

Hydroxyisohexyl 3-cyclohexene carboxaldehyde-known as Lyr[®]: quantitative aspects and risk assessment of an important fragrance allergen

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Hydroxyisohexyl 3-cyclohexene carboxaldehyde, also known as Lyr[®], is a fragrance ingredient identified as the cause of contact allergic reactions in 2–3% of eczema patients undergoing patch testing. Lyr[®] has been included in the standard patch test series in many clinics due to its importance as an allergen. It has been used without restrictions in cosmetic products, until now. In the present study, the dose–response relationship of Lyr[®] contact allergy was studied with doses relevant for normal exposure in cosmetic products. 18 eczema patients, who previously had given a positive patch test to Lyr[®] 5% petrolatum, were included along with 7 control subjects. All cases were tested with a serial dilution of Lyr[®] in ethanol 6% to 6 p.p.m and subjected to a 2-week, repeated open application test with a low dose of Lyr[®] in ethanol. In the case of no reaction, this was followed by another 2 weeks of testing with a higher dose. The test was performed at the volar aspect of the forearm. In 16 of 18 cases (89%), a positive use test developed, 11 reacting to the low and 5 to the high concentration. None reacted to the vehicle control of ethanol applied to the contralateral arm. All controls were negative to both the test solutions of Lyr[®] and the ethanol control. The difference between the test and the control group was statistically significant (Fisher's test, $P < 0.001$). It is concluded that Lyr[®] at the current usage levels is inducing sensitization in the community. The same levels were shown to elicit allergic contact dermatitis in almost all sensitized individuals. A significant reduction in usage concentrations is recommended to prevent contact allergic reactions.

Key words: cosmetics; elicitation; fragrance; hydroxyisohexyl-3-cyclohexene carboxaldehyde; Lyr[®]; prevention; risk assessment; sensitization. © Blackwell Munksgaard, 2003.

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Hydroxyisohexyl 3-cyclohexene carboxaldehyde, also known as Lyr[®], is a fragrance ingredient widely used in fragrance compositions in cosmetics. Initial testing in animals and experimental induction studies in humans was negative (1). However, a systematic investigation in a European research collaboration showed that Lyr[®] was a frequent cause of allergic contact dermatitis in eczema patients. 2.7% gave a positive patch test reaction to Lyr[®], and in only 60% of these Lyr[®]-hypersensitive individuals, there was a simultaneous contact allergy to fragrance mix in the standard series (2). Lyr[®] was then tested by the German Contact Dermatitis Research Group, and 1.9% of eczema patients showed a positive patch

test to Lyr[®] 5% petrolatum. Half of those were found to be of clinical relevance (3). This leads to the inclusion of Lyr[®] in the standard patch test series in Germany and in many other clinics. Recently, Lyr[®] was also identified as a sensitizer in an animal experiment, the local lymph node assay (4).

In outlining a European, EU-sponsored research program for prevention of contact allergy to fragrance ingredients, Lyr[®] became a natural target of research.

In the present study, the elicitation profile of Lyr[®] on patch and use testing will be described in order to evaluate the safety profile of current exposure in relation to allergic contact dermatitis.

Patients and Methods

Test subjects

18 eczema patients, who on a prior occasion, had given at least a + reaction (erythema and infiltration) to Lyrall® 5% pet. on patch testing, were included in the study. Control subjects were eczema patients, who had given a negative patch test to Lyrall® 5% pet. on a prior occasion. Persons below the age of 18, pregnant women and subjects with active eczema were excluded from the study. The study was approved by the local ethical committees, and all participants were included after informed consent. Dermatological clinics in Malmö, Dortmund, Odense and Gentofte participated. Characteristics of the participants are summarized in Table 1.

Test substances

Lyrall® with the assigned INCI name hydroxyisohexyl 3-cyclohexene carboxaldehyde, chemical name: 3-cyclohexene-1-carboxaldehyde, 4-(4-hydroxy-4-methylpentyl), CAS. no. 31906-04-4, was used for patch and use testing. The substance was delivered from Hermal Chemie as a courtesy. The purity by GC-MS was >99%.

