

Volatility of Fragrance Chemicals: Patch Testing Implications

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Background: Diagnostic and predictive patch testing to determine contact allergy due to fragrance materials requires applying a fixed dose of material to the skin. This dose can be affected by the volatile nature of fragrances; little data exist on how the loss of fragrance dose due to volatility affects patch testing.

Objective: (1) To evaluate pH dependence and evaporation rates of two fragrance chemicals, geraniol, citronellol, and a common fragrance solvent, diethyl phthalate (DEP) and (2) Assess implications for predictive patch-testing methods for fragrances.

Method: pH analysis of each material at 1% for three values (4.0, 5.0, 7.0) was done over 40 hours. Volatility experiments for each material, nonradiolabeled and radiolabeled, were conducted over a 24-hour period, taking readings at six time points (5 minutes, 15 minutes, 40 minutes, 1 hour, 3 hours, and 24 hours).

Results: Evaporation rates were not sensitive to pH shifts from 4.0 to 7.0. Evaporation rates for nonradiolabeled materials were low: after 24 hours, geraniol lost 8.9%, citronellol 27.0% and DEP 14.5%. The volatility data for radiolabeled materials demonstrated that geraniol loses up to 39% of its dose, citronellol loses up to 26%, and DEP up to 14% within 40 minutes.

Conclusion: The tendency of fragrance materials to evaporate can impact the dose being applied to the patch and therefore the result of the patch and ultimately the decision-making process regarding that fragrance material's safety. These data, developed with DEP, utilized in a predictive sensitization assay cannot be generalized.

THE PHYSICOCHEMICAL UNIQUENESS of fragrance materials adds complexity when dermal absorption is being determined. Because of the lack of data on individual fragrance materials, dermal exposure has often been incorrectly assessed owing to reliance on the conservative assumption in toxicologic modeling of 100% dermal absorption.^{1,2} This approach is inconsistent with research on dermal absorption that has found it to be largely dependent on a compound's characteristics, namely, lipophilicity, molecular weight, and volatility.³

Fragrance materials evaporate from skin to some degree. This is the main way in which they impart aroma, via evaporation into the air, making them detectable as fragrance. Because volatile materials evaporate into the environment, diagnostic and predictive patch-test procedures need to adequately measure and account for the amount of material lost by evaporation before and during application of the patch. The effective skin exposure dose drops as the fragrance material is absorbed into the skin

and as it evaporates from the skin.⁴ Since fragrance regulations are based largely on dosing data published from clinical patch-test studies, starting levels of volatile fragrance materials need to be accurate. This is true for whatever type of vehicle is used (petrolatum, olive oil, water, etc), as well as the type of protocol being followed.

In addition to regulations, risk assessments for fragrance materials rely on the accurate evaluation of the dose metrics of exposure. The induction and elicitation of contact allergy depend on the dose applied, measured as quantity of fragrance per unit of area.^{5,6} If evaporation upon dosing occurs, the material lost will directly affect the ability to elicit reactions, thereby making exposure measurements inaccurate. For diagnostic patch testing of fragrances, evaporation of dose should be measured, as should loss via evaporation from semioclusive patches sometimes used in predictive testing.

No detailed studies characterize citronellol and geraniol (two commonly used fragrance materials) and diethyl phthalate (DEP) (a diluent used in many predictive studies as a vehicle when mixed with ethanol). This research measures the volatility of these fragrance compounds in an aqueous vehicle under controlled conditions to learn how factors such as pH affect volatility and with the longer range goal of determining the effects of fragrance volatility on dermal absorption and kinetics. Gaining this informa-

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tion should help more accurately determine a toxicologic exposure profile for more accurate risk assessment of these fragrance materials and those that are similar in chemistry and volatility characteristics.⁷

Materials and Methods

Model Compounds

Aqueous solutions of radiolabeled geraniol ($1\text{-}^{14}\text{C}$) (molecular weight, 153.25; 0.05 mCi/mL; purity, 99%; specific activity, 55 mCi/mmol), citronellol ($1\text{-}^{14}\text{C}$) (molecular weight, 156.27; 0.05 mCi/mL; purity, 99%; specific activity, 52 mCi/mmol), and DEP (carbonyl- ^{14}C) (molecular weight, 224.24; 0.1 mCi/mL; purity, 99%; specific activity, 55 mCi/mmol) were purchased from American Radiolabeled Chemicals, Inc. (St. Louis, MO). DEP was chosen because it is the vehicle recommended by the Research Institute for Fragrance Materials (RIFM) for patching in a 1:3 mixture with ethanol; however, little is known of its effects on fragrance compounds.⁷

Nonradiolabeled geraniol natural isolate (98.5% purity) was obtained from Citrus and Allied Essences Ltd (Lake Success, NY), Berjé (Bloomfield, NJ) provided nonradiolabeled citronellol natural isolate (95% purity), and DEP (100% purity) was obtained from Univar USA (Redmond, WA).

