

Quantitative patch and repeated open application testing in hydroxyisohexyl 3-cyclohexene carboxaldehyde sensitive-patients

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Objective: To identify the concentration of the fragrance compound hydroxyisohexyl 3-cyclohexene carboxaldehyde (INCI) (HICC) that is sufficiently low not to cause an allergic reaction in patients with proven sensitization.

Methods: Repeated open application testing (ROAT) in 64 subjects with 2 preparations (perfume and cream) in different concentration (0.005–2.5%). Confirmatory patch testing with four preparations in two different concentrations (2.5% and 5%).

Results: The concentrations of HICC being tolerated by 90% of those sensitized to HICC are estimated as <88.2 ppm (cream) and <270 ppm (perfume) equivalent to 1.2 µg/cm² (perfume) and 4.9 µg/cm² (cream). Patch test preparations differed with regard to sensitivity (88.5–98.1%) and specificity (37.5–87.5%) against the ROAT result as external criterion. ROAT concentrations and the reaction strength in patch testing were inversely correlated (Kendall's tau-b: 0.69), both indicating the existence of different degrees of susceptibility.

Conclusion: To protect 90% (50%) of people sensitized, the use concentration should be in the range of 0.009–0.027% (0.18–0.34%), depending on the product type. Taking into account these results, excessive concentrations should be avoided, as this would continue to sensitize people. Close monitoring is indispensable to prove the efficacy of any recommendations aiming to prevent induction.

Key words: allergic contact dermatitis; CAS 31906-04-4; dose-response relationship; elicitation; hydroxyisohexyl 3-cyclohexene carboxaldehyde; NOEL. © John Wiley & Sons A/S, 2009.

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Certain fragrance compounds must be considered important contact allergens, particularly those contained in the fragrance mixes (FM) (1, 2). Over

the last decade, further fragrances were identified as important sensitizers (3). In particular, the synthetic fragrance hydroxyisohexyl 3-cyclohexene

carboxaldehyde (HICC) had been noted to be an allergen of interest in the past (4–11). Routine patch testing in Europe has shown that 1.5–3% of eczema patients have positive reactions to HICC (6, 8, 9, 12–14), whereas frequencies of sensitization appear to be lower in North America (15). The obvious importance of this fragrance compound as a contact allergen prompted the German Contact Dermatitis Research Group (DKG) and later the European Society of Contact Dermatitis (ESCD) to include HICC in the patch test baseline series (8, 16), as well as in the newly developed FM II (17–20). Patch tested in the baseline series since 2005, the Information Network of Departments of Dermatology (IVDK) reported 4.7% positive reactions to the FM II, HICC itself contributing about 40% to this frequency (20).

In addition to the evident impact of HICC derived from clinical epidemiology, quantitative aspects of exposure were addressed. A use test (repeated open application test, ROAT) with two different concentrations (0.5% and 3% with ethanol as vehicle) was conducted in 18 patients allergic to HICC as diagnosed by prior patch testing (10). In 16 of 18 cases (89%) a positive use test developed, 11 reacting to the low and 5 to the high concentration. In an experimental elicitation study, a deodorant containing 200, 600 and 1200 ppm HICC was applied in the axillae. 9/14 reacted in this ROAT very early, after less than 2 weeks of exposure, to a deodorant preparation containing 200 ppm HICC (11). Finally the Scientific Committee on Consumer Products (SCCP) of the European Commission (Health & Consumer Protection Directorate-General) reviewed the available data on (human and animal) skin sensitization and elicitation studies and exposure data, and concluded that the provided experimental data did not (yet) demonstrate the highest level for the safe use of HICC in cosmetics (21).

From this background, the present study addresses the objective to find a maximum non-eliciting concentration used in two applications (perfume and leave-on cosmetic) in HICC sensitized patients. 'Non-eliciting' in this context means that not more than 10% of those sensitized will be elicited under use conditions. We therefore conceived two use-related tests (ROAT), each with five concentrations of HICC (0.005%, 0.01%, 0.1%, 0.5%, and 2.5%). The concentration of 0.005% (50 ppm) HICC was chosen as the lowest concentration, because we are unaware of cases elicited by such a low concentration in a ROAT. The highest concentration was chosen as it is very probable that almost all sensitized patients will react to such a concentration, according to a former ROAT with HICC (10). Two different vehicles were chosen as both application forms are

mainly used for HICC. Furthermore, possible vehicle effects on the ROAT-outcome, if present, could be detected and quantified.

Patients and Methods

Study patients were recruited at dermatological centres that cooperate with the IVDK (www.ivdk.org). The IVDK is a multicentre project collecting clinical and patch test data from 49 departments of dermatology in Germany, Austria and Switzerland. Methods of the IVDK have been described (2, 22). Fourteen departments participated in this study. Additionally one department from Barcelona/Spain (Hospital del Mar, Universitat Autònoma) joined the study.

Inclusion criteria for the participation were at least a + reaction to HICC at reading D3 on routine patch testing, registered within the last 2 years; 2 weeks should have been elapsed after the last patch test before starting the ROAT; age >18 years, and signed informed consent.

Exclusion criteria were: age <18 years, systemic or local immuno-suppressant treatment, known corticosteroid allergy, immuno-compromising disease, current eczema or recent UV light exposure in the test area, and pregnancy.

The study had been approved by the ethics committee of the University of Göttingen (No. 21/3/05, dated 4th April 2005).

Materials

Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC), CAS 31906-04-4, was used for patch and use testing.

Two types of products containing HICC were tested in two ROATs in parallel:

- A solution in 96% ethanol to mimic alcoholic perfume products. The solutions were prepared with five different concentrations of HICC (0.005%, 0.01%, 0.1%, 0.5% and 2.5%).
- A cream containing 15% glyceryl stearate in water as vehicle containing the test substance. The creams were prepared with the same five concentrations (0.005%, 0.01%, 0.1%, 0.5% and 2.5%).

The vehicles without HICC (ethanol 96% and 15% glyceryl stearate in water, respectively) served as negative controls. The products had been prepared and delivered by International Flavor & Fragrances Inc, Hilversum, NL (IFF), in polypropylene droplet bottles and 10 ml syringes. The concentration was checked in one total set of preparations by an independent laboratory (Hermal/Reinbeck). The concentrations of HICC in the ethanol preparations were slightly higher (<5%), the concentrations in the cream were as indicated, or, in one

preparation (0.5% cream) lower by 12%. During the study period the material was not replaced by new preparations. At the end of the study (17 months after delivery of materials), the concentration of a total set was again determined by Hermal/Reinbeck. The values found in the ethanol preparation were all slightly higher than the nominal values (excess <4.8%). In the cream preparations, the value found in the two lower concentrations was slightly higher, but in the higher concentrations (0.1%, 0.5% and 2.5%), the concentration were lower by 30–50%. These preparations were taken from a batch stored in Göttingen, the ethanol preparations stored in the refrigerator, the cream under room conditions with large temperature deviations.

Use test

The ROAT with HICC in ethanol ('perfume') and with HICC in a cream ('cream') were performed on $3 \times 3 \text{ cm}^2$ areas on the volar side of the *left* (perfume) and the *right* (cream) forearm. Each test area had been assigned to one of the test preparations (marked in red) or the control preparation (blue). Test and control areas were allocated randomly to the proximal or distal skin areas. The participants were given four preparations (two with and two without HICC) and strictly instructed to apply two drops of the perfume (equivalent to 0.04 ml) and a suitable dose ($\sim 0.5 \text{ ml}$) of the cream as well as the respective control preparations 2 $\times$ daily (morning and evening) always on the same area by gently

spreading the material on the areas with a finger. The test sites were allowed to dry for 15 min before covered with clothing. Patients were advised to avoid contact with water for the following 2 h. If a reaction occurred in a test area, further exposure was stopped.

The application of the same preparations was continued for 2 weeks or until an erythematous reaction occurred (see below). If there was no reaction after 2 weeks, the participants were given the preparations at the next higher concentration to be used for another 2 weeks. Hence, the theoretical maximum period of application was 10 weeks (Fig. 1). Patients were instructed to stop further applications if symptoms (redness) in the test area occurred, and to consult the physician on the same day (or, if not possible, to document the day of the occurrence of the symptoms). In case that one ROAT turned positive and the other remained negative, the former was stopped and the latter was to be continued. Both ROATs were evaluated at least weekly (see Fig. 1), or earlier at the request of the patient, with assessment of the following morphological features: erythema, papules/infiltration, vesicles and extent of area involved. The readings were classified according to the recommendations of Johansen et al. (23, 24). The ROAT was considered as positive in cases of (spotty) erythema of at least 25% of the test area along with homogeneous infiltration or papules regardless of the number.

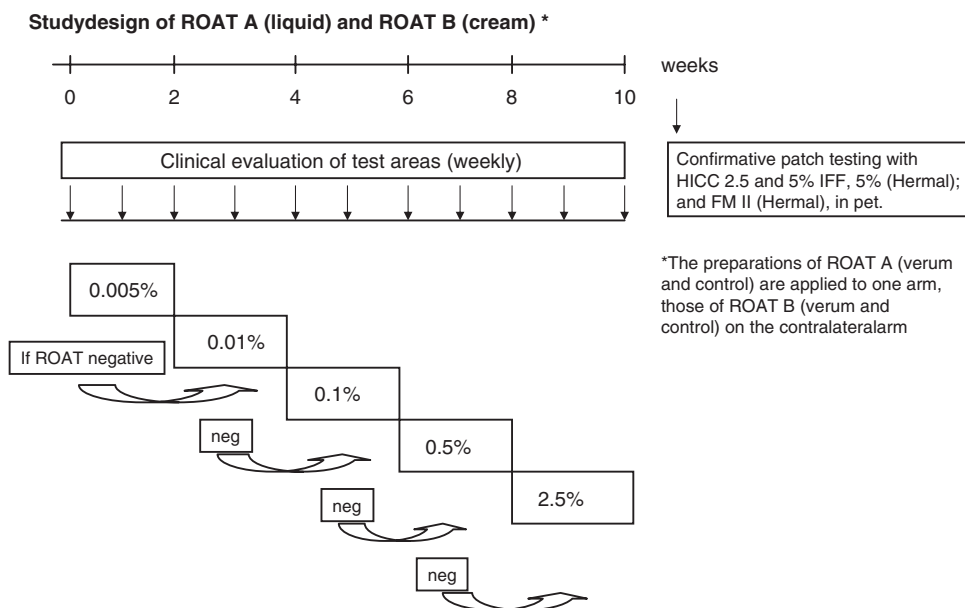


Fig. 1. ROAT A: ethanol with different concentrations; ROAT B: Cream with different concentrations. The concentrations in both vehicles are identical, namely 0.005%, 0.01%, 0.1%, 0.5% and 2.5%. If the lower concentration is tolerated during a period of 2 weeks, the next higher concentration is applied for further 2 weeks. In case that 1 ROAT turns positive and the other remains negative, the former will be stopped and the latter will be continued.

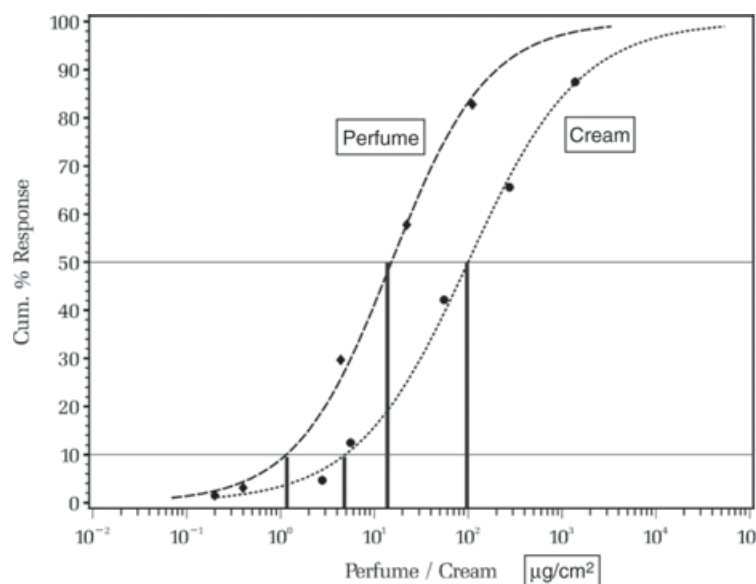


Fig. 2. Relationship between cumulative response and increasing amounts as $\mu\text{g}/\text{cm}^2$ of perfume and cream, resp. The 10% and 50% thresholds for no response are indicated. (1.2 and 4.9 $\mu\text{g}/\text{cm}^2$; 15.2 and 99.5 $\mu\text{g}/\text{cm}^2$) The different responses to perfume and cream by a factor of 4.1–6.5 are probably due to matrix effects. Note: Calculations are based on doses in the 2 products used in this study, and not on doses found in standard commercial products.