Patch testing

Patch testing was performed with a 10-fold serial dilution of Lyrall® from 6% w/v to 6 p.p.m. in ethanol and with ethanol as control patch. The patches were applied to the upper back using Finn Chambers® (Epitest, Tuusula, Finland) with filter discs affixed with Scanpor® (Norgesplaster A/S, Vennessla, Norway). 15 µl of solution was applied to each patch. The test was left for 2D. Readings were performed on D2, D3 and D7 using the ICDRG's scale (5). The threshold response was defined as the weakest concentration giving a visible skin response in a continuous line

of patch test reactions starting with 6% w/v in ethanol. The threshold response data were incorporated into a logistic dose-response model (6).

Use testing

A repeated open application was made using the volar aspect of the lower arm. A test area of 3 × 3 cm of each arm was marked. 2 drops of a solution of Lyrall® in ethanol, equivalent to 30 mg (7), were applied. First, a concentration of 0.5% w/v Lyrall® was used on one area twice daily for 2 weeks; in the case of no reaction, applications were continued with 3% w/v Lyrall® in ethanol for another 2 weeks. On the contralateral site, ethanol was applied to a similar area and in a similar fashion. Applications were made by use of polypropylene droplet bottles (ApodanNordic, Copenhagen, Denmark) in a randomized and blinded fashion. Bottles were weighed prior to and after use. Test sites were evaluated weekly, and new sets of bottles with test solution and vehicle were issued.

Termination of the study

Readings of the use test sites were made by a use test reading scale (8), slightly modified due to the small test area (Fig. 1). A positive reaction was defined as erythema covering at least 25% of the test area and papule(s), regardless of number. The score for a positive reaction was thus at least 5 points. The test participants were encouraged to continue until at least this goal was reached. If no reaction had occurred in 4 weeks, the study was terminated.

Statistics

Fisher's test was used for comparison between 2 groups. A logistic dose-response model was used for analyzing the patch test data (6).

Table 1. Characteristics of patients and controls

	Test group (n = 18)	Control group (n = 7)
	Lyrall®-sensitized individuals	Lyrall®-negative patients
Female	16	5
Male	2	2
Age in years (median, range)	47.5 (28–70)	41 (24–56)
Duration of eczema	5 years (1–25)	3 years (1–10)
Hand eczema	11	3
Facial eczema	4	1
Axillary eczema	4	0
Atopic dermatitis		
Present	3	2
Previous		1

1. Involved area of application:					
	0	1–24%	25–49%	50–89%	90–100%
	0	1	2	3	4
2. Erythema					
Involvement				Strength	
None	Spotty	Homogenous		Weak	Medium Strong
0	1	2		1	2 3
3. Papules					
				Homogenous infiltration	
None	<5	5–10	>10		
0	1	2	3		4
4. Vesicles					
None	<5	5–10	>10		Confluent
0	1	2	3		4

Fig. 1. Modified scale for reading use test results. The minimum requirement for a positive test is marked in the figure, equivalent to that of 5 points.

Results

Patch testing

1 or more reactions to the dilution patch series were found in all cases but 1 (17/18). The patient completely negative on repeat testing had previously given a ++ reaction on patch testing and had abstained from using perfumed products. The dose-response curve is shown in Fig. 2. From this curve, it can be calculated that the dose of Lyr[®] eliciting a reaction in 10% of the group was 29 p.p.m and 50% elicited to 662 p.p.m., 95% confidence limits 7–69 p.p.m and 350–1250 p.p.m. This corresponds to 0.9 µg/cm² and 20 µg/cm², respectively, given the application of 15 µl solution to 0.5 cm² of patch test area using Finn Chambers[®] (Epitest).