Stock Solution

A solution of each compound was made with a 2% concentration (weight per volume [w/v]) of each radiolabeled fragrance in stock nonradiolabeled material. The stock solution was made to approximate a dose volume of 10 μL per application, containing approximately 0.5 μCi radioactivity.

pH Analysis

To characterize each material for pH effects, each of the three nonradiolabeled samples was added to a 10 mL vial of deionized water prepared at three pH values (4.0, 5.0, and 7.0). Each was diluted to 1% (w/v). pH readings were taken with an Orion 3-Star pH meter (Thermo Electron Corporation, Waltham, MA) at each of the following time points: 15 minutes, 40 minutes, 1 hour, 2 hours, 4 hours, and 20 hours. Vials were tightly capped after each reading to prevent evaporation. Samples were kept in a temperature-controlled hood at $22^\circ\text{C} \pm 2^\circ\text{C}$.

Volatility Experiments for Nonradiolabeled Materials

The relative volatility of the nonradiolabeled fragrance materials was determined by adding 10 μL of each material to a 20 mm molded plastic chamber (Hill Top Research, Cincinnati, OH). The beginning weight of each dosed chamber was determined with an accu-225D analytical balance (Fisher Scientific, Pittsburgh, PA) and recorded. Each time point was run in triplicate for 5 minutes, 15 minutes, 40 minutes, 1 hour, 3 hours, and 24 hours. The chambers were left open to allow for volatilization. At the end of each time point, the end weight was recorded and the percent loss calculated. All experiments were done in an open room at $26^\circ\text{C} \pm 2^\circ\text{C}$ and a relative humidity of $41\% \pm 2\%$.

Volatility Experiments for Radiolabeled Materials

The relative volatility of the radiolabeled fragrance materials was determined by adding 10 μL of each material to a plastic disk (area = 2 cm^2). The weight of the syringe before and after dosing was recorded to obtain an accurate dose amount. After dosing, the disks were placed on a stable surface open to the environment. Each time point was run in triplicate for 5 minutes, 15 minutes, 40 minutes, 1 hour, 3 hours, and 24 hours. After the desired time point was reached, the disks were removed with forceps and added to scintillation vials. A scintillation cocktail (Universol, MP Biomedicals, Irvine, CA) was added to each vial, and the vials were placed into the counter.

Scintillation Counting

Background control samples and test samples were counted in a computer-controlled liquid scintillation analyzer (Tri-Carb 2900TR, PerkinElmer, Inc., Waltham, MA).

Results

For pH effects at 1% in water, none of the materials showed significant variation in pH value from the buffered solution over 20 hours. This indicates that they were not sensitive to pH shifts from 4.0 to 7.0.

Evaporation rates for the nonradiolabeled materials were low. After 24 hours, geraniol showed a loss of 8.9%, citronellol showed a loss of 27.0% and DEP lost 14.5%. As

was expected, evaporation (noted as percent loss) increased as time elapsed (Fig 1).

Radiolabeled material volatility data was analyzed for significant differences. For geraniol, the most weight loss (87.8%) occurred after 21 hours. For citronellol, 83.8% of weight loss occurred after 4 hours (Fig 2). For each material, note the trend of increasing loss of mass with increasing time. This loss is assumed to be due to volatilization.

DEP showed unusual activity. It increased in overall recovery up until 3 hours, and then evaporation loss started, the majority (68.9%) after 4 hours. Another drop of evaporation occurred at 6 hours, and another maximum loss (55.5%) was reached after 21 hours (see Fig 2).

The mean values, standard deviations, and percent loss of each of the three materials at each given time point are shown in Table 1.