Patch testing

After termination of the ROATs, a confirmatory patch test was done with HICC 2.5% and 5% in petrolatum (both from IFF), FM II (containing HICC 2.5%) and HICC 5% (both from Hermal, Reinbeck/D). Patch tests were performed in accordance with the recommendations of the International Contact Dermatitis Research Group (ICDRG) (25) and the DKG (26).

Data analysis

In addition to descriptive analyses, Kendall's tau-b with accompanying 95% confidence interval (CI) was calculated as measure of correlation of ordered categorical data (ROAT and patch test result, each aggregated to an ordinal variable with three levels). A logistic function (curve) (Formula 1) was fitted to the observed quantitative relationship between ROAT dose as estimated from the two prototypically formulated products used in this study and the probability of cumulative response for each of the two ROATs (Fig. 2). The respective ROAT dose yielding a 10% and a 50% cumulative response, respectively, was derived from the fitted logistic curve. All statistical analyses were performed with the SAS software package (version 9.1, SAS Institute, Cary, NC).

$$f(z) = \frac{1}{1 + e^{-z}} \quad (1)$$

Results

Determination of the no-observed or low-observed effect level (NOEL/LOEL) for HICC

In 15 departments of dermatology joining the IVDK, and in one department from the university of

Barcelona a total of 67 patients were included in the study and the ROAT was performed between 1/2006 and 3/2007. After exclusion of three cases, two reacting to the control and one reacting allergic to the marker, 64 subjects (median age: 46.5 (18–65), 68% women) were suitable for further evaluation. The test results to the different concentrations are presented in Table 1. In total, 56 patients reacted to the cream and 53 to the perfume. Considering the low concentrations (50 and 100 ppm), 8/64 = 12.5% (exact 95% CI: 5.6–23.2%) reacted to the cream and 2/64 = 3.1% (exact 95% CI: 0.38–10.8%) reacted to the perfume in low concentrations (50 or 100 ppm). Although the concentrations in both vehicles were the same, the amount of material (cream: 0.5 ml; perfume: 0.04 ml, assuming that the subjects had followed the instructions), which was applied to the 9 cm^2 test area, differed, thus yielding different doses per cm^2 , see Table 1. For example: 0.5 ml (applied amount) of a cream containing 100 ppm contain 50 μg , which are applied to an area of 9 cm^2 (the application area of 3 × 3 cm). This gives 5.6 $\mu\text{g}/\text{cm}^2$. The relationship between the cumulative response and increasing amounts as $\mu\text{g}/\text{cm}^2$ of perfume and cream is shown in a logistic curve fitted to the observed cumulative response data (Fig. 2). The 10% thresholds for no response are 1.2 $\mu\text{g}/\text{cm}^2$ and 4.9 $\mu\text{g}/\text{cm}^2$ for the perfume and the cream, respectively; and the 50% thresholds are 15.2 and 99.5 $\mu\text{g}/\text{cm}^2$. The concentrations being tolerated by 90% (50%) of those sensitized to HICC are thus estimated as <88.2 ppm (<1791 ppm) for the cream and <270 ppm (<3420 ppm) for the perfume (Fig. 2).

As it cannot be excluded that the concentration in the preparations changed over time, for which the

Table 1. Reactions to ROAT with hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) in different concentrations (%) / different amounts applied ($\mu\text{g}/\text{cm}^2$) as cream (upper panel) and perfume (lower panel) in 64 patients previously found sensitized to HICC. The amount applied was not measured but estimated, assuming that the instructions of application were followed by the subjects

Cream						
Concentration (%)	0.005	0.01	0.1	0.5	2.5	Neg.
Amount applied ^a ($\mu\text{g}/\text{cm}^2$)	2.8	5.6	55.6	277.8	1388.9	
Responders	3	5	19	15	14	8
<i>N</i> cumulative responders	3	8	27	42	56	
% Cumulative responders	4.7	12.5	42.2	65.6	87.5	
Perfume						
Concentration (%)	0.005	0.01	0.1	0.5	2.5	Neg.
Amount applied ^a ($\mu\text{g}/\text{cm}^2$)	0.2	0.4	4.4	22.2	111.1	
Responders	1	1	17	18	16	11
<i>N</i> cumulative responders	1	2	19	37	53	
% Cumulative responders	1.6	3.1	29.7	57.8	82.8	

^aAmount applied per application over a variable time (maximum 2 weeks per concentration level, see Fig. 1).

non-exhaustive analysis of one set of preparations gives some indication, we compared the results of different study periods (1/2006 to 9/2006 and 10/2006 to 3/2007), which, however, yielded no evidence of temporal effects: Reaction rates to the low concentrations (1–3) in both periods were 20% and 23% in the cream group, and 5% (both periods) in the perfume group, respectively.

Inverse correlation between ROAT concentration and the reaction strength in patch testing

At the end of the ROAT, in 60 patients a confirmatory patch test was done with the 2.5% and 5% preparations from IFF and the FM II (containing HICC 2.5%) whereas HICC 5% from Hermal was used in $n = 58$.

Of those who reacted to the cream-ROAT and were subsequently patch tested, the positive ROAT cases ($n = 52$) were confirmed by patch testing (any of the preparations) in $n = 50$ (96.2%). Of those who reacted to the perfume ROAT and were subsequently patch tested, the positive ROAT cases ($n = 49$) were confirmed by patch testing (any of the preparations) in $n = 48$ (98.0%). Out of the eight non-responders to the ROAT (cream) (Table 1), four were also negative to the confirmatory patch test (Table 2), indicating that some were false positive to HICC at the screening patch test. The reaction pattern of different patch test concentrations in cream-ROAT positive and negative cases is shown in Fig. 3. When using the ROAT results as external criterion for 'false positive' or 'false negative' patch test results, it is obvious that the increase in concentration from 2.5% to 5% yields less false negative reactions, but this beneficial effect goes along with slightly more false positive patch test cases. As well, the number of ++ reactions increased. Testing with the FM II (2.5% HICC) differs considerably from patch testing with 2.5%. In the former, there was only one false negative patch

Table 2. ROAT and patch testing with a **5% commercial preparation of hydroxyisohexyl 3-cyclohexene carboxaldehyde** (Hermal). Relation between reactions to lower (1: 0.005; 2: 0.01; 3: 0.1) or higher (4: 0.5; 5: 2.5) concentrations (%) and reactions to strength of patch test reactions regardless of patch test concentration in confirmatory patch testing. Patch testing was done in $n = 58$

Cream				
ROAT response/patch test	1–3	4–5	Neg	
++/++ ++	21	4	3	25
+	5	18	4	26
Neg		3 ^a	7	7
	26	25	7	58

^aOne of the three patients did react to a different patch test preparation (see results).

Mantel-Haenszel Chi-square test for linear association: $P < 0.0001$. Kendall's tau-b as measure of correlation of ordered categorical data: 0.69 (95% CI: 0.55–0.83).

test reaction, and less +, but stronger (++/++ ++ +) reactions.

Furthermore, there is a substantial inverse correlation between the ROAT concentrations and the reaction strength in patch testing. The lower the ROAT concentrations leading to reactions, i.e. the lower the individual elicitation threshold, the stronger were the reactions in confirmatory patch testing; Mantel-Haenszel Chi-square test for linear association: $P < 0.0001$ (Tables 2 and 3). For the commercial preparations 5% HICC and FM II the degree of correlation with the quantitative ROAT outcome is: 0.69 (95% CI: 0.55–0.83) and 0.48 (95% CI: 0.29–0.67), respectively (as expressed by Kendall's tau-b as measure of correlation of ordered categorical data).

Accordingly, the sensitivity and specificity of the different patch test preparations, considering the cream-ROAT outcome as external criterion ('gold standard'), differ (Table 4). With sensitivity increasing, the specificity decreases, although the differences were not significant. In this context, the FM II, containing not only HICC, but four other fragrance allergens, cannot be compared with

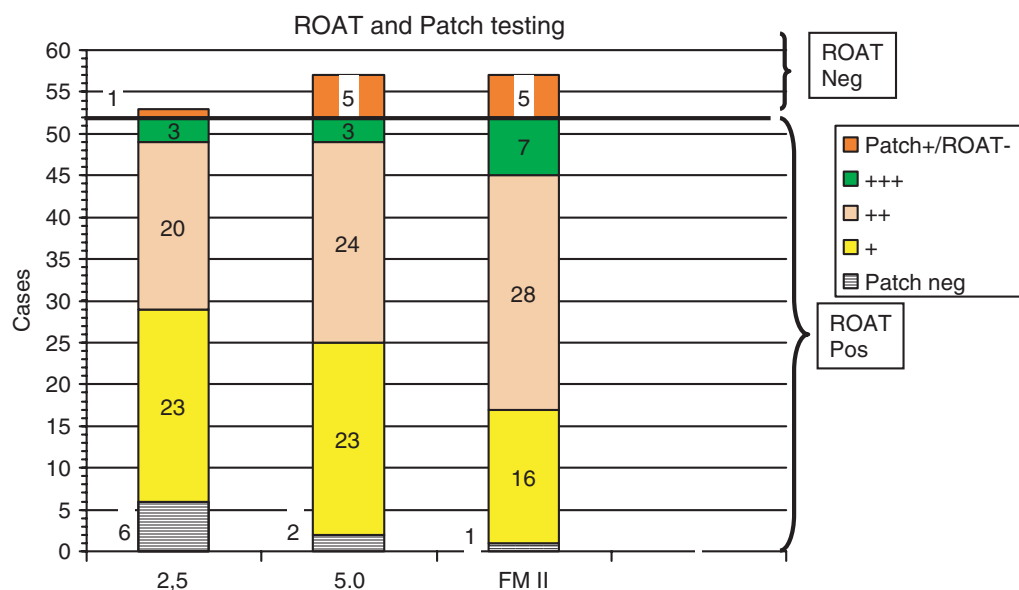


Fig. 3. Results of ROAT with the cream and patch testing with different concentrations of hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) (2.5%, 5% and fragrance mix II (containing 2.5%). There were 2 preparations of HICC 5% (IFF and Hermal). If there were discordant strengths (5/60), the stronger reaction was considered.

Table 3. ROAT and patch testing with a commercial preparation of the **fragrance mix II** (Hermal) containing 2.5% hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC). Relation between reactions to lower (1: 0.005; 2: 0.01; 3: 0.1) or higher (4: 0.5; 5: 2.5) concentrations (%) and reactions to strength of patch test reactions regardless of patch test concentration in confirmatory patch testing. Patch testing was done in $n = 60$

Cream				
ROAT response/patch test	1–3	4–5	Neg	
+++ / +++	21	13		34
+	6	11	5	22
Neg		1	3	4
	27	25	8	60

Mantel-Haenszel Chi-square test for linear association: $P < 0.0001$. Kendall's tau-b as measure of correlation of ordered categorical data: 0.48 (95% CI: 0.29–0.67).

the other three test preparations with pure HICC regarding the seemingly low specificity, as positive reactions to the FM II may be due to other ingredients.

Discussion

The aim of the study was to identify the concentration of the fragrance compound HICC that is sufficiently low not to cause an allergic reaction in patients with proven sensitization, or at least only in a very low proportion of these. For this purpose, two preparations of the fragrance, each with graded concentration of HICC, were applied in a stepwise ROAT with increasing concentrations (until a positive ROAT reaction was achieved or the respective maximum concentration of the preparation was reached).

One of the main results is that the concentrations being tolerated by 90% and by 50% of those sensitized to HICC are estimated as <88.2 ppm and <1791 ppm for the cream and <270 ppm and <3420 ppm for the perfume. Expressed as amount applied per square centimetre, the 10% thresholds for no response are $1.2 \mu\text{g}/\text{cm}^2$ for the perfume and $4.9 \mu\text{g}/\text{cm}^2$ for the cream; and the 50% thresholds are $15.2 \mu\text{g}/\text{cm}^2$ and $99.5 \mu\text{g}/\text{cm}^2$ (Fig. 2). In total, 56/64 (cumulative response: 87.5%) reacted to the cream and 53/63 (82.8%) to the perfume. In the ROAT with 0.5% and 3% in ethanol done by Johansen et al., 11/18 (61%) reacted to the low concentration and the cumulative response to both concentrations was 89% (16/18) (10). Thus, the proportions of positive reactions to 0.5% (61% and 57.8%) as well as the overall outcome of both studies are very similar. In contrast, in another ROAT study with HICC (published after the initiation of our study), using deodorants with graded HICC concentrations in the axillae, a higher susceptibility was shown, as 9/14 already reacted to the deodorant preparation with 200 ppm HICC (11). This was an unexpected finding, as, at that time, 200 ppm were considered safe (10). Apparently, the specific anatomical and physiological characteristics of the site, with semi-occlusion, may have increased the absorption of the material. This is supported by another experimental study, in which was shown that skin penetration of HICC was enhanced under occlusion by a factor of >3 (27). In comparison, evaporation from other, unoccluded sites may

Table 4. Sensitivity and specificity (accompanied by exact 95% CIs) of different patch test preparations of hydroxyisohexyl 3-cyclohexene carboxaldehyde using the positive reactions of patch testing and ROAT outcomes (positive versus negative) (see Tables 2 and 3). The ROAT served as external criterion

	2.5% (IFF)	5% (IFF)	5% (Hermal)	Fragrance mix II (Hermal)
Sensitivity	88.5% (76.6–95.7%)	96.2% (86.8–99.5%)	94.1% (83.8–98.8%)	98.1% (89.7–100.0%)
Specificity	87.5% (47.4–99.7%)	50.0% (15.7–84.3%)	57.1% (18.4–90.1%)	37.5% (8.5–75.5%)

go along with a lesser sensitization or elicitation risk (28, 29).

Both studies addressed the question as to the concentration that would be tolerated by >10% sensitized based on the results of quantitative patch testing. As thresholds 29 and 25 ppm, respectively, were found, corresponding to 0.9 and 0.8 µg/cm², respectively, given the application of 15 µg solution to 0.5 cm² of test area. Although patch testing and ROAT results are not directly comparable, this would be close to the threshold we found with the perfume preparations in the ROAT (1.2 µg/cm²).

Apparently the vehicle itself influences the threshold of tolerance. Approximately half of the patients exposed to the perfume did not react to the same concentration after the same time of application, compared to HICC in a cream base. For instance, more patients (12.5% versus 3.1%) reacted to the cream at low concentrations (50 or 100 ppm). However, although the concentrations in both vehicles were the same, the amount of material applied to identically sized areas differed (cream: 0.5 ml; perfume: 0.04 ml). Comparing the dose–response curves, the thresholds for the cream would be higher by a factor of 4–7 (Fig. 2). The higher dose tolerated is probably due to the vehicle effect of the emulsion type versus ethanol: Because of a lower polarity and a higher lipophilicity permeation is better with ethanol as vehicle, whereas permeation from a vehicle of the emulsion type is much poorer (30, 31). This finding emphasizes the necessity to consider the formulation of the product when defining safety limits.

Patch testing

The majority of patients diagnosed by previous patch testing as sensitized to HICC reacted to at least one concentration of the ROAT preparations; and almost all ROAT positives were confirmed in confirmatory patch testing, indicating that there were very probably no false positive ROAT cases in our group of patients.

Using the ROAT outcome as external criterion, the sensitivity and specificity of the different patch test preparations differed, with the 5% preparation giving the optimal results, whereas the 2.5% preparation suffered from a number of false negative cases, i.e. reduced sensitivity. This could confirm

the doubts of Frosch et al. wondering whether the 2.5% HICC contained in the FM II is a sufficiently high test concentration (19). Clearly, the 5% HICC test preparation is preferable over the 2.5% if tested as single compound. However, regarding the sensitivity of the FM II with regard to HICC, our results are reassuring, as the sensitivity of the 5% preparations and of the FM II are in the same range, probably due to synergistic effects of the mix preparation compensating for the lower concentration (2.5%) used in the mix (32). Furthermore, there was also a significant and quite good correlation (Kendall's tau-b = 0.69) between reaction strength in patch testing and the reactivity dose in the ROAT (Table 2), a finding quite similar to the result of Jørgensen et al. ($r = 0.71$) (11). This study again confirms the usefulness of ROATs to evaluate and identify the optimal patch test preparations (33, 34).

Considerations with regard to safety and exposure

To estimate the public health impact of sensitization to HICC, an extrapolation of patch test results to an estimated frequency of sensitization in the general population according to the 'Clinical Epidemiology–Drug Utilization Research' (CE-DUR) model may be helpful (35, 36).

Based on the annual sales data of patch test materials (supplied by the four main manufacturers present on the German market) the number of applications of the standard series in German patients was calculated for the two periods (1998–1999 and 2000–2007) as approximate average of 487 500 and 391 800 patch tests per year, respectively. Based on these figures and after applying the correction factor for a 'medium case' scenario the number of patients with suspected contact allergy, eligible for patch testing, is derived as 9 328 338, for the 10-year period, i.e. an 'estimated total patch test population'. Based on a frequency of sensitization in the clinical population of 2.5% (14, 37), the 10 years prevalence of sensitization would be 233 209, equal to 0.28% of the 82 million population of Germany. With a threshold of 90 ppm (0.009%) HICC (cream) or 270 ppm (0.027%) (perfume), 2300 (0.028% of the population) would theoretically be at risk of elicitation, i.e., a relapse of allergic contact dermatitis. With a threshold of <1791 ppm (0.18%) for the cream and <3420 ppm

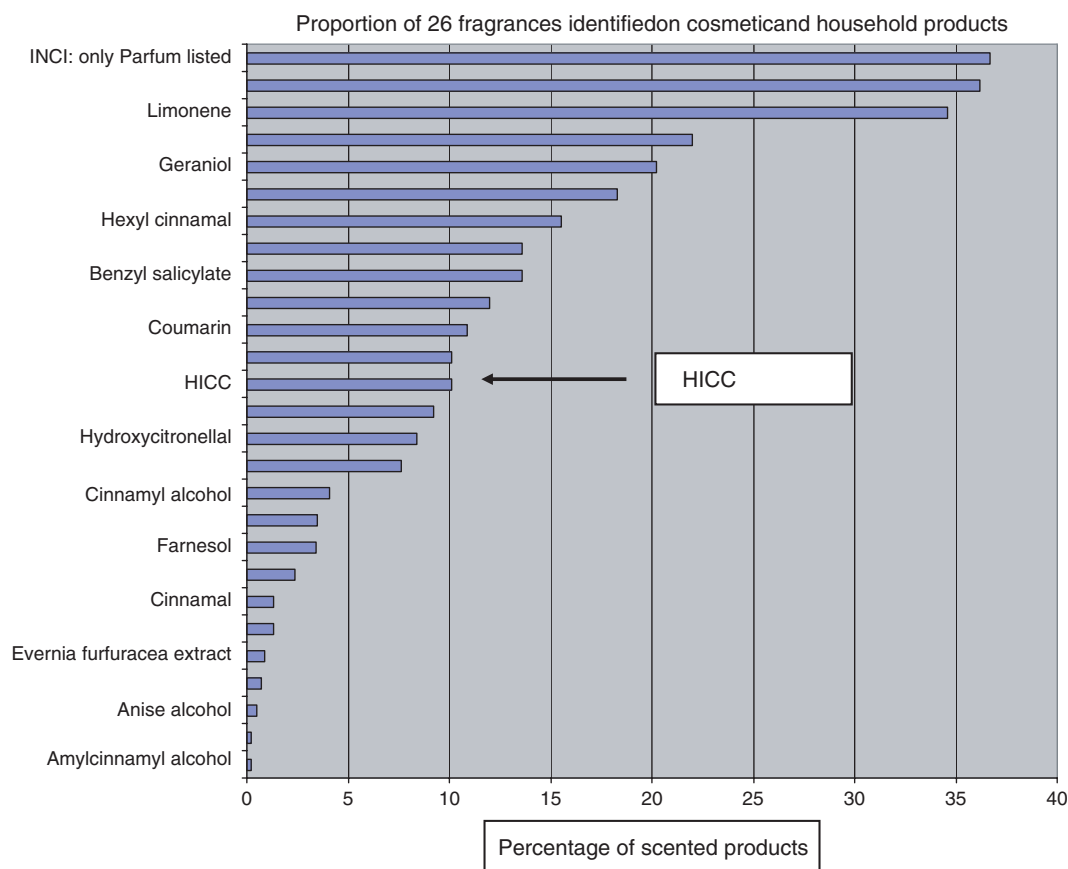


Fig. 4. INCI labelling of fragrances on 3000 randomly selected cosmetic and household products in Germany, 2006 and 2007. Total number of scented products: 2207. Products containing hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC): $n = 223$; percentage of products containing HICC 10.1% (Data kindly obtained from G. Mildau G. Chemisches und Veterinär-Untersuchungsamt Karlsruhe 2008 (personal communication)).

(0.34%) for the perfume, 116 605 (0.14%) would theoretically be at risk, i.e. if all people sensitized would continue to use products containing HICC exceeding the threshold concentrations. One should bear in mind that these thresholds refer to the two prototypical product types used in this study, and may be different (e.g. lower) in real products. The above range of concentrations is far lower than that recommended by International Fragrance Association (IFRA) standards in 2003 namely, 1.5% and in 2008 1% for hand creams and 1.5% for hydroalcoholic products for unshaved skin (38, 39), which are the product categories relevant for the current study. These recommendations were meant, however, to prevent induction and not elicitation and thus concentration thresholds are not directly comparable.

The question remains how HICC has become one of the most important fragrance allergens (12). Three factors should be considered: The sensitizing potency of a molecule, the chances of being exposed (e.g. through a frequent presence in products) and the concentration during exposure.

- (1) HICC was found to be a weak sensitizer. In several human repeated insult patch (HRIPT), in doses up to the $4000 \mu\text{g}/\text{cm}^2$, a sensitizing dose was not identified (21), and the LLNA EC3 value was 17.1% corresponding to $4275 \mu\text{g}/\text{cm}^2$ (10).
- (2) According data from a consumer magazine HICC was found to be present in 10% of cosmetic and household products (Fig. 4), and in about 18% of various cosmetics (Table 5). In both statistics, fine fragrances were not considered. In certain product categories, however, HICC seemed to be used quite often (after shave, deodorants for men, skin care creams) (Table 5).
- (3) With regard to the concentration of exposure, there are different types of data, which are based on systematic and sporadic analyses (Table 6). Products with high concentrations comprise fine fragrances and, to a lesser degree, deodorants. Domestic products do not seem to be a major source of exposure to HICC. In the 1990 s, the concentration in fine fragrances exceeded in average 3%, in the years after 2000

Table 5. Categories (selected from a total of $n = 37$) of cosmetic products and proportion of products containing hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) within each category according to labelling in 2005. Between 20 and 30 products (mean: 22.8) were considered within each category. Number of products screened: $n = 843$; Number of products containing HICC: $n = 151$ (18%). Only the leading categories with a proportion of >20% (16/37) of products containing HICC are shown. Data from K. Schumacher, consumer magazine 'Oekotest' (personal communication)

Product category	% of products with HICC
After shave	52
Deodorant for men (spray)	42.3
Skin care	42.1
Face creams ('intensive')	34.8
Anti-aging	34.6
Deodorant roll-on	30.8
Deodorant sprays	30.8
Styling mousse	30.8
Hand creams	30.0
Acne products	29.4
Make up	29.4
Face moisturizer mask	27.8
Sun creams	26.3
Body lotion	24.2
Shower gels for men	24.0
Moisturizers	23.3

they were lower, but still exceeding 0.1% and 0.5% in some cases (Table 6).

Considering the relative impact of all three factors, it seems that products with a high concentration (fine fragrances, after shave) and products used

under conditions enhancing sensitization (deodorants) may be regarded as the main reasons for the relatively high sensitization rate, and not the potency of the molecule or the overall frequency of exposure. Furthermore, the rare cases elicited by an unexpected, extremely low concentration may be explained by very high concentrations during induction—in view of the inverse relationship between dose of induction and dose of elicitation (45). The stable frequencies of sensitization up to 2007 cannot be explained by the high concentrations during the 1990s—even 12–20% were cited (15). Rather, they may indicate that the concentrations used in certain products during the last 5 years were too high.

Conclusion

What can be learnt from the findings of our study? To protect 90% (50%) of people already sensitized, the use concentration should be in the range of 0.009–0.027% (0.18–0.34%), depending on the product type. Moreover, evidence regarding elicitation thresholds as the one presented here can serve as a starting point for the definition of thresholds which are 'safe' regarding induction. Above all too high concentrations will not only continue to sensitize people, but will sensitize to a high degree, so that they will react to even small amounts of allergen exposure. A readjustment of the IFRA standard is needed (39).

Table 6. Exposure data to hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) [modified from (10)]

Product type	Concentration range (%)	Frequency in products	Time	Reference
<i>Systematic studies</i>				
Fine fragrances ^a	0.0058–0.2058%	18/25 (72%)	<2007	(40)
	>0.01	12/18		
	>0.1%	3/18		
Fine fragrances ^a	0.07–0.78	13/41	<2007	Schmidt (personal communication)
	>0.5%	7/13		
Fine fragrances ^a	Above 1% (average 3.2%)	43%	<1990	(41)
Deodorants g/100 ml	0.0001–0.1874	53% (37/73)	1996	(42)
'Vapo' sprays	0.0001–0.1874	59% (13/23)	<1998	(42)
	median 0.0387			
Aerosol sprays	0.0006–0.1606	77% (17/22)	<1998	(42)
	median: 0.0210			
Roll-on	0.0001–0.1822	27% (7/28)	<1998	(42)
	median: 0.0582			
Children's cosmetics/toys			<1999	(43)
Creams	0.033	2/4	<1999	
Fine fragrances	0.054–0.630	6/7	<1999	
Domestic/occupational ^b	0.0036–0.0103	10% (5/59)	<2001	(44)
	mean: 0.00748 (+/–0.00239)			
<i>Occasional analysis</i>				
Fine fragrances	0.083–3.832	13 products	<2005	(19)
Fine fragrances	3.67–3.83%	2 products	<2003	(10)
Fine fragrances	0.12–2.34	3 products	<1999	(6)
After Shave	0.06	1 product	<1999	(6)
Lotions	0.02–0.2	2 products	<1999	(6)
Deodorants	0.02–0.56	3 products	<1999	(6)

^a Fine fragrances: Parfum, eau de parfum, eau de toilette, eau de cologne.

^b Soap (bars/liquid), surface cleaners, detergents for hand wash, dish wash, etc.

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References

1. Frosch P J, Johansen J D, White I R. *Fragrances. Beneficial and Adverse Effects*. Berlin, Heidelberg, New York, Springer, 1998.
2. Schnuch A, Geier J, Uter W et al. National rates and regional differences in sensitization to allergens of the standard series. Population adjusted frequencies of sensitization (PAFS) in 40000 patients from a multicenter study (IVDK). *Contact Dermatitis* 1997; 37: 200–209.
3. Frosch P J, Johansen J D, Menné T et al. Further important sensitizers in patients sensitive to fragrances. I. Reactivity to frequently used chemicals. *Contact Dermatitis* 2002; 47: 78–85.
4. Militello G, James W. Lyrall: a fragrance allergen. *Dermatitis* 2005; 16: 41–44.
5. Frosch P J, Pilz B, Andersen K E et al. Patch testing with fragrances: results of a multicenter study of the European Environmental and Contact Dermatitis Research Group with 48 frequently used constituents of perfumes. *Contact Dermatitis* 1995; 33: 333–342.
6. Frosch P J, Johansen J D, Menné T et al. Lyrall® is an important sensitizer in patients sensitive to fragrances. *Br J Dermatol* 1999; 141: 1076–1083.
7. Hausen B M, Bottenbruch S. Lyrall®-Allergie. *Akt Dermatol* 2001; 27: 310–314.
8. Geier J, Brasch J, Schnuch A, Lessmann H, Pirker C, Frosch P J. Lyrall® has been included in the patch test standard series in Germany. *Contact Dermatitis* 2002; 46: 295–297.
9. Baxter K F, Wilkinson S M, Kirk S J. Hydroxymethyl pentylcyclohexene- carboxaldehyde (Lyrall) as a fragrance allergen in the UK. *Contact Dermatitis* 2003; 48: 117–118.
10. Johansen J D, Frosch P J, Svedman C, Andersen K E, Bruze M, Pirker C, Menné T. Hydroxyisohexyl 3-cyclohexene carboxaldehyde- known as Lyrall: quantitative aspects and risk assessment of an important fragrance allergen. *Contact Dermatitis* 2003; 48: 310–316.
11. Jorgensen P H, Jensen C D, Rastogi S, Andersen K E, Johansen J D. Experimental elicitation with hydroxyisohexyl-3-cyclohexene carboxaldehyde-containing deodorants. *Contact Dermatitis* 2007; 56: 146–150.
12. Schnuch A, Geier J, Uter W, Frosch P J. Sensitization to 26 fragrances to be labelled according to current European regulation. *Contact Dermatitis* 2007; 57: 1–10.
13. Nardelli A, Carbonez A, Ottoy W, Drieghe J, Goossens A. Frequency of and trends in fragrance allergy over a 15-year period. *Contact Dermatitis* 2008; 58: 134–141.
14. Braendstrup P, Johansen J D. Hydroxyisohexyl 3-cyclohexene carboxaldehyde (Lyrall®) is still a frequent allergen. *Contact Dermatitis* 2008; 59: 187–188.
15. Belsito D V, Fowler J F, Sasseville D, Marks J G, DeLeo V A, Storrs F J. Delayed-type hypersensitivity to fragrance materials in a select North American population. *Dermatitis* 2006; 17: 23–28.
16. Bruze M, Andersen K E, Goossens A. Recommendation to include fragrance mix2 and hydroxyisohexyl 3-cyclohexene carboxaldehyde (Lyrall) in the European baseline patch test series. *Contact Dermatitis* 2008; 58: 129–133.
17. Frosch P J, Pirker C, Rastogi S et al. The new fragrance mixII—test results of a multicentre European study. *Contact Dermatitis* 2004; 50: 149.
18. Frosch P J, Pirker C, Rastogi S C et al. Patch testing with a new fragrance mix detects additional patients sensitive to perfumes and missed by the current fragrance mix. *Contact Dermatitis* 2005; 52: 207–215.
19. Frosch P J, Rastogi S C, Pirker C et al. Patch testing with a new fragrance mix- reactivity to the individual constituents and chemical detection in relevant cosmetic products. *Contact Dermatitis* 2005; 52: 216–225.
20. Geier J, Lessmann H, Uter W, Schnuch A. Experiences with the fragrance mixII—the German perspective. *Contact Dermatitis* 2006; 55(Suppl. 1): 12.
21. Scientific Committee on Consumer Products (SCCP). Opinion on Hydroxyisohexyl 3-cyclohexene carboxaldehyde (sensitisation only). Adopted 7 December 2004. SCCP/0838/04 2004.
22. Uter W, Schnuch A, Geier J, Frosch P J. Epidemiology of contact dermatitis: the information network of the departments of dermatology (IVDK) in Germany—a surveillance system on contact allergies. *Eur J Dermatol* 1998; 8: 36–40.
23. Johansen J D, Bruze M, Andersen K E et al. The repeated open application test: suggestions for a scale of evaluation. *Contact Dermatitis* 1998; 39: 95–96.
24. Johansen J D, Andersen K E, Menné T. Quantitative aspects of isoeugenol contact allergy assessed by use and patch tests. *Contact Dermatitis* 1996; 34: 414–418.
25. Fregert S. *Manual of Contact Dermatitis. On behalf of the International Contact Dermatitis Research Group and the North American Contact Dermatitis Group*, 2nd edition. Copenhagen, Munksgaard Publishers, 1981.
26. Schnuch A, Aberer W, Agathos M, Brasch J, Frosch P J, Richter G. Leitlinien der Deutschen Dermatologischen Gesellschaft (DDG) zur Durchführung des Epikutantests mit Kontaktallergenen. *Hautarzt* 2001; 52: 864–866.
27. Bhatia S P, Lalko J, Brain K R, Green D M, Api A M. In-vitro human skin penetration of the fragrance material HMPCC. *Toxicologist* 2007; 96: 432 (Abs 2091).
28. Bronaugh R L, Wester R C, Bucks D, Maibach H I, Sarason R. In vivo percutaneous absorption of fragrance ingredients in rhesus monkeys and humans. *Food Chem Toxicol* 1990; 28: 369–373.
29. Saiyasombati P, Kasting G B. Two-stage kinetic analysis of fragrance evaporation and absorption from skin. *Int J Cosmet Sci* 2003; 25: 235–243.
30. Smith C K, Hotchkiss S A M. *Allergic Contact Dermatitis. Chemical and Metabolic Mechanisms*. London, Taylor and Francis, 2001.
31. Cal K. How does the type of vehicle influence the in vitro skin absorption and elimination kinetics of terpenes? *Arch Dermatol Res* 2006; 297: 311–315.
32. Johansen J D, Skov L, Volund A, Andersen K, Menné T. Allergens in combination have a synergistic effect on the elicitation response: a study of fragrance-sensitized individuals. *Br J Dermatol* 1998; 139: 264–270.
33. Schnuch A, Kelterer D, Bauer A et al. Quantitative patch and repeated open application testing in Methylidibromoglutaronitrile-sensitive patients—results of the IVDK. *Contact Dermatitis* 2005; 52: 197–206.
34. Villarama C D, Maibach H I. Correlations of patch test reactivity and the repeated open application test (ROAT)/provocative use test (PUT). *Food Chem Toxicol* 2004; 42: 1719–1725.
35. Schnuch A, Uter W, Geier J, Gefeller O. Epidemiology of contact allergy: an estimation of morbidity employing the clinical epidemiology and drug utilisation research (CE-DUR) approach. *Contact Dermatitis* 2002; 47: 32–39.
36. Thyssen J P, Uter W, Schnuch A, Linneberg A, Johansen J D. 10-year prevalence of contact allergy in the general population in Denmark estimated through the CE-DUR method. *Contact Dermatitis* 2007; 57: 265–272.
37. Schnuch A. Der IVDK - eine kurze Synopsis. *Akt Dermatologie* 2009; 35: 94–98.

38. IFRA. International Fragrance Association. IFRA standards (37th amendment). 3- and 4- (4-Hydroxy-4-menthylpentyl)-3-cyclohexene-1-carboxaldehyde (HMPCC), April 2003. Available at: www.ifraorg.org (last accessed 16 January 2009).
39. IFRA. International Fragrance Associations. IFRA standards (43rd amendment). 3- and 4- (4-Hydroxy-4-menthylpentyl)-3-cyclohexene-1-carboxaldehyde (HMPCC), April 2003. Available at: www.ifraorg.org (last accessed 16 January 2009).
40. Rastogi S C, Johansen J D, Bossi R. Selected important fragrance sensitizers in perfumes—current exposures. *Contact Dermatitis* 2007; 56: 201–204.
41. Fenn R S. Aroma chemical usage trends in modern perfumery. *Perfumer Flavorist* 1989; 14: 1–10.
42. Rastogi S C, Johansen J D, Frosch P et al. Deodorants on the European market: quantitative chemical analysis of 21 fragrances. *Contact Dermatitis* 1998; 38: 29–35.
43. Rastogi S C, Johansen J D, Menné T et al. Contents of fragrance allergens in children's cosmetics and cosmetic-toys. *Contact Dermatitis* 1999; 41: 84–88.
44. Rastogi S C, Heydorn S, Johansen J D, Basketter D A. Fragrance chemicals in domestic and occupational products. *Contact Dermatitis* 2001; 45: 221–225.
45. Hostynek J J, Maibach H I. Thresholds of elicitation depend on induction conditions. Could low level exposure induce sub-clinical allergic states that are only elicited under the severe conditions of clinical diagnosis? *Food Chem Toxicol* 2004; 42: 1859–1865.

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