

Hydroxyisohexyl 3-Cyclohexene Carboxaldehyde (Lyal) in Patch Test Preparations Under Varied Storage Conditions

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Background: The common practice of preparing patch tests in advance has recently been called into question by researchers. It has been established that fragrance compounds are volatile and their testing efficacy may be affected by storage conditions and preparation. Allergens in fragrance mix I rapidly decrease in concentration after preapplication to test chambers.

Objectives: This study aimed to investigate the volatility of hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC) in petrolatum when stored in test chambers and to explore the correlation between vapor pressure and allergen loss in petrolatum during preparation and storage.

Methods: Standardized HICC in petrolatum was prepared and stored in IQ Chambers and Finn Chambers with covers at 5°C, 25°C, and 35°C, and concentration was analyzed at intervals for up to 9 days using gel permeation chromatography.

Results: Changes in HICC concentrations were not statistically significant at 8 hours at 5°C, 25°C, and 35°C. After 9 days, HICC concentrations were found to fall approximately 30% when stored at 35°C, 10% at 25°C, and less than 5% at 5°C. There was no significant difference between IQ and Finn chambers.

Conclusions: Hydroxyisohexyl 3-cyclohexene carboxaldehyde concentrations are more stable in petrolatum than many other studied fragrance allergens, but HICC is still at risk for decreasing concentration when exposed to ambient air or heat for prolonged periods.

WHAT'S ALREADY KNOWN ABOUT THIS TOPIC?

Patch tests are often prepared in advance. Recent studies have investigated fragrances in fragrance mix I and selected acrylates/methacrylates and found that concentrations may fall to an extent that impacts efficacy if prepared in advance and exposed to ambient air for extended periods of time.

WHAT DOES THIS STUDY ADD?

This study investigates HICC—the most frequent sensitizer in fragrance mix II—and finds HICC concentrations in prepared patch tests to be stable at short intervals and when stored at 5°C;

however, HICC concentrations are at risk for significant loss if exposed to ambient temperatures or heat for prolonged periods. Increased evidence supporting a relationship between vapor pressure and concentration loss is discussed.

Hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC), also known as Lyal, is the most frequent sensitizer in fragrance mix II and is a common additive in perfumes, lotions, and other beauty products.^{1,2} In some settings, clinicians prepare patch tests up to a week in advance or order patient-specific patch tests to be mailed by outside laboratories. Patch tests may experience prolonged exposure to temperatures above storage recommendations and contact with ambient air during storage or transport. The purpose of this study was to investigate the volatility of HICC suspended in petrolatum at different temperatures and in different chambers to determine whether prolonged contact with ambient air could affect its concentration, potentially impacting diagnostic efficacy.

MATERIALS AND METHODS

Standardized 5.0% wt/wt HICC in petrolatum was prepared (International Flavors & Fragrances, Union Beach, NJ; 97.5% pure stabilized with 0.1% butylated hydroxytoluene; Vasilinum Album Snowwhite USP/NF Qual. E; Apoteket, Göteborg, Sweden).

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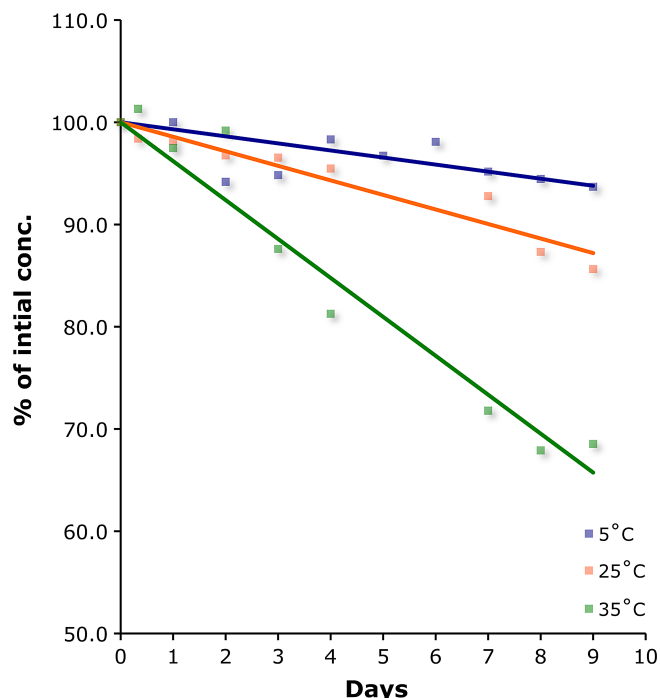


Figure 1. Percentage of initial concentration of HICC in IQ Chamber.