Discussion

With the new European Union's Registration, Evaluation and Authorization of Chemicals (REACH) legislation, industry, dermatologists, toxicologists, and regulators seek a nonanimal-based model to predict skin sensitization potential.⁸ One option is quantitative structure activity relationship (QSAR), a modeling method based on the potential of compounds to cause skin sensitization based on a given set of structural and chemical characteristics. QSAR uses the knowledge of mechanisms of skin sensitization to predict fragrance compound behaviors on the basis of mathematical modeling of experimental rate constants, statistics based on local lymph node assay data, or expert knowledge-based computer programs.^{9,10}

In order to create the models relied on for QSAR for fragrance materials and make reliable dermal exposure assessments with other toxicologic models, parameters of

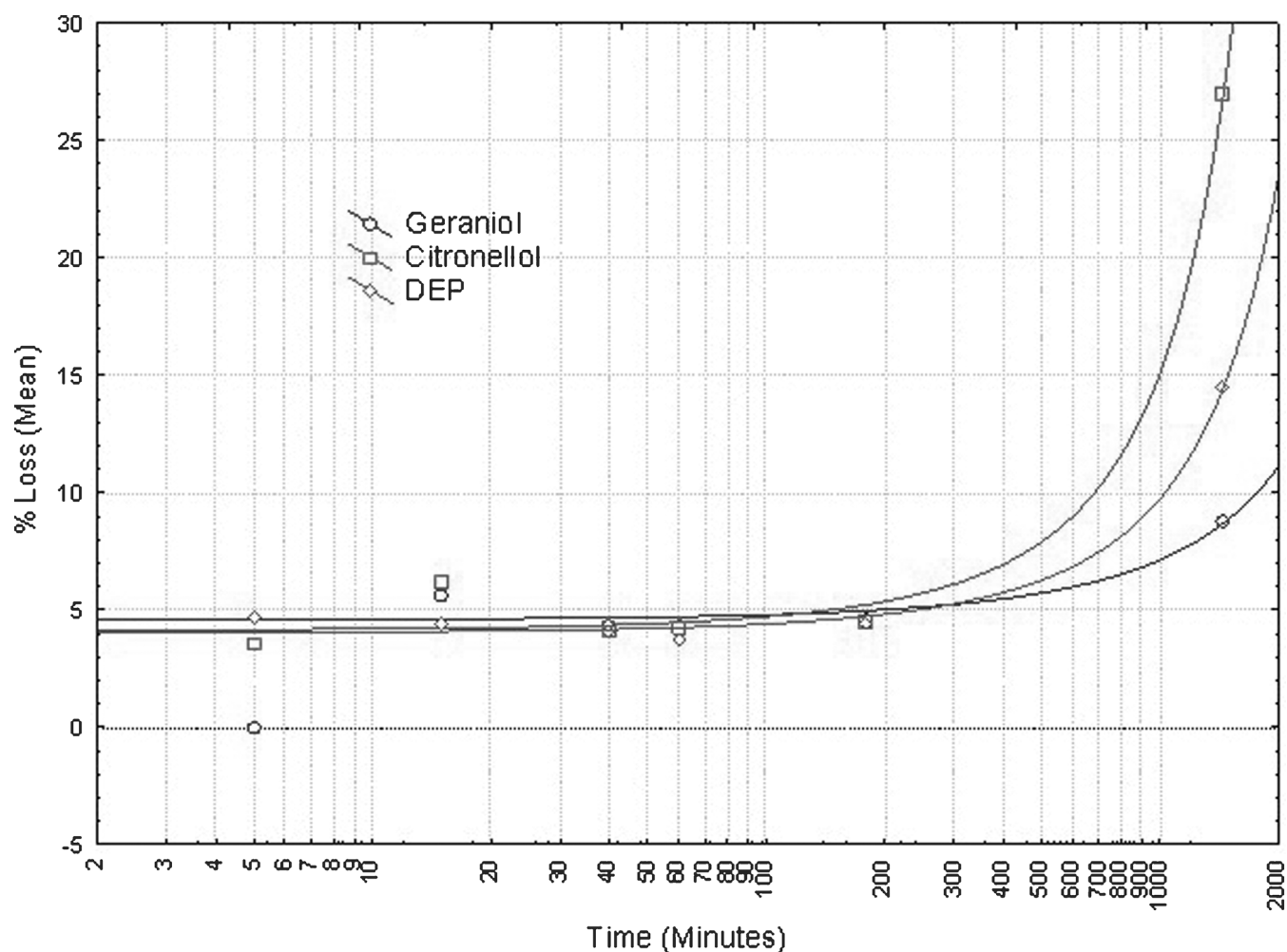


Figure 1. Evaporation rates (percent loss) for each nonradiolabeled fragrance compound, plotted against time on a logarithmic time scale. DEP = diethyl phthalate.