Use testing

In 16 of 18 cases (89%), a positive use test developed by the given definitions. In 11 cases, the use test became positive to the low concentration (0.5% Lyr[®] in ethanol), and in 5, the use test became positive to the high concentration (3.0% Lyr[®] in ethanol). The 2 most sensitive patients began to give a reaction after only 2 applications. None reacted to the vehicle control of ethanol applied to the contralateral arm. All controls were negative to both the test solutions of Lyr[®] and the ethanol control. The difference between the test and the control group is statistically significant (Fisher's test, $P < 0.001$). The doses of Lyr[®] used per cm² per application in the different groups are illustrated in Figs 3 and 4. Amongst cases becoming positive to the lowest dose (weeks 1 and 2) the median amount applied was 15.3 µg Lyr[®]/cm²/application (range 3.4–22.2); in the cases becoming negative, weeks 1 and 2, had used 12.3 µg/

cm²/application (range 8.6–22). The 5 cases becoming positive to the highest dose had used 126.2 µg Lyr[®]/cm²/application (range 40.5–226.2) in weeks 3 and 4.

The amounts applied by normal use of deodorants/eau de toilette or parfums are 5, 25 and 300 µg/cm²/application, respectively, given the conditions of exposure, that are listed in Table 3.

Use test readings

The median day of termination due to a positive use test was D9 (range 3–28). The median score of use

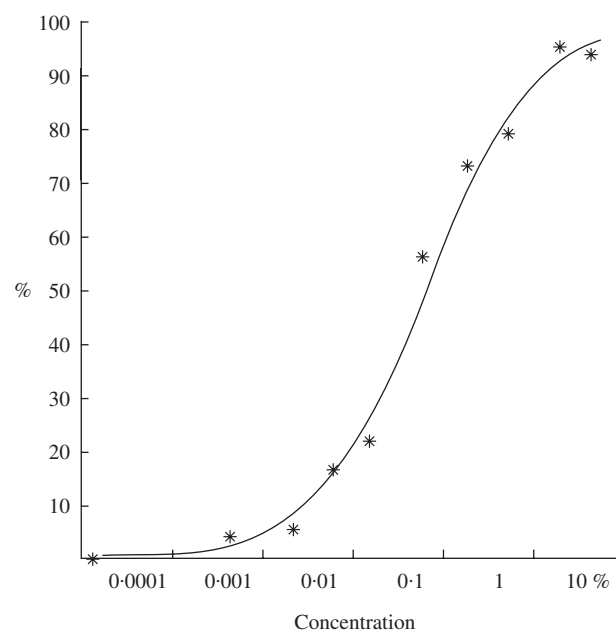


Fig. 2. The dose-response curve obtained by patch testing with a serial dilution of Lyr[®] in ethanol from 6% w/v to 6 p.p.m. The dose eliciting a response in 10% of the test group was 29 p.p.m., equivalent to 0.9 µg/cm², and the dose eliciting in 50% of individuals was 662 p.p.m.

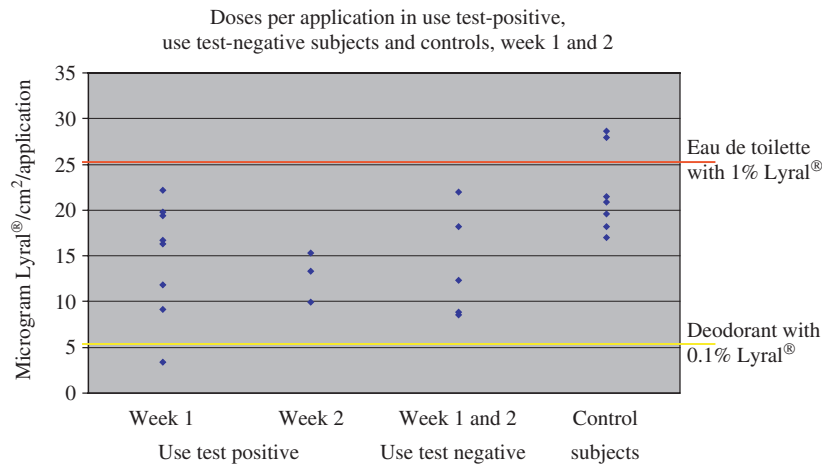


Fig. 3. The amount of Lyrall®/cm²/application is given for different groups: the group giving a positive use test, week 1 or 2, the cases positive to Lyrall® on patch testing with a negative use test, week 1 and 2 and the controls. In the diagram, the theoretical amount of Lyrall® used per application of an eau de toilette with 1% Lyrall® (red line) and of a roll-on deodorant with 0.1% Lyrall® is given (yellow line).

test reaction amongst those reacting to the lowest dose (0.5% Lyrall®) was median 10 (range 7–17), and amongst those reacting to the highest dose, the median score was also 10 (range 8–16) when the test was terminated. In 9 cases, there was full involvement of the test area, 5 cases showed homogeneous infiltration (Fig. 5) and 6 had a follicular pattern with many papules, and in 10 cases, vesicles were present.

Use test/patch test relationship

The threshold on patch testing to Lyrall® was median 0.06% (range 2–<0.0006%) in the group becoming positive on use testing to the low concentration and median 2% (range 6–0.002%) in the group becoming positive on use testing to the high concentration.

Discussion

Lyrall® is recognized as a frequent cause of contact allergy (2, 3), and the impression from the clinical epidemiological experience is that it is a moderately potent allergen. Lyrall® has a similar structure-activity-relationship to hydroxycitronellal (9), which is one of 8 fragrance ingredients of the diagnostic test for fragrance allergy, the fragrance mix. While hydroxycitronellal has been recognized as an allergen for many years (10) and restricted due to its allergenic properties to 1% in finished products by the International Fragrance Association (IFRA) (11), no restrictions have been applied to Lyrall® until recently (12). The many years of uncontrolled exposure to Lyrall® have produced many cases of contact

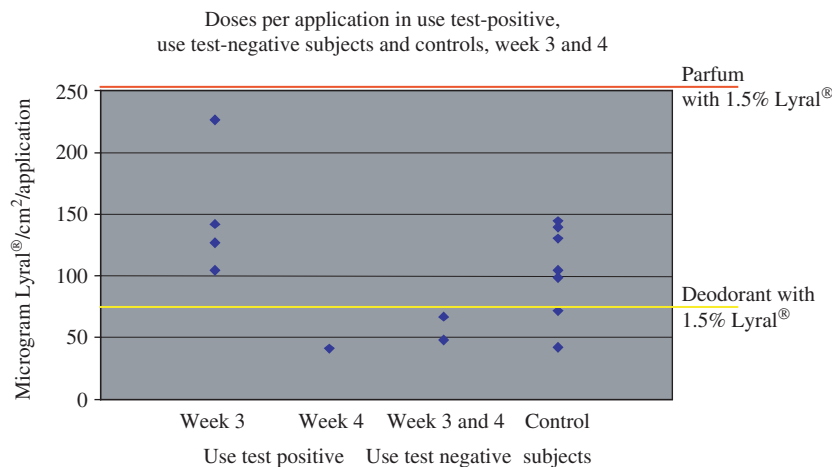


Fig. 4. The amount of Lyrall®/cm²/application is given for different groups: the group giving a positive use test, week 3 or 4, the cases positive to Lyrall® on patch testing, but who have a negative use test, weeks 3 and 4 and the controls. In the diagram, the theoretical amount of Lyrall® used per application of a parfum with 1.5% Lyrall® is indicated by a red line above the diagram, as the amount used may be much larger, e.g. 300 µg/cm²/application. The amount of Lyrall® per application of a deodorant with 1.5% Lyrall® is indicated by a yellow line (see Table 3).

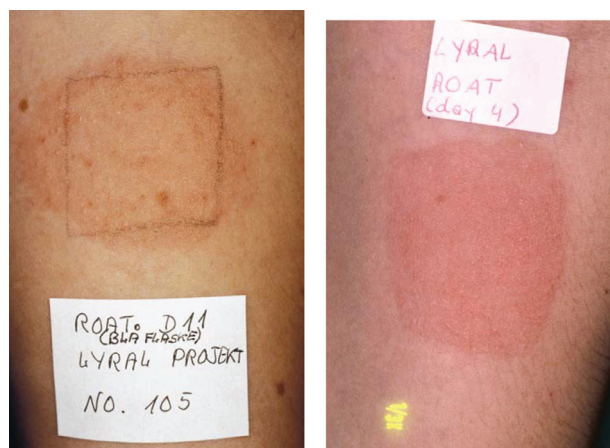


Fig. 5. Positive use tests to repeated applications of Lyr[®] in ethanol.

allergic reactions leading to inclusion in the standard patch test series, e.g. in Germany (3).

In the current study, the dose–response relationships of Lyr[®] were studied in sensitized individuals and compared with the exposure situation from the use of fine fragrances (eau de toilette/parfum) and deodorants. The study shows that sensitized individuals will respond with eczema to realistic exposure levels of Lyr[®]. In 89% (16/18 cases), an allergic eczema developed to a repeated open application of a Lyr[®] solution, whereas none out of 7 controls responded ($P < 0.001$). The levels used were calculated based on published exposure information. A survey of marketed products showed that Lyr[®] was present in 46% of fine fragrances at a concentration $> 1\%$ and with an average concentration of 3.2% (13). In case studies, Lyr[®] has been identified in fine fragrances at concentrations of 0.1–3.8% (Table 2). The current study simulated the usage range of an eau de toilette with 1% Lyr[®] and a parfum with 1.5% Lyr[®] (Table 3). In this usage range 11 of 18 (61%) reacted to exposures lower than the ‘model’ eau de toilette exposure and 16 of 18 to exposures lower than a ‘model’ parfum (Figs 3 and 4).

For comparison, results from a newly developed risk assessment model for induction of contact

allergy give a sensitization reference dose for Lyr[®] of $10 \mu\text{g}/\text{cm}^2$ in a fine fragrance, corresponding to 0.3% (Table 3). The calculation of the sensitization reference dose is based on the principles, which have been used by industry toxicologists to estimate risk for another fragrance ingredient, cinnamal (14). The sensitization reference dose signifies that exposures exceeding this limit are likely to induce sensitization, which clearly is the case with Lyr[®] at the current usage levels in fine fragrances.

A European survey has shown that deodorants contain up to 0.18% Lyr[®] (15). The content of 0.1% Lyr[®] in a deodorant corresponds to an exposure of $5 \mu\text{g}/\text{cm}^2$ /application (16). However, there is no published information on the variation in use of deodorants. It is likely that the amount will differ according to the type of deodorant (roll-on, spray and stick) and that abraded skin in the axilla due to shaving may have an additional effect. The exposure to deodorants is in some ways more similar to the occluded patch test than the repeated open application test. On patch testing, 10% reacted to around $1 \mu\text{g}/\text{cm}^2$, a fivefold lower dose than a usual deodorant exposure. Translated into percentage this is 0.02% Lyr[®] in a deodorant. It has been shown for nickel that even though the individual reactivity varies over time, the cumulated dose–response curves do not change significantly (17). Even dose–response curves from patch testing obtained in different clinics show limited variations (18). The nickel regulation, which has proven successful in preventing nickel allergy (19), is based on dose–response curves obtained in sensitized individuals. The limit used in the regulation corresponds to the nickel release/ cm^2 /week, which elicits eczema in about 10% of sensitized individuals. The appropriate safety factors and long-term low-dose experiments in the axilla have not been carried out to suggest an appropriate level for use of Lyr[®] in deodorants, and there is no published information on the principles for calculating a sensitization reference dose for deodorants. However, it must be assumed that the safety factors would be greater than for a fine

Table 2. Exposure data for Lyr[®]

Product type	Frequency in products	Concentration range	Reference
Fine fragrances	46%	Above 1% (average concentration 3.2%)	(13)
Deodorants	53%	1–1874 p.p.m.	(16)
Domestic products	10%	36–103 p.p.m.	(21)
Aftershave	All case reports	0.06%	(2)
Fragrances		0.12–2.34%	
Deodorants		0.02–0.56%	
Lotions		0.02–0.2%	
Fine fragrances	Two cases	3.67–3.83%	Rastogi 2003 (unpublished)

Table 3. Risk assessment for sensitization to Lyrall[®]

Potency LLNA: EC3 value = 17.1% corresponds to 4275 µg/cm ² Classification as a weak sensitizer gives default no-effect level* for use in risk assessment: 1000 µg/cm ²	(4) (14)
Safety factors Fragrances: 10 for inter-individual variability, 1 for matrix [†] and 10 for exposure patterns: ×100 Deodorants: undetermined	(14)
Exposures Fragrance, eau de toilette, exposure by pump spray: 2.6 µg/cm ² Lyrall [®] in product used at concentration 1% (0.01 × 2600 µg/cm ²) = 26 µg/cm ² Lyrall [®] in product used at concentration 1.5% (0.015 × 2600 µg/cm ²) = 39 µg/cm ² Fragrance in parfum, exposure by drop application: 20 mg/cm ² Lyrall [®] in product used at concentration 1.5% (0.015 × 20 000 µg/cm ²) = 300 µg/cm ² Fragrance in anti-perspirant exposure: 5 mg/cm ² Lyrall [®] in product used at concentration 0.1% (0.001 × 5000 µg/cm ²) = 5 µg/cm ² Lyrall [®] in product used at concentration 1.5% (0.015 × 5000 µg/cm ²) = 75 µg/cm ²	(22) (16)
Induction Sensitization reference dose for fragrances: 10 µg/cm² Sensitization reference dose for deodorant: <10 µg/cm ² (unknown) Margin of safety eau de toilette (1% Lyrall [®]): 10/26 µg/cm ² : 0.38 (1.5% Lyrall [®]): 10/39 µg/cm ² : 0.26 Margin of safety parfum (1.5% Lyrall [®]): 10/300 µg/cm ² : 0.03 Margin of safety antiperspirant (1.5% Lyrall [®]): 10/75 µg/cm ² : 0.13	Interpretation <1: not sufficiently safe <1: not sufficiently safe <1: not sufficiently safe <1: not sufficiently safe
Elicitation thresholds based on dose-response patch test data: 1 µg/cm²	
Induction and elicitation 'safe(r)' concentrations': Eau de toilette: 0.03–0.3% Parfum: 0.005–0.05% Deodorants: 0.02–<0.2%	

LLNA, Local lymph node assay.

*The potency classification in the LLNA is used to group substances and assign a theoretical no-effect level in humans, which can be used in risk assessments.

†The choice of safety factors is based on the publication (14). The safety factor is 1, as there is no difference between the matrix in use of the product (fine fragrance) and in the animal experiment.

fragrance, which again would mean a level well below 10 µg/cm².

Benchmarking to an experimental study of deodorant exposure with the chemically similar substance hydroxycitronellal is in general agreement with the dose–response data from patch testing with Lyrall[®]. The study showed that all test subjects reacted to roll-on deodorants containing 0.032–0.32% hydroxycitronellal on repeated exposure in the axilla. A level of hydroxycitronellal below 0.03% in deodorants was recommended (20). In April 2003, an IFRA standard was issued restricting Lyrall[®] to 1.5% in finished products, giving the producers a year to comply (12). This concentration is well above the described sensitization and elicitation reference doses and finds no support in the currently available data.

It can be concluded that Lyrall[®] at the current usage levels is inducing sensitization in the community. The same levels have been shown to elicit allergic contact dermatitis in almost all sensitized individuals. This also applies when the possible effect of the newly established IFRA restriction is considered. Results from the current experiment and a risk assessment model suggest that a safer level of exposure would be in the range of 1–10 µg/cm², corresponding to 0.02–0.3% depending on the type of product. A significant reduction in usage concentrations is recommended to reduce the number of contact allergic reactions to Lyrall[®] in the community.

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