This standard was applied to both IQ Chambers (Chemotechnique Diagnostics, Vellinge, Sweden) and Finn Chambers with Finn Chamber covers (SmartPractice, Phoenix, Ariz). The covers are not airtight. Patch test preparations were stored at 5°C, 25°C, and 35°C, in a refrigerator, cupboard, and oven, respectively. Thermometers were used to ensure consistent temperature. There was no significant airflow in the refrigerator, cupboard, or oven. Humidity was not measured. The concentration of HICC was measured in triplicate using gel permeation chromatography (GPC) every hour for 8 hours and at intervals up to 9 days. At appropriate intervals, the petrolatum was collected, and 10 μ g was placed into a glass test tube and was dissolved in 5 mL of dichloromethane high-pressure liquid chromatography-grade (Fisher Scientific, Leicestershire, United Kingdom). The solubility of HICC in dichloromethane is sufficient for extraction. The test tube was capped, stirred using an orbital mixer, then filtered either using a disposable syringe and filter or a glass pipette with compressed cotton. The filtered solution was analyzed by GPC system consisting of an LDC Gradient Master and 2 LDC Constametric III pumps (Laboratory Data Control, Riviera Beach, Fla). A Rheodyne 7725 injector (Rheodyne/ IDEX Health & Science, Rohnert Park, Calif) was used, allowing the injection volume of 20 μ L. A UV1000 variable wavelength detector (Thermo Separation Products, Waltham, Mass) was used at 240 nm with dichloromethane as the mobile phase. A flow rate of 0.3 mL/min was used through 3 consecutive GPC analytic columns packed with styrene-divinylbenzene copolymer (2 columns 3 μ m, 50Å from Showa Denko, Kanagawa, Japan, and 1 column 5 μ m, 100Å from Polymer Standard Service, Mainz, Germany).

RESULTS

Hydroxyisohexyl 3-cyclohexene carboxaldehyde concentrations were shown to decrease when stored in preprepared patch test chambers (Figs. 1 and 2). At 8 hours, there was no statistically significant fall in concentration at any temperature. There was no significant difference between the IQ Chamber and Finn Chamber with covers. After 9 days, the concentration of HICC fell approximately 30% when stored at 35°C, 10% when stored at 25°C, and less than 5% when stored at 5°C. Standard deviations and coefficients of variation were calculated for each sample. The mean (range) value of all SDs was 3.9 (0.8–10.9), and mean (range) value of all coefficients of variation was 4.8 (1.1–15.7).

DISCUSSION

Our methods for evaluating allergens for concentration loss when prepared and stored in a simulated clinical setting are sufficiently similar to other allergen storage volatility studies to produce useful comparisons.^{3–6} Our data differ from a previous study that showed that most fragrance allergens in fragrance mix I decreased significantly in concentration within the first 24 hours.³ Of note, amyl cinnamal, which has the lowest vapor pressure of the fragrance allergens in fragrance mix I was stable at 8 hours, and significant decrease in concentration was not seen. Of other allergens tested in preprepared IQ chambers at room temperatures, triethylene glycol diacrylate had the lowest vapor pressure and also showed no loss of concentration at 8 hours.

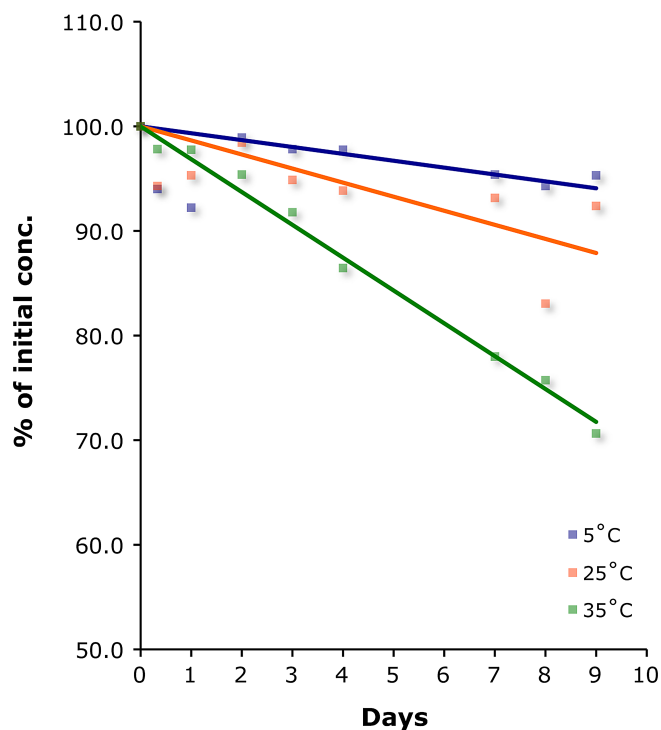


Figure 2. Percentage of initial concentration of HICC in Finn Chamber with cover.

TABLE 1. The Stability of Selected Allergens Suspended in Petrolatum in IQ Chambers Arranged by Vapor Pressure

Allergens	Vapor Pressure, mm/Hg	Mowitz et al	Goon et al	Mose et al	Our Results
		Approximate % of Initial Concentration After 8 h at 23°C	Approximate % of Initial Concentration After 8 h at 23°C	Approximate % of Initial Concentration After 8 h at 22°C	Approximate % of Initial Concentration After 8 h at 25°C
MMA	36.9		50	50	
2-HEMA	0.159		80	85	
2-HPA	0.115		40	70	
Cinnamal	0.0289	55		75	
Cinnamyl alcohol	0.018	80			
Geraniol	0.0128	70			
EGDMA	0.0121		80		
Eugenol	0.00975	75		85	
Isoeugenol	0.00518	95			
Hydroxycitronellal	0.00323	80			
Amyl cinnamal	0.0023	100			
HICC	0.000086			99*	
TREGDA	0.000095		100		

*SD, 1.6.

2-HEMA, 2-hydroxyethyl methacrylate; 2-HPA, 2-hydroxypropyl acrylate; EGDMA, ethylene glycol dimethacrylate; MMA, methyl methacrylate; TREGDA, triethylene glycol diacrylate.

It has been discussed in prior articles that allergens with high vapor pressures may be more likely to decrease in concentration when stored in a clinical setting.^{3,5} Our data combined with prior studies show a qualitative trend of increasing vapor pressure and decreased allergen concentrations in preprepared storage conditions. Thus, it is useful for clinicians and researchers to appreciate evidence that an objective physical property of an allergen—vapor pressure—may increase suspicion for volatility and loss of allergen with prepreparation and storage (Table 1).

Our data show HICC to be more stable than other studied fragrance allergens. Significant decreases in allergen concentration were not seen at 8 hours; however, preprepared tests with HICC in petrolatum are at risk for decreasing allergen content when exposed for prolonged periods to ambient air or heat. Hydroxyisohexyl 3-cyclohexene carboxaldehyde has the lowest vapor pressure of all the allergens in fragrance mix I and II. For this reason, we suspect that HICC is the most stable of the fragrance allergens in petrolatum. The volatility of the remaining fragrances in fragrance mix II in petrolatum, as well as other allergens with high vapor pressures, warrants further research.

Although a previous study found decreased allergen loss in Van der Bend Chambers as compared with IQ Chambers,⁵ our data found no significant difference in HICC loss between the 2 chambers tested—Finn Chambers with covers and IQ Chambers. Our results suggest that Finn Chamber covers and reclosing of IQ Chambers after initial allergen application does little to prevent volatile allergen loss.

The clinical practice of preparing patch tests in advance or ordering patient-specific patch tests through the mail without refrigeration may risk false negatives. Although more stable than other fragrances, HICC may also be at risk for allergen loss if stored improperly. To ensure proper concentrations, patch tests with volatile allergens should not be prepared in advance.

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