34 | | 38 | | 40 | | 41 | | 45 | | 49 | | 52 | | 53 | | 54 | | 57 | | 58 | | 59 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Original Patch Test | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | |
| Reaction | | COM | NOM | COM | NOM | COM | NOM | COM | NOM | COM | NOM | COM | NOM | COM | NOM | COM | NOM | COM | NOM | COM | NOM | COM | NOM | COM | NOM | COM | NOM | COM | NOM | COM | NOM |
| DEP | 100% | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| DEP/EtOH | 2/98% | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| EtOH | 100% | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| OM | 2,0% | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 1,32% | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ? | 0 | 0 | 0 | 0 | 0 |
| | 0,67% | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ? | 0 | 0 | 0 | 0 | 0 |
| | 0,22% | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ? | 0 | 0 | 0 | 0 | 0 |
| | 0,074% | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 0,025% | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ? | 0 | 0 | 0 | 0 | 0 |
| | 0,0082% | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 0,0027% | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 0,00091% | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 0,0003% | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 0,0001% | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 0,00003% | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ROAT | | No. of applications until positive reaction | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| COM | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | |
| NOM | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | |
| DEP/EtOH VEH1 | 2/98% | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | |
| DEP/EtOH VEH2 | 2/98% | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | |
| Amount used | | P.A. | Total | P.A. | Total | P.A. | Total | P.A. | Total | P.A. | Total | P.A. | Total | P.A. | Total | P.A. | Total | P.A. | Total | P.A. | Total | P.A. | Total | P.A. | Total | P.A. | Total | P.A. | Total | P.A. | Total |
| COM | | 0,1564 | 0,7820 | 0,0270 | 0,3780 | 0,0277 | 1,1630 | 0,0586 | 2,4628 | 0,0762 | 3,2010 | 0,084 | 0,76 | 0,0426 | 0,9790 | 0,0485 | 2,0380 | 0,1025 | 0,6150 | 0,0326 | 0,4570 | 0,0853 | 3,3270 | 0,0292 | 1,2250 | 0,0712 | 2,9890 | 0,0511 | 0,5110 | 0,0470 | 1,9720 |
| NOM | | 0,0635 | 2,6660 | 0,0263 | 1,1030 | 0,0314 | 1,3190 | 0,0542 | 2,2780 | 0,0935 | 3,9270 | 0,0819 | 3,4390 | 0,0432 | 1,8140 | 0,0471 | 1,9800 | 0,0432 | 1,8130 | 0,0237 | 0,997 | 0,0705 | 2,9620 | 0,0286 | 1,2030 | 0,0493 | 2,0690 | 0,0266 | 1,1190 | 0,0537 | 2,2570 |
| DEP/EtOH VEH1 | 2/98% | 0,0751 | 3,1530 | 0,0494 | 2,0750 | 0,0323 | 1,3570 | 0,0501 | 2,1030 | 0,1115 | 4,6840 | 0,0570 | 2,3960 | 0,0404 | 1,6950 | 0,0378 | 1,5860 | 0,0459 | 1,9270 | 0,0275 | 1,1570 | 0,0627 | 2,6340 | 0,0338 | 1,4200 | 0,0629 | 2,6430 | 0,032 | 1,327 | 0,0574 | 2,4100 |
| DEP/EtOH VEH2 | 2/98% | 0,0820 | 3,4420 | 0,0474 | 1,9913 | 0,0589 | 2,4740 | 0,0276 | 1,1590 | 0,0501 | 2,1060 | 0,0832 | 3,4950 | 0,0872 | 3,6620 | 0,0349 | 1,4640 | 0,0465 | 1,9520 | 0,0256 | 1,0770 | 0,0574 | 2,4100 | 0,0268 | 1,1270 | 0,0599 | 2,5150 | 0,0297 | 1,2470 | 0,0535 | 2,2470 |

# Oak Moss Allergics

| Participant | | 4 | | 15 | | 28 | | 32 | | 33 | | 35 | | 36 | | 37 | | 44 | | 3 | | 5 | | 30 | | 11 | | 19 | | 21 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Original patch test | | + | | + | | + | | + | | + | | + | | + | | + | | + | | ++ | | ++ | | ++ | | ++ | | ++ | | ++ | |
| Reaction | | COM | NOM | COM | NOM | COM | NOM | COM | NOM | COM | NOM | COM | NOM | COM | NOM | COM | NOM | COM | NOM | COM | NOM | COM | NOM | COM | NOM | COM | NOM | COM | NOM | COM | NOM |
| DEP | 100% | 0 | 0 | ? | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ? | 0 | 0 | 0 | 0 | 0 | + | +++ | + |
| DEP/EtOH | 2/98% | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ? | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ? | 0 |
| EtOH | 100% | 0 | 0 | ? | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| OM | 2,0% | +++ | ++ | ++ | + | ++ | 0 | +++ | 0 | 0 | 0 | + | 0 | ++ | ? | + | + | ++ | ? | ++ | + | + | 0 | 0 | 0 | ++ | + | ++ | ? | ++ | 0 |
| | 1,32% | ++ | ++ | +++ | + | + | 0 | ++ | 0 | 0 | 0 | + | 0 | ++ | ? | + | + | ++ | +++ | +++ | + | ++ | ? | 0 | 0 | +++ | + | ++ | 0 | +++ | 0 |
| | 0,67% | ++ | + | ++ | + | + | 0 | ++ | 0 | 0 | 0 | 0 | 0 | ++ | ? | + | ++ | ++ | ++ | ++ | + | + | ? | 0 | 0 | +++ | ? | + | 0 | ++ | 0 |
| | 0,22% | +++ | + | ++ | ? | ? | 0 | ++ | 0 | 0 | 0 | + | 0 | + | 0 | 0 | 0 | + | ? | ++ | + | + | + | 0 | 0 | ++ | 0 | + | + | ++ | 0 |
| | 0,074% | + | 0 | ++ | ? | 0 | 0 | + | 0 | 0 | 0 | 0 | 0 | + | 0 | 0 | 0 | 0 | ? | ++ | ? | ++ | ? | 0 | 0 | + | 0 | + | ? | +++ | 0 |
| | 0,025% | + | 0 | + | ? | 0 | 0 | + | 0 | 0 | 0 | 0 | 0 | + | 0 | 0 | 0 | ++ | 0 | + | 0 | + | + | 0 | 0 | + | 0 | + | ? | +++ | + |
| | 0,0082% | ? | 0 | + | ? | 0 | 0 | ? | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | + | 0 | ? | ? | 0 | 0 | + | 0 | ++ | ++ | ++ | + |
| | 0,0027% | 0 | 0 | ? | 0 | 0 | 0 | ? | 0 | 0 | 0 | 0 | ? | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ? | 0 | 0 | ? | 0 | ? | 0 | 0 | 0 |
| | 0,00091% | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ? | 0 | 0 | 0 | 0 | 0 |
| | 0,0003% | 0 | 0 | ? | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ? | 0 | 0 | 0 | 0 | 0 | ? | 0 | 0 | 0 |
| | 0,0001% | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | + | ? | 0 | 0 | 0 | 0 | 0 | + | ? | ? |
| | 0,00003% | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | + | 0 | 0 | 0 | 0 | 0 | 0 | +++ | 0 |
| ROAT | | No. of applications until positive reaction | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| COM | | 14 | | 10 | | - | | 5 | | - | | - | | 25 | | - | | 39 | | 28 | | 14 | | - | | 14 | | 14 | | 5 | |
| NOM | | - | | 33 | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | 28 | | - | |
| DEP/EtOH VEH1 | 2/98% | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | |
| DEP/EtOH VEH2 | 2/98% | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | |

Negative patch tests

| Amount used | | P.A. | Total | P.A. | Total | P.A. | Total | P.A. | Total | P.A. | Total | P.A. | Total | P.A. | Total | P.A. | Total | P.A. | Total | P.A. | Total | P.A. | Total | P.A. | Total | P.A. | Total | P.A. | Total | P.A. | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| COM | | 0,1924 | 0,9620 | 0,0220 | 0,5490 | 0,0857 | 3,6000 | 0,0057 | 0,2410 | 0,0925 | 3,8870 | 0,0760 | 3,1940 | 0,0583 | 2,4470 | 0,0808 | 3,3920 | 0,0511 | 0,7160 | 0,0611 | 0,8550 | 0,0134 | 0,5640 | 0,0478 | 1,3370 | 0,0147 | 0,6180 | 0,0466 | 0,6520 | 0,0173 | 0,2420 |
| NOM | | 0,0781 | 3,2810 | 0,0755 | 2,4920 | 0,0835 | 3,5080 | 0,0458 | 1,9250 | 0,0748 | 3,1420 | 0,0677 | 2,8430 | 0,1290 | 5,4170 | 0,0751 | 3,1560 | 0,0665 | 2,7950 | 0,0782 | 3,2841 | 0,0554 | 2,3250 | 0,0543 | 2,2810 | 0,0553 | 2,3220 | 0,0558 | 1,5620 | 0,0659 | 2,7680 |
| DEP/EtOH VEH1 | 2/98% | 0,0619 | 2,5980 | 0,1089 | 4,5750 | 0,0955 | 4,0130 | 0,0474 | 1,9890 | 0,0595 | 2,4970 | 0,0855 | 3,5890 | 0,1031 | 4,3300 | 0,1005 | 4,2200 | 0,0642 | 2,6970 | 0,0817 | 3,4300 | 0,1016 | 4,2670 | 0,0451 | 1,8960 | 0,0596 | 2,5020 | 0,0542 | 2,2750 | 0,0773 | 3,2470 |
| DEP/EtOH VEH2 | 2/98% | 0,0857 | 3,6000 | 0,0606 | 2,5440 | 0,0808 | 3,3940 | 0,0532 | 2,2330 | 0,0708 | 2,9720 | 0,0792 | 3,3270 | 0,1442 | 6,0580 | 0,0760 | 3,1930 | 0,0638 | 2,6800 | 0,0679 | 2,8534 | 0,0968 | 4,0640 | 0,0589 | 2,4740 | 0,0495 | 2,0800 | 0,0683 | 2,8680 | 0,0656 | 2,7540 |

| Participant | | 22 | | 26 | | 27 | | 29 | | 39 | | 42 | | 43 | | 46 | | 47 | | 48 | | 50 | | 55 | | 56 | | 60 | | 51 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Original Patch Test | | ++ | | ++ | | ++ | | ++ | | ++ | | ++ | | ++ | | ++ | | ++ | | ++ | | ++ | | ++ | | ++ | | ++ | | +++ | |
| Reaction | | COM | NOM | COM | NOM | COM | NOM | COM | NOM | COM | NOM | COM | NOM | COM | NOM | COM | NOM | COM | NOM | COM | NOM | COM | NOM | COM | NOM | COM | NOM | COM | NOM | COM | NOM |
| DEP | 100% | 0 | 0 | 0 | 0 | ? | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ++ | ++ | 0 | 0 | ? | 0 | ++ | ? | + | 0 | 0 | 0 | + | 0 | 0 | ? |
| DEP/EtOH | 2/98% | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ? | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| EtOH | 100% | 0 | 0 | ? | ? | 0 | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ? | ? | 0 | 0 | 0 | 0 | 0 |
| OM | 2,0% | ++ | ? | + | 0 | +++ | ? | 0 | 0 | +++ | 0 | +++ | + | +++ | ++ | +++ | ++ | + | 0 | +++ | + | +++ | +++ | +++ | + | +++ | ++ | +++ | +++ | +++ | + |
| | 1,32% | + | 0 | + | 0 | +++ | + | 0 | 0 | +++ | ? | ++ | + | +++ | + | +++ | +++ | + | 0 | +++ | + | +++ | +++ | +++ | + | +++ | + | ++ | + | +++ | + |
| | 0,67% | 0 | 0 | + | 0 | +++ | + | 0 | 0 | ++ | 0 | ++ | ? | +++ | ++ | +++ | +++ | 0 | 0 | +++ | 0 | +++ | ++ | +++ | + | +++ | + | ++ | +++ | +++ | ? |
| | 0,22% | + | 0 | + | 0 | ++ | 0 | 0 | 0 | ++ | ? | + | ? | +++ | + | +++ | ++ | 0 | 0 | +++ | ? | +++ | ? | ++ | ? | ++ | ? | ++ | + | ++ | ? |
| | 0,074% | 0 | 0 | ? | 0 | ++ | 0 | 0 | 0 | +++ | 0 | + | 0 | ++ | + | +++ | ++ | 0 | 0 | ++ | 0 | +++ | + | ++ | 0 | ++ | 0 | ++ | + | +++ | 0 |
| | 0,025% | ? | 0 | 0 | 0 | + | 0 | 0 | 0 | +++ | 0 | + | ? | ++ | + | +++ | ++ | 0 | 0 | ++ | 0 | +++ | + | ++ | 0 | ? | 0 | ++ | 0 | ++ | 0 |
| | 0,0082% | 0 | 0 | 0 | 0 | + | 0 | 0 | 0 | 0 | 0 | + | 0 | + | ? | ++ | + | 0 | 0 | + | 0 | +++ | + | ++ | 0 | ? | 0 | + | ? | ++ | 0 |
| | 0,0027% | 0 | 0 | ? | 0 | + | 0 | 0 | 0 | ? | 0 | 0 | 0 | + | 0 | +++ | ? | 0 | 0 | ? | 0 | + | 0 | + | 0 | 0 | 0 | + | 0 | + | 0 |
| | 0,00091% | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ++ | ? | 0 | 0 | 0 | ? | ? | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ? | 0 |
| | 0,0003% | 0 | 0 | 0 | 0 | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ++ | 0 | 0 | 0 | 0 | 0 | ? | 0 | ? | 0 | 0 | 0 | ? | 0 | ? | 0 |
| | 0,0001% | 0 | 0 | ? | 0 | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | + | 0 | 0 | 0 | 0 | ? | 0 | ? | ? | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 0,00003% | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | + | 0 | 0 | 0 | 0 | 0 | + | 0 | 0 | 0 | 0 | 0 | 0 | ? | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ROAT | | No. of applications until positive reaction | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| COM | | - | | 14 | | 14 | | - | | 28 | | 9 | | 5 | | 14 | | - | | 4 | | 5 | | 14 | | 23 | | 6 | | 5 | |
| NOM | | - | | - | | - | | - | | - | | 14 | | 28 | | 30 | | - | | - | | 11 | | - | | - | | - | | - | |
| DEP/EtOH VEH1 | 2/98% | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | |
| DEP/EtOH VEH2 | 2/98% | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | | - | |

Positive DEP

| Amount used | | P.A. | Total | P.A. | Total | P.A. | Total | P.A. | Total | P.A. | Total | P.A. | Total | P.A. | Total | P.A. | Total | P.A. | Total | P.A. | Total | P.A. | Total | P.A. | Total | P.A. | Total | P.A. | Total | P.A. | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| COM | | 0,0537 | 2,2560 | 0,0232 | 0,9730 | 0,0237 | 0,9950 | 0,0490 | 2,0590 | 0,0627 | 0,8780 | 0,0211 | 0,8870 | 0,0260 | 0,3640 | 0,0199 | 0,8360 | 0,0378 | 1,5880 | 0,0058 | 0,2440 | 0,0091 | 0,3820 | 0,0238 | 0,9990 | 0,0370 | 1,5520 | 0,0087 | 0,3670 | 0,0558 | 0,2790 |
| NOM | | 0,0478 | 2,0060 | 0,0911 | 3,8250 | 0,0804 | 3,3760 | 0,0439 | 1,8430 | 0,0843 | 3,5410 | 0,0909 | 1,2730 | 0,0489 | 1,3700 | 0,0943 | 2,8290 | 0,0297 | 1,2460 | 0,0371 | 1,5570 | 0,0251 | 1,0540 | 0,0727 | 3,0540 | 0,0705 | 2,9620 | 0,0523 | 2,1970 | 0,0365 | 1,5310 |
| DEP/EtOH VEH1 | 2/98% | 0,0482 | 2,0250 | 0,0676 | 2,8390 | 0,0580 | 2,4340 | 0,0697 | 2,9290 | 0,1093 | 4,5900 | 0,1031 | 4,3290 | 0,0452 | 1,8980 | 0,0844 | 3,5460 | 0,0319 | 1,3410 | 0,0606 | 2,5460 | 0,1120 | 4,7050 | 0,0585 | 2,4590 | 0,0560 | 2,3520 | 0,0501 | 2,1050 | 0,0352 | 1,4790 |
| DEP/EtOH VEH2 | 2/98% | 0,0573 | 2,4060 | 0,0752 | 3,1570 | 0,1090 | 4,5800 | 0,0527 | 2,2120 | 0,0844 | 3,5440 | 0,0713 | 2,9940 | 0,0456 | 1,9150 | 0,0823 | 3,4580 | 0,0320 | 1,3450 | 0,0525 | 2,2070 | 0,1175 | 4,9340 | 0,0727 | 3,0520 | 0,0525 | 2,2050 | 0,0410 | 1,7240 | 0,0372 | 1,5610 |

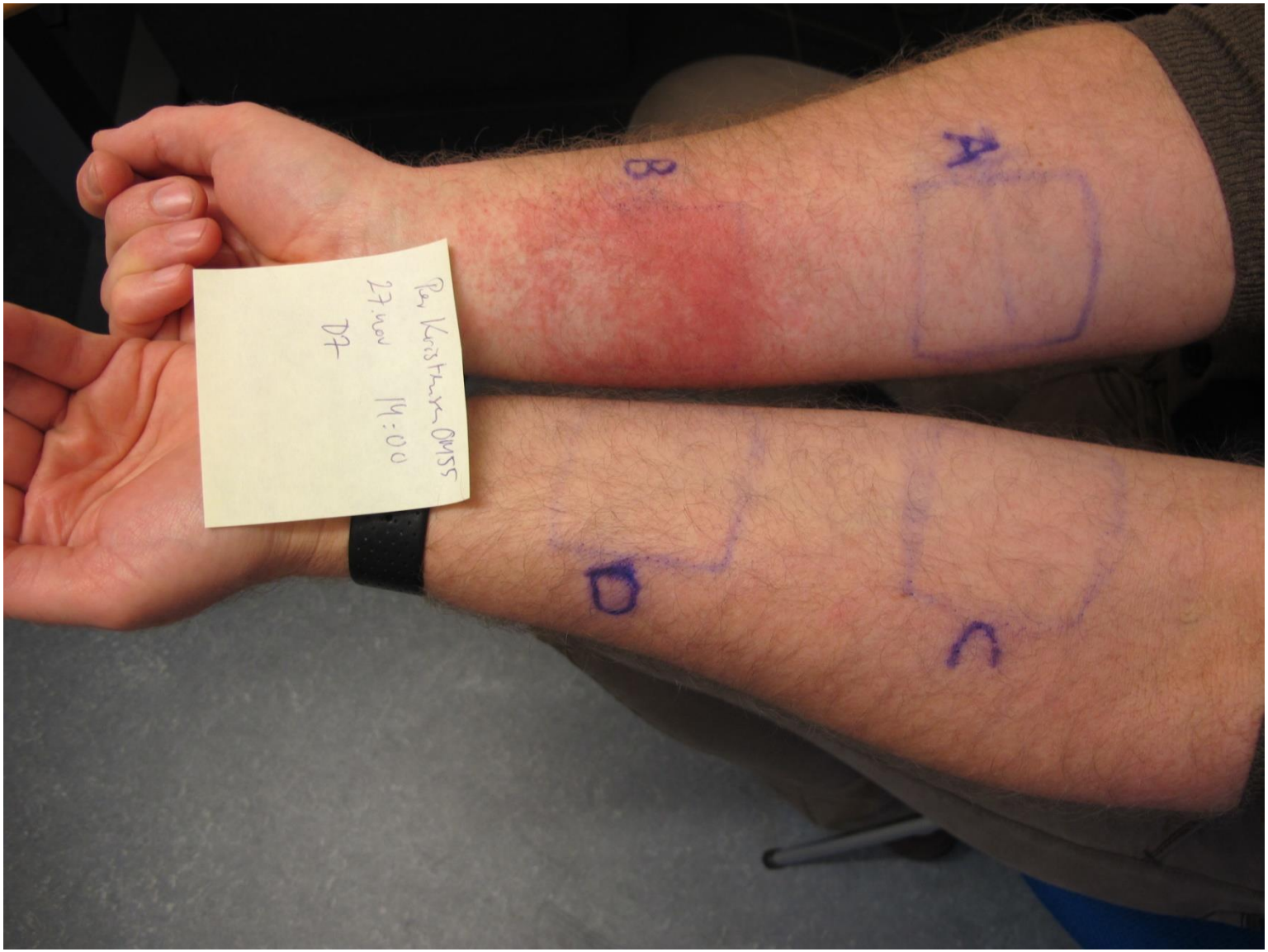
# Results – patch tests

•16/27 with positive reactions to both COM and NOM reacted to COM at a significantly lower concentration than to NOM (Wilcoxon matched-pairs signed-ranks test, P = 0,0078).

Dermatological Investigations Scandinavia™

OUH Odense Universitetshospital Svendborg Sygehus

# Positive response to Classic Oak Moss



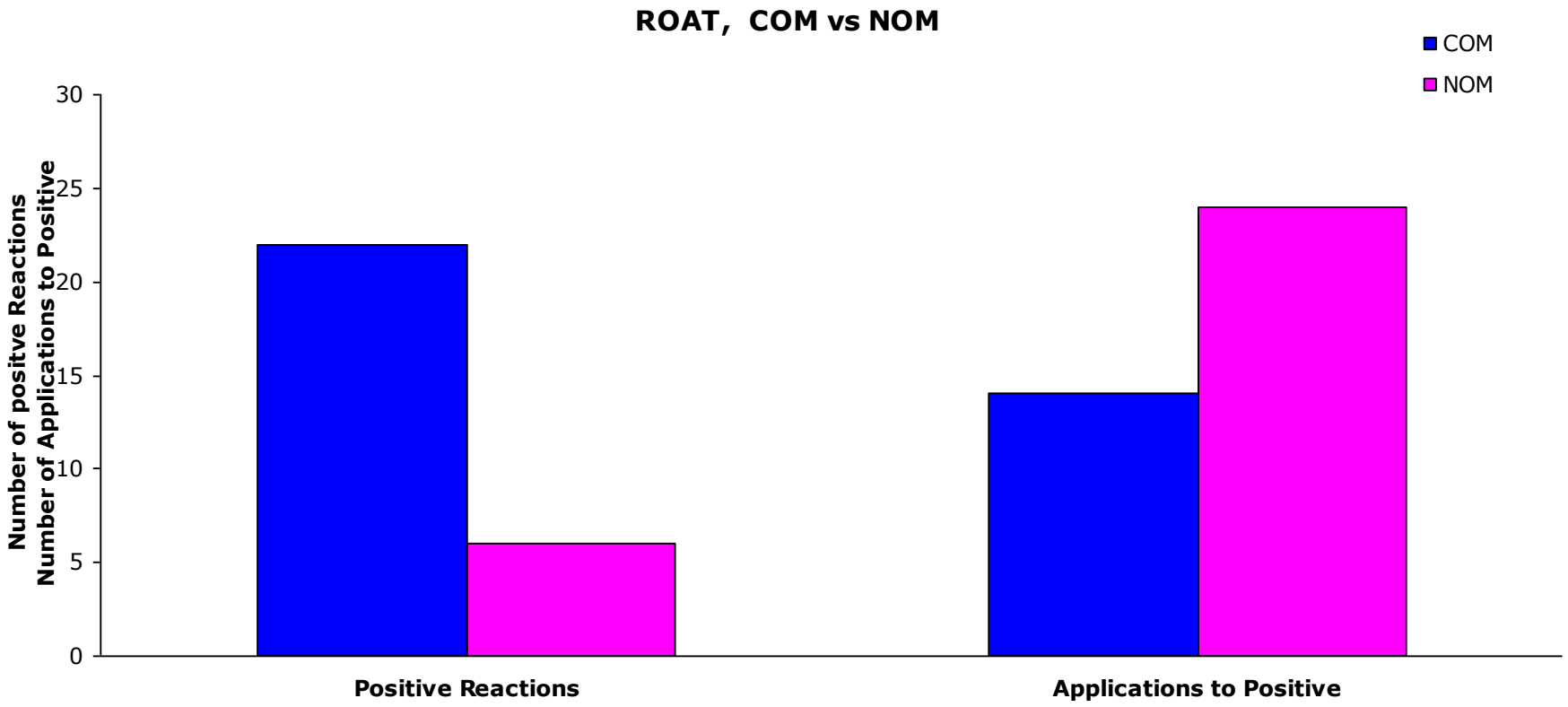
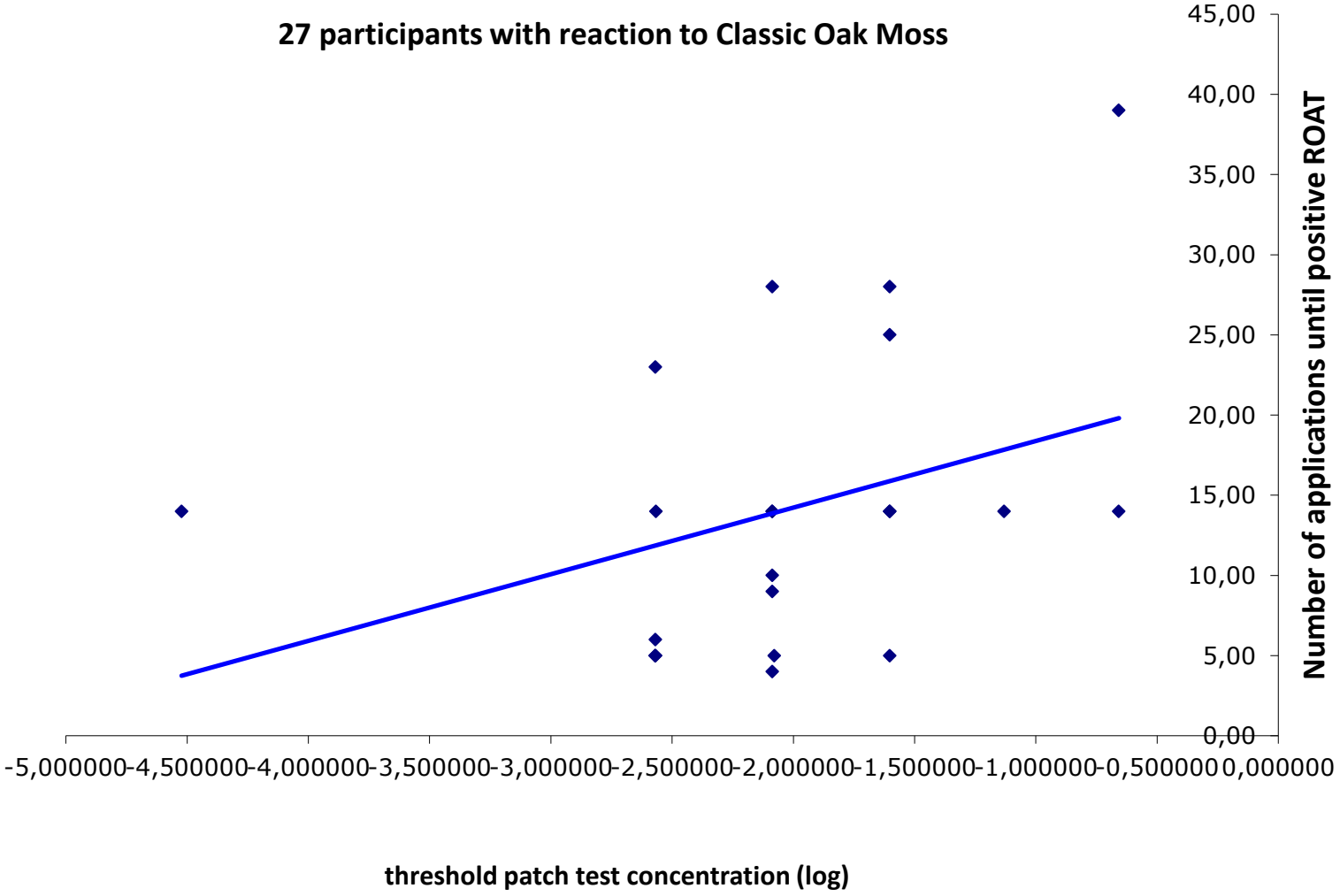



**Figure 1. Outcome of ROAT with Classic and New Oak Moss**

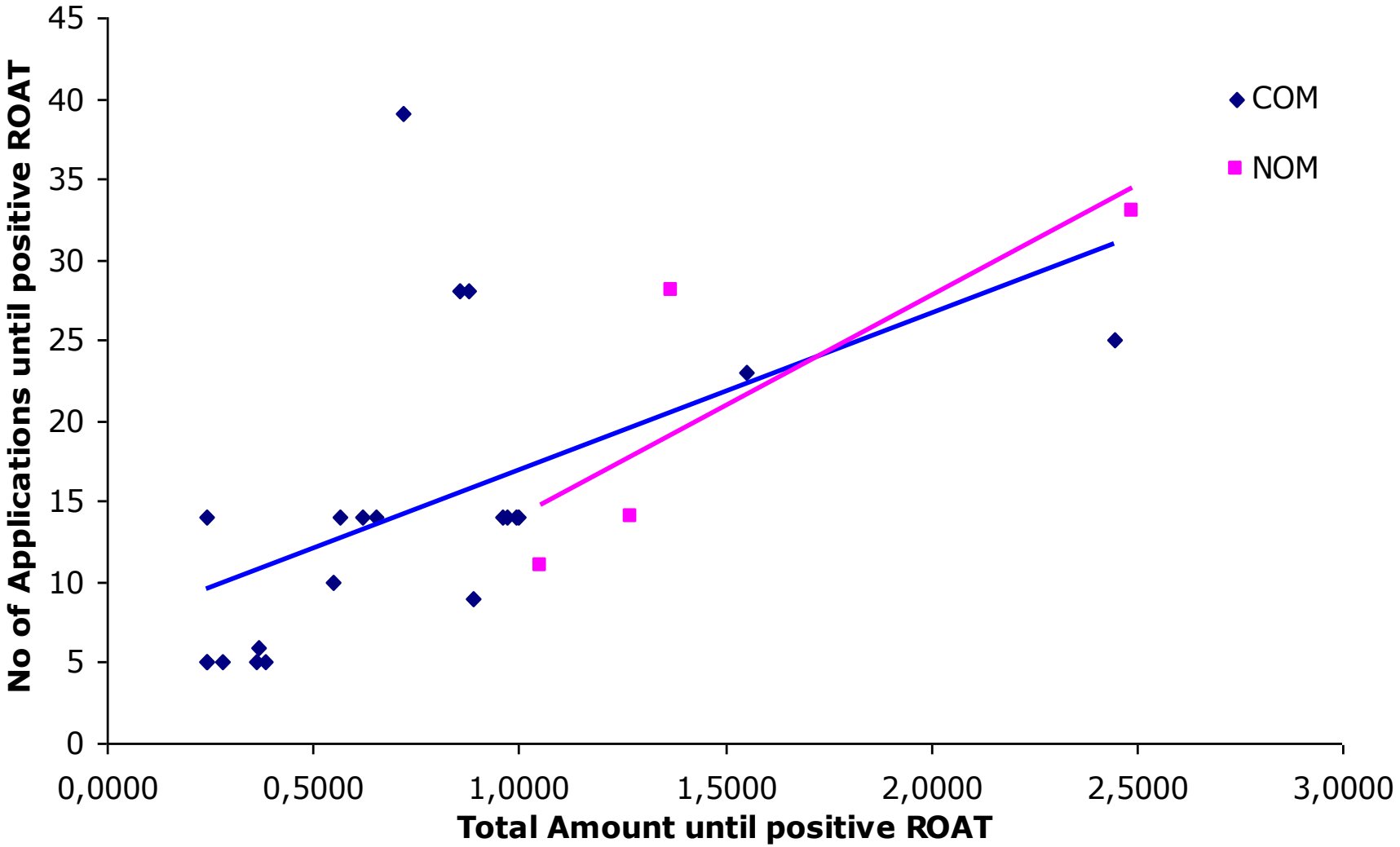
Neither Controls nor known Allergics reacted to vehicle

No Controls reacted to neither COM nor NOM

22/30 Allergics reacted to COM, and 6 of these also reacted to NOM, nobody reacted to NOM alone.




The number of applications until a positive ROAT in relation to the threshold patch test concentration




Relationship between Total amount of OM used to provoke a positive reaction and Number of Applications until positive ROAT

**Conclusions**

New oak moss absolute (NOM) elicited significantly less allergic contact dermatitis in previously oak moss sensitive participants in a human experimental exposure model compared to classic oak moss absolute (COM).

NOM appears to be less likely to induce OM-allergy in previous non-allergic persons, and significantly less likely to elicit an allergic response in persons with previously diagnosed OM allergy

Patch and ”use” tests and diagnose perfume allergy

Current challenges:

- Improved screening test materials
- Test with patient´s own products when possible
- Patch test concentrations
- More dose-response tests in volunteers with documented fragrance allergy