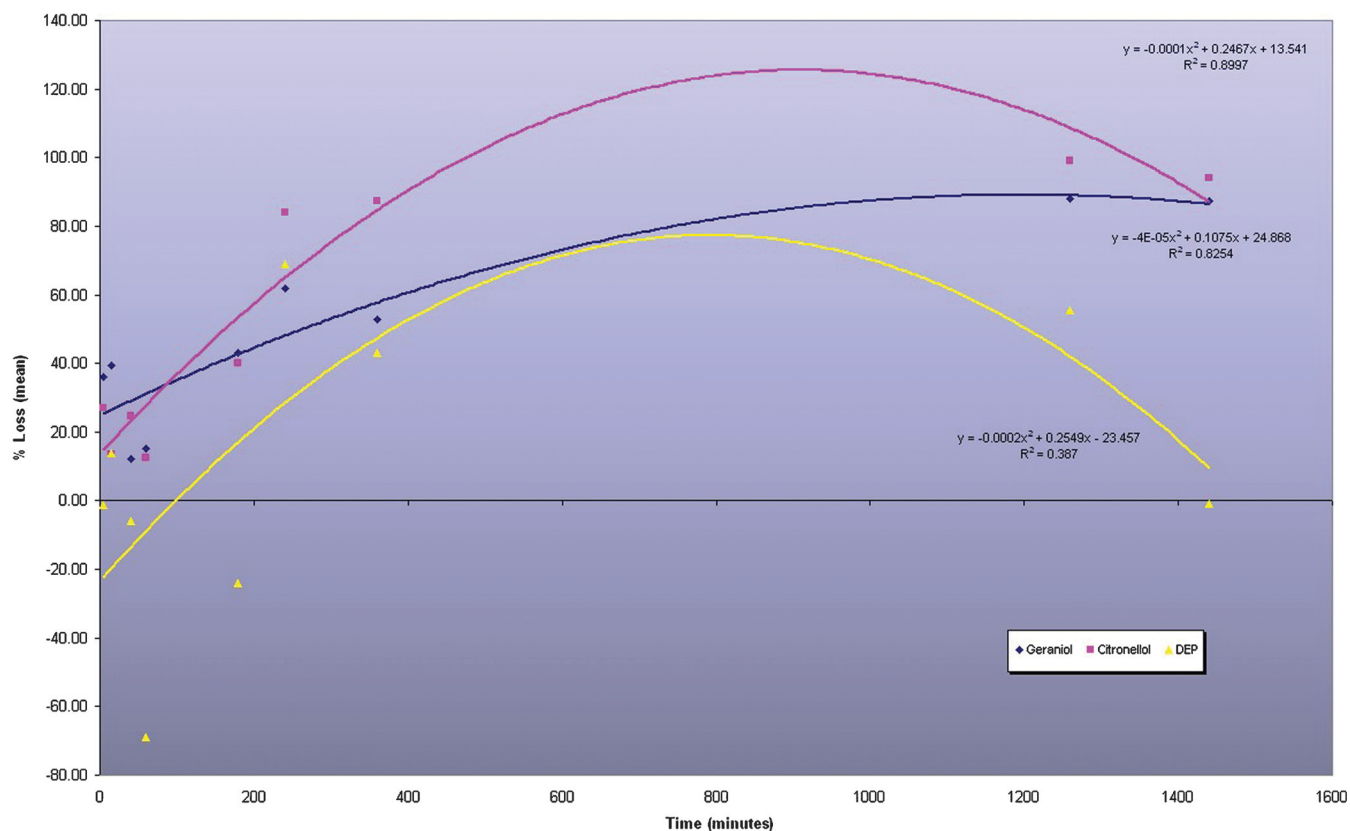


Figure 2. Evaporation profiles of geraniol, citronellol, and diethyl phthalate (DEP) over 24 hours.

dermal absorption and metabolism must be determined. The ability to make these determinations has improved since the early studies on dermal absorption. The science of dermal absorption was originally devised to investigate exposures to occupational hazards but has since been recognized for its usefulness in drug and cosmetics assessments.¹¹ It is a complex science, as dermal absorp-

tion depends on factors that include material polarity, the membrane/water partition coefficient (K_m) of the permeating material, molecular weight, and the material's octanol/water partition coefficient (K_{ow}), as well as the skin barrier and stratum corneum integrity.^{12–15} Extensive research on the effects of these factors on other volatile chemicals have determined that temperature, humidity,

Table 1. Evaporation of Radiolabeled Geraniol, Citronellol, and Diethyl Phