

Hydroxyisohexyl 3-cyclohexene carboxaldehyde allergy: relationship between patch test and repeated open application test thresholds

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Summary

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Conflicts of interest

None declared.

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Background Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) is a synthetic fragrance ingredient. Case reports of allergy to HICC appeared in the 1980s, and HICC has recently been included in the European baseline series. Human elicitation dose-response studies performed with different allergens have shown a significant relationship between the patch-test threshold and the repeated open application test (ROAT) threshold, which mimics some real-life exposure situations. Fragrance ingredients are special as significant amounts of allergen may evaporate from the skin.

Objectives The study aimed to investigate the relationship between elicitation threshold doses at the patch test and the ROAT, using HICC as the allergen. The expected evaporation rate was calculated.

Materials and methods Seventeen HICC-allergic persons were tested with a dilution series of HICC in a patch test and a ROAT (duration up to 21 days). Seventeen persons with no HICC allergy were included as control group for the ROAT.

Results The response frequency to the ROAT (in $\mu\text{g HICC cm}^{-2}$ per application) was significantly higher than the response frequency to the patch test at one of the tested doses. Furthermore the response rate to the accumulated ROAT dose was significantly lower at half of the doses compared with the patch test. The evaporation rate of HICC was calculated to be 72% over a 24-h period.

Conclusions The ROAT threshold in dose per area per application is lower than the patch test threshold; furthermore the accumulated ROAT threshold is higher than the patch test threshold, which can probably be explained by the evaporation of HICC from the skin in the open test.

Hydroxyisohexyl-3-cyclohexene carboxaldehyde (HICC, CAS number: 31906-04-4) is a synthetic fragrance ingredient. It has been used for more than 30 years¹ in consumer products such as deodorants, lotion, perfumes and household products. The initial testing of the allergenic potential of HICC in animal and human studies was negative.² Case reports of allergy to HICC began to appear in the 1980s,³⁻⁵ and the incidence of allergy to HICC among consecutive patch-tested patients is 1.9–2.8% in different European studies.^{1,6-8} HICC was included in the European baseline series in 2008⁹ and is one of the 26 fragrance ingredients which has to be labelled on cosmetics products, according to the EU legislation, because of its allergenic potential. Following the demonstration of HICC as a significant problem in consumers, HICC was identi-

fied as an allergen in an animal test: the local lymph node assay.¹⁰ Since 2003 the International Fragrance Association has had a standard on the use of HICC with a recommended maximum concentration of 1.5% (<http://www.ifraorg.org/Home/Code,+Standards+Compliance/IFRA+Standards/page.aspx/56>, accessed 23 February 2009). This limit was set to prevent induction of allergy, and product labelling was introduced to help people already sensitized to avoid exposure. But because a considerable number of individuals are already sensitized, and some might not be diagnosed, and those who are diagnosed have difficulties in reading the labelling and identifying the allergens to which they are allergic,^{11,12} a maximum limit should also prevent elicitation. Exposure studies showed that among sensitized patients 11 of 18 (61%)

reacted to a repeated open application test (ROAT) of 0.5% HICC¹³ and nine of 14 (64%) reacted to a repeated deodorant exposure of 0.02% HICC,¹⁴ so more than half of sensitized individuals are not protected by the current recommendations. Such data call for a more systematic risk assessment of allergens, according to the real-life exposure. A central point is the transformation of elicitation patch-test data into more realistic exposure situations. Several human elicitation dose-response studies^{13,15–21} performed with different allergens have shown a significant relationship between patch-test threshold and the ROAT threshold, which mimics some real-life exposure situations. Knowledge of the real-life allergen hazard can be used in preventive strategies and has proven effective with legislation regarding chromium,²² nickel^{23–26} and methyldibromoglutaronitrile.^{27–29}

The results from two experimental elicitation studies^{30,31} performed by the authors have shown that significantly more allergic persons reacted in a ROAT, compared with the same dose in the patch test, when testing the nonvolatile compounds nickel and methyldibromoglutaronitrile. When looking at the accumulated dose in the ROAT, approximately the same number of persons reacted compared with the same dose in the patch test. The original ROAT³² is designed to last only 7 days, but because many allergens are often applied for long time periods, we chose a duration of 3 weeks.

The aim of this experimental study was to investigate if the results from the nickel and methyldibromoglutaronitrile studies also apply for volatile compounds such as fragrance ingredients by using HICC as the allergen. The physical properties of volatile compounds are different from many other allergens, because they evaporate from the skin surface,³³ meaning that the accumulated allergen exposure over time might be lower in an open test compared with an occluded test. In this study, the elicitation dose-response in single occluded exposure was compared with the repeated open exposure with HICC. Furthermore an estimate of the expected absorption and evaporation of HICC from the skin was calculated using a diffusion/evaporation model.³³

Materials and methods

Test subjects

The inclusion criteria were age over 18 years, and a former positive standard patch test to HICC 5% in pet., with a positive reaction defined by at least 1+ according to the International Contact Dermatitis Research Group (ICDRG) criteria.³⁴ The exclusion criteria were active eczema, systemic immunosuppressive therapy, ultraviolet light on the test area within 3 weeks of the testing, pregnancy, breastfeeding and not being able to cooperate. Seventeen patients with contact allergy to HICC, demonstrated by a positive standard patch test to HICC within the last 6 years at the Department of Dermatology, Gentofte Hospital, University of Copenhagen, Denmark, or at the skin clinic, Rødovre, Denmark, were enrolled in the study after oral and written consent. The test subjects

were aged 22–64 years (mean 47.8 years). Fourteen women and three men participated in the study.

Control subjects

Inclusion and exclusion criteria were the same as for the test subjects, except for the required negative patch test to HICC. Seventeen control persons, all responders to an advertisement, were enrolled in the study after oral and written consent. Two of the control subjects withdrew from the study before the start-up of the ROAT, so 15 control subjects, eight women and seven men, completed the study. The control subjects were aged 23–66 years (mean 37.1 years).

Study design and materials

The study was performed with the same methodology as previously described for nickel³⁰ and methyldibromoglutaronitrile.³¹ The patch-test material was HICC in 70% ethanol with 30% water and the control was 70% ethanol with 30% water. The HICC was kindly donated by the Research Institute for Fragrance Materials (Woodcliff Lake, NJ, U.S.A.) and was produced by International Flavors and Fragrances Inc. (New York, NY, U.S.A.), lot no. SM/8059062. The doses were chosen so that the dose per single application in the ROAT was present in the patch test, except for the highest ROAT concentration. The accumulated 1-week, 2-week and 3-week doses from the ROAT were also present in the patch test in order to facilitate a comparison of the threshold for the two test methods (Table 1). Except for the highest ROAT concentration, the doses were chosen so that they were similar to the doses in the nickel and methyldibromoglutaronitrile studies. The placement of the different test concentrations in both tests was randomized and blinded for the investigator and the subjects. After termination of the study the randomization code was broken. The study was performed according to the Helsinki II declaration and the study was approved by the local ethics committee (the capital region of Denmark, KA04032).

Patch test

Each test subject was tested with the following dilution series of HICC in 70% ethanol with 30% water: [in μg HICC cm^{-2} skin (μg HICC cm^{-2}): 1500, 300, 150, 100, 50, 25, 15, 10, 5, 3.57, 1.5, 1, 0.5, 0.357, 0.1786, 0.0893, 0.0357, 0.0179, 0.0022 and one blank containing the vehicle only. Fifteen microlitres of each dilution was applied on a filter paper in a small Finn Chamber of 0.5 cm^2 (Smart Practice Finland OY, Tuusula, Finland) on Scanpor[®] tape (Actavis Norway AS, Norgesplaster, Vennesla, Norway).

The patch test was applied on day 0, occluded for 2 days and the reactions were observed on days 2, 3 and 7, but only the second reading was used for the statistical calculations. The reactions were also strongest on day 3. The reading scale for the patch test was based on the ICDRG reading scale, and further developed by Hindsén and Bruze³⁵ in order to

Table 1 Study design. The dilution series in the patch test and the equivalent exposure using the ROAT

Patch-test dilution series ($\mu\text{g HICC cm}^{-2}$)	Equivalent ROAT exposure ($\mu\text{g HICC cm}^{-2}$)
1500	Accumulated 3-week dose (35.7 per app.) in the ROAT
300	
150	Accumulated 3-week dose (3.57 per app.) in the ROAT
100	Accumulated 2-week dose (3.57 per app.) in the ROAT
50	Accumulated 1-week dose (3.57 per app.) in the ROAT
25	
15	Accumulated 3-week dose (0.357 per app.) in the ROAT
10	Accumulated 2-week dose (0.357 per app.) in the ROAT
5	Accumulated 1-week dose (0.357 per app.) in the ROAT
3.57	Highest middle dose per application in the ROAT (3.57)
1.5	Accumulated 3-week dose (0.0357 per app.) in the ROAT
1	Accumulated 2-week dose (0.0357 per app.) in the ROAT
0.5	Accumulated 1-week dose (0.0357 per app.) in the ROAT
0.357	Lowest middle dose per application in the ROAT (0.357)
0.1786	
0.0893	
0.0357	Lowest dose per application in the ROAT (0.0357)
0.0179	
0.0022	

ROAT, repeated open application test; HICC, hydroxyisohexyl-3-cyclohexene carboxaldehyde; **bold**, doses also present in the ROAT.

recognize smaller differences in the allergic reactions. The scale used was a modification of this scale to include weaker reactions. The scale was as follows: 0 = no reaction; 1 = few papules with no erythema, no infiltration; 2 = faint erythema with no infiltration or papules; 3 = faint erythema with few

papules and no homogeneous infiltration; 4 = erythema, homogeneous infiltration; 5 = erythema, infiltration and a few papules; 6 = erythema, infiltration and papules; 7 = erythema, infiltration, papules and a few vesicles; 8 = intensive erythema, infiltration and vesicles. The threshold concentration was defined as the weakest concentration giving a visible reaction on day 3 in a continuous line of patch-test reactions, starting from the highest test concentration. The same investigator did all readings.

Repeated open application test

Each test subject was tested on four to five areas on the volar aspect of the forearms on areas of 3×3 cm. Twenty microlitres was applied twice daily using a micropipette (Acura 815, 20 μL , Buch & Holm A/S, Herlev, Denmark) with a fixed volume. The test subjects received four to five numbered containers every week. The containers were stored in refrigerators to minimize evaporation. Each container contained a numbered bottle for each day. Each number referred to a specific area. The solutions were spread on the area with the tip of the pipette and allowed to dry by evaporation. The subjects were instructed thoroughly, both orally and manually, in using the pipette. They also received written instructions. The areas were tested with a dose per application of either 35.7, 3.57, 0.357 or 0.0357 $\mu\text{g HICC cm}^{-2}$. These doses per application correspond to accumulated 1-week doses of 500, 50, 5 and 0.5 $\mu\text{g HICC cm}^{-2}$, accumulated 2-week doses of 1000, 100, 10 and 1 $\mu\text{g HICC cm}^{-2}$ and accumulated 3-week doses of 1500, 150, 15 and 1.5 $\mu\text{g HICC cm}^{-2}$, respectively (Table 2). Because only a few test subjects reacted to 3.57 $\mu\text{g HICC cm}^{-2}$ in the first weeks of the study, it was decided to add 35.7 $\mu\text{g HICC cm}^{-2}$ to the ROAT 3 weeks after the beginning of inclusion. Thus test subjects numbers 501, 505, 506, 507, 510 and 511 were not tested with the 35.7 $\mu\text{g HICC cm}^{-2}$ because they already had a reaction to 3.57 $\mu\text{g HICC cm}^{-2}$ at the time that the high dose was added to the study. The fifth area was a control site exposed to 70% ethanol with 30% water. The control subjects were tested on two areas with 0.0357 $\mu\text{g HICC cm}^{-2}$ and 70% ethanol, respectively. The reactions were read on all subjects at days 7, 14 and 21 of exposure routinely and additionally if a reaction occurred. In the case of a score of 5 or above (based on the

Table 2 The dose per application and accumulated dose after 1, 2 and 3 weeks in the ROAT

ROAT, dose per application ($\mu\text{g HICC cm}^{-2}$)	Number of applications after 1 week	Total accumulated dose after 1 week ($\mu\text{g HICC cm}^{-2}$)	Number of applications after 2 weeks	Total accumulated dose after 2 weeks ($\mu\text{g HICC cm}^{-2}$)	Number of applications after 3 weeks	Total accumulated dose after 3 weeks ($\mu\text{g HICC cm}^{-2}$)
35.7	14	500	28	1000	42	1500
3.57	14	50	28	100	42	150
0.357	14	5	28	10	42	15
0.0357	14	0.5	28	1	42	1.5

ROAT, repeated open application test; HICC, hydroxyisohexyl-3-cyclohexene carboxaldehyde.

involved area, erythema, number of papules and number of vesicles), the exposure to that area was terminated. If no reaction occurred, or in the case of a score below 5, the exposure was terminated on day 21. The same investigator did the reading of the reactions on all the subjects using a ROAT reading scale developed previously.³⁶ The threshold concentration was defined as the weakest concentration giving a visible reaction, which was also present at the end of week 3 (if the exposure had not been terminated) or the weakest concentration giving a reaction with a score of 5 or above.

Evaporation

Evaporation of HICC from unoccluded skin in the ROAT was estimated by the method of Kasting and Saiyasombati.³³ Calculations were conducted for the doses and dose regimen employed in the ROAT assuming an indoor environment, no rub-off, wash-off or chemical degradation during the exposure time and a skin temperature of 32 °C. Physical properties of HICC relevant to this calculation were estimated at skin temperature as follows. A vapour pressure of 5.045×10^{-3} mmHg (torr) was estimated from the boiling point of 120–122 °C at 133 Pa (1 torr) (<http://www.chemblink.com/products/31906-04-4.htm>, accessed 23 February 2009). The method used was Method 1, Eqn 14–25, from Lyman *et al.*³⁷ A calculated log octanol/water partition coefficient (CLOGP) of 2.15 was obtained using the CLOGP program V. 2.0, 1999 (BioByte, Inc., Claremont, CA, U.S.A.). Water solubility was estimated to be 4.71 g L^{-1} using the method of Jain and Yalkowski³⁸ and the calculated CLOGP. A density of 0.994 g cm^{-3} was obtained (see <http://www.chemblink.com/products/31906-04-4.htm>, accessed 23 February 2009).

Statistics

Standard logistic regression analysis was used to estimate the dose–response relation in the patch test. The elicitation dose ED_{xx} , which predicts a dose that will elicit a reaction in 50%, 25%, 10%, 5% and 1% of subjects, respectively, was calculated and a fitted dose–response curve for the patch test was drawn. A comparison of elicitation reactions to the dose per application in the ROAT ($\mu\text{g HICC cm}^{-2}$) and the elicitation reactions to the corresponding dose in the patch test ($\mu\text{g HICC cm}^{-2}$) was performed using McNemar's test. As $35.7 \mu\text{g HICC cm}^{-2}$ was not present in the patch test, $50 \mu\text{g HICC cm}^{-2}$ was used for the comparison. A fitted dose–response curve for the patch test and the dose–response curve for the ROAT, expressed in dose per application, was calculated and drawn. In addition the elicitation reactions to the accumulated 1-week, 2-week and 3-week doses in the ROAT ($\mu\text{g HICC cm}^{-2}$) were compared with the elicitation reactions to the corresponding doses in the patch test ($\mu\text{g HICC cm}^{-2}$) also using McNemar's test. As 500 and $1000 \mu\text{g HICC cm}^{-2}$ were not present in the patch test, the nearest doses were used. The dose difference did not have any practical consequences because 100% of subjects had a

positive patch-test reaction to $150 \mu\text{g HICC cm}^{-2}$ and would therefore also have had a positive reaction to 500 and $1000 \mu\text{g HICC cm}^{-2}$. A fitted dose–response curve for the patch test and the 1-week, 2-week and 3-week accumulated dose–response curves for the ROAT was drawn. When the patch test and the ROAT dose–response reactions were compared, only the results from the 16 subjects who participated in both tests were used.

Results

Patch test

Seventeen HICC-allergic persons participated in the patch test. All the test subjects had a reaction to at least one of the patch test HICC concentrations (Table 3). The lowest threshold concentration observed was $0.0022 \mu\text{g HICC cm}^{-2}$. This person was excluded from the ROAT because of a beginning flare reaction at the original site of the HICC eczema (itching, no visible eczema). The lowest concentration in the patch test giving a + reaction (according to the ICDRG criteria) in this person was $50 \mu\text{g HICC cm}^{-2}$. Two persons had a weak reaction (score = 1) to the ethanol patch. The threshold for these two persons was $50 \mu\text{g HICC cm}^{-2}$.

The calculated dose with a response frequency of 50% (ED_{50}) was $11.1 \mu\text{g HICC cm}^{-2}$; ED_{10} was $0.662 \mu\text{g HICC cm}^{-2}$ and

Table 3 Results of patch testing with HICC in all 17 test subjects (A) and in the 16 test subject who also performed the ROAT (B)

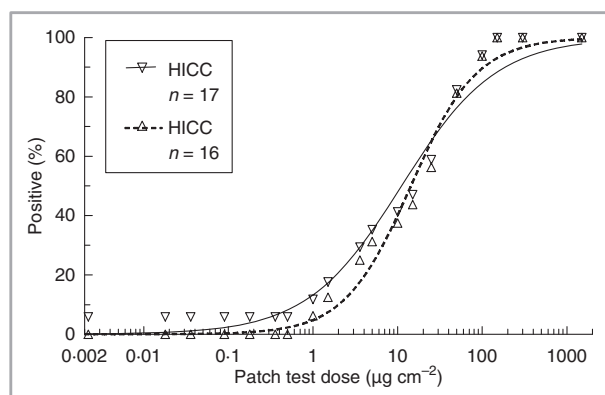
Patch test conc. ($\mu\text{g HICC cm}^{-2}$)	No. of test subjects reacting (A)	% reacting	No. of test subjects reacting (B)	% reacting
1500	17	100	16	100
300	17	100	16	100
150	17	100	16	100
100	16	94.1	15	93.8
50	14	82.4	13	81.3
25	10	58.8	9	56.3
15	8	47.1	7	43.8
10	7	41.2	6	37.5
5	6	35.3	5	31.3
3.57	5	29.4	4	25
1.5	3	17.6	2	12.5
1	2	11.8	1	6.3
0.5	1	5.9	0	0
0.357	1	5.9	0	0
0.1786	1	5.9	0	0
0.0893	1	5.9	0	0
0.0357	1	5.9	0	0
0.0179	1	5.9	0	0
0.0022	1	5.9	0	0

ROAT, repeated open application test; HICC, hydroxyisohexyl-3-cyclohexene carboxaldehyde. **Bold**, corresponds to the dose per application, the accumulated 1-week, 2-week and 3-week doses in the ROAT.

Table 4 Predicted doses (ED_{xx}) ($\mu\text{g HICC cm}^{-2}$) from the patch test with 95% confidence limits

Probability (%)	Patch test calculated dose-response		
	ED_{xx}	Upper limit	Lower limit
50	11.1	33.1	3.41
25	2.71	7.79	0.478
10	0.662	2.35	0.052
5	0.254	1.1	0.011
1	0.031	0.225	0.0003

HICC, hydroxyisohexyl-3-cyclohexene carboxaldehyde.

**Fig 1.** Observed (triangles) and calculated logistic dose-response curve from the patch test for all 17 subjects participating in the patch test (solid line) and for the 16 subjects who also participated in the ROAT (broken line).

ED_1 was $0.031 \mu\text{g HICC cm}^{-2}$ (Table 4). The fitted dose-response curve for all 17 test subjects and for the 16 test subjects who also participated in the ROAT is shown in Figure 1, and it is seen that they are very close to one another.

Repeated open application test

Sixteen HICC-allergic persons were included in the ROAT. Fifteen of the 16 test subjects had a reaction to $35.7 \mu\text{g HICC cm}^{-2}$ within the 3 weeks of exposure. The test subjects who were not tested with the highest dose, because they already had a reaction to the lower dose, were counted as positive to the highest, as it is expected that if one has a reaction to a lower dose, then this person will also react to the higher dose. Twelve of 16 had a reaction to $3.57 \mu\text{g HICC cm}^{-2}$, three of 16 had a reaction to $0.357 \mu\text{g HICC cm}^{-2}$, none had a reaction to $0.0357 \mu\text{g HICC cm}^{-2}$, and none had a reaction to the ethanol control site (Table 5). Seventeen control subjects were included in the study, but two chose to withdraw from the study, so 15 participated in the whole study. None had a reaction to either $0.0357 \mu\text{g HICC cm}^{-2}$ or the ethanol control site.

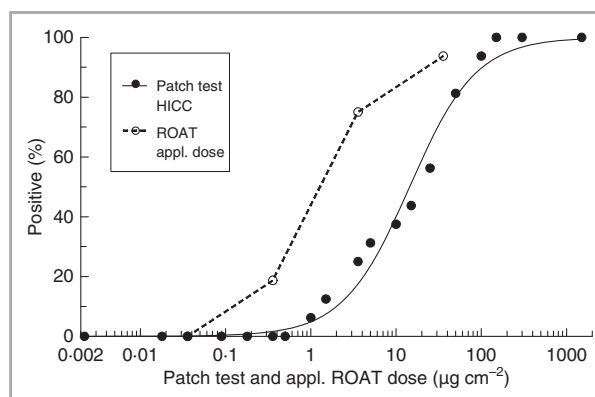
Table 5 The response frequency in the patch test compared with the response status in the ROAT (the dose per application) after 3 weeks

Dose ($\mu\text{g HICC cm}^{-2}$)	Patch test; response frequency (%)	ROAT; response frequency after 3 weeks (%)		P-value (McNemar's test)
35.7 ^a	81.3	93.8		0.32
3.57	25	75		0.0047
0.357	0	18.8		0.083
0.0357	0	0		nd

^a50 $\mu\text{g HICC cm}^{-2}$ in the patch test. ROAT, repeated open application test; HICC, hydroxyisohexyl-3-cyclohexene carboxaldehyde; nd, not defined.

Comparison of the patch test and the repeated open application test

The response frequencies of the dose per application in the ROAT were compared with the response frequencies to the same dose in the patch test. When $35.7 \mu\text{g HICC cm}^{-2}$ was applied per application in the ROAT, 15 of 16 (93.8%) had a reaction within the 3 weeks of exposure. In the patch test 13 of 16 (81.3%) had a reaction to $50 \mu\text{g HICC cm}^{-2}$. This difference in response frequencies was not statistically significant. When $3.57 \mu\text{g HICC cm}^{-2}$ was applied per application in the ROAT, 12 of 16 (75%) had a response within the 3 weeks of exposure, whereas in the patch test four of 16 (25%) had a reaction; this difference was statistically significant ($P = 0.0047$). When $0.357 \mu\text{g HICC cm}^{-2}$ was applied per application in the ROAT three of 16 (18.8%) had a reaction, and in the patch test none had a reaction to this dose; this difference was not statistically significant ($P = 0.083$). None had a reaction to $0.0357 \mu\text{g HICC cm}^{-2}$ in either test (when only the 16 subjects participating in both tests are included). These results are shown in Table 5 and illustrated by Figure 2. Even

**Fig 2.** The fitted dose-response curve for the patch test ($n = 16$) (solid line) and the observed response after 3 weeks of exposure to the dose per application in the ROAT (broken line).

though only one of the ROAT responses was significantly different from the patch test, it is seen that the ROAT curve is displaced to the left compared with the patch test curve, indicating that the dose per application needed to elicit a reaction in the ROAT was lower than the dose needed to elicit a reaction in the patch test. Then we added up the doses applied everyday in the ROAT to an accumulated 1-week, 2-week and 3-week dose. For each week a curve for the ROAT response was drawn using the response frequency from the accumulated weekly doses for the four different concentrations. These dose–responses were compared with the equivalent doses in the patch test. In half of the doses (5, 10, 15, 50, 150 and 500 $\mu\text{g HICC cm}^{-2}$) there was a significantly higher response frequency in the patch test compared with the ROAT. These results are shown in Table 6 and illustrated by Figure 3. It can

Table 6 The response frequency in the patch test compared with the response status in the ROAT (the accumulated dose after 1, 2 and 3 weeks, respectively)

Dose ($\mu\text{g HICC cm}^{-2}$)	Patch test; response frequency in %	ROAT; response frequency in %	P-value (McNemar's test)
0.5 ^a	0	0	nd
1 ^b	6.3	0	0.32
1.5 ^c	12.5	0	0.16
5 ^a	31.3	6.3	0.046
10 ^b	37.5	12.5	0.046
15 ^c	43.8	18.8	0.046
50 ^a	81.3	31.3	0.0047
100 ^b	93.8	62.5	0.059
150 ^c	100	75	0.046
500 ^a	nt	62.5	0.014
1000 ^b	nt	93.8	0.32
1500 ^c	100	93.8	0.32

^aAccumulated 1-week dose. ^bAccumulated 2-week dose. ^cAccumulated 3-week dose. ROAT, repeated open application test; HICC, hydroxyisohexyl-3-cyclohexene carboxaldehyde; nd, not defined; nt, not tested (assumed 100%).

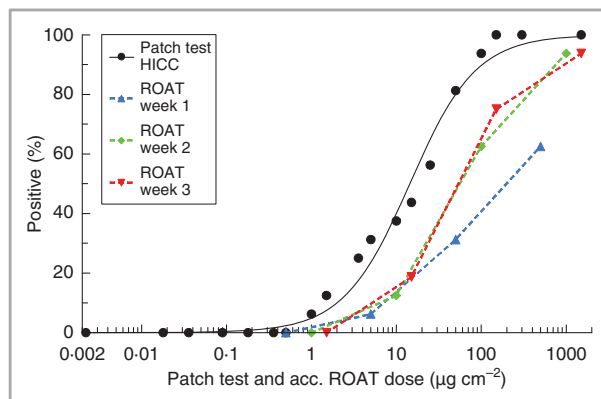


Fig 3. The fitted dose–response curve for the patch test ($n = 16$) and the 1-week, the 2-week 3-week accumulated ROAT doses.

be seen that the accumulated ROAT dose–response curve is displaced to the right compared with the patch test dose–response curve. This indicates that the response to the accumulated dose in the ROAT was lower than the response to the corresponding dose in the patch test.

Evaporation

The calculations indicated that 72–75% of HICC applied to unoccluded skin every 12 h would evaporate and 25–28% would be absorbed over the range of doses evaluated in the ROAT. This calculation assumes no chemical degradation of the compound on the skin surface or within the stratum corneum.

Discussion

The induction and elicitation of allergic contact dermatitis are dependent on several exposure conditions such as the concentration of allergen, frequency of exposure,¹⁷ exposure site,^{39,40} duration of application,^{41,42} type of exposure such as occluded or open exposure,⁴³ and individual degree of sensitivity.⁴⁴ In this study we wanted to find out if the results from two other experimental studies using nonvolatile compounds (nickel and methyldibromo glutaronitrile)^{30,31} could be reproduced when using a volatile compound by investigating the elicitation dose–response under standardized conditions in the patch test compared with ROAT using HICC as the allergen.

In the patch test the ED_{10} was 0.66 $\mu\text{g HICC cm}^{-2}$. This correlates well with the results from two other HICC studies where the ED_{10} was 0.85 $\mu\text{g HICC cm}^{-2}$,¹³ and 1.17 $\mu\text{g HICC cm}^{-2}$,¹⁴ respectively. The ED_{10} in our study is slightly lower than the ED_{10} from these two studies, which might be because two of the test subjects were very reactive, one of those had a beginning flare of eczema, so they probably represent one end of the elicitation spectrum. When the same amount of HICC was applied in the ROAT and the patch test, more sensitized individuals reacted in the ROAT. This was statistically significant at only one of the doses, but still the dose–response curve for the ROAT was visually displaced to the left compared with the patch test, as illustrated by Figure 2. This means that a person can be reactive at a repeated open application in spite of a negative finding in the patch test to that dose. This finding correlates well with the nickel³⁰ and methyldibromo glutaronitrile³¹ studies and is supported by other experimental studies with HICC¹⁴ and methyldibromo glutaronitrile.⁴⁵ The reason why a higher response is seen to the ROAT compared with the patch test could be that repeated exposures result in accumulation of allergen or that the immune system might react to the accumulated exposure over time. Studies with *para*-phenylenediamine,^{41,46,47} methyldibromo glutaronitrile⁴⁸ and isoeugenol¹⁷ have shown that accumulated exposure over time is probably an important factor in the elicitation response and the induction of contact allergy.

From the results in the two studies with the nonvolatile compounds^{30,31} we would expect that when we add up

the dose applied everyday in the ROAT to an accumulated 1-week, 2-week and 3-week dose, the response to these doses would be approximately the same as the response to the corresponding doses in the patch test, but this was not what we found in the HICC study. In this study we found that the response to the accumulated dose in the ROAT was lower than the response to the corresponding dose in the patch test. This was significant in half of the doses. An explanation of this might be that volatile compounds evaporate from the skin; therefore the actual accumulated dose over time in an open test with a fragrance may be lower than the dose in the occluded patch test, where the evaporation is limited. According to the calculated evaporation of HICC applied as in the ROAT, approximately 72–75% will evaporate. If this is true *in vivo*, the actual accumulated dose over time in the open test is much lower than the dose calculated by adding up the dose applied everyday. An *in vitro* study⁴⁹ using radiolabelled HICC has shown that only 13.8% of radioactivity associated with a single dose of ¹⁴C-HICC evaporated from a skin sample during 24 h compared with 9.5% in the occluded test, a much lower value than estimated in our study. A study investigating the concentration stability of fragrance test preparations applied in test chambers stored at room temperature has shown that the fragrance allergen concentration decreases rapidly, indicating a high evaporation rate at room temperature.⁵⁰ Many deodorants contain fragrances; and the exposure when using a deodorant must be considered as semi-occluded; therefore the evaporation is limited. In a repeated deodorant exposure study¹⁴ 100% of allergic persons had a positive reaction to a median dose per application of 0.79 µg HICC cm⁻², whereas in our study only 75% reacted to a ROAT dose of 3.57 µg HICC cm⁻². Furthermore, patients with fragrance allergy report deodorant sprays to be the most frequent cause of the first time rash,⁵¹ so evidence speaks for evaporation as an important factor when looking at fragrance allergy. We would expect that a repeated semi-occluded exposure, such as deodorant exposure, gives a higher risk of elicitation and probably also sensitization compared with an open exposure, because of the limited evaporation.

In conclusion, the ROAT threshold in dose per area per application is lower than the patch-test threshold, which is supported by two other studies with other allergens using the same methodology. Furthermore the response to the accumulated ROAT dose is lower than the response to the corresponding dose in the patch test, which can probably be explained by the evaporation of HICC from the skin in the open test. The knowledge of the relationship between the patch test (which is an easy test to perform) and the ROAT (which mimics some real-life exposure situations) is important for the understanding of how patch-test data can be used as a tool in risk assessment and thereby in preventive strategies.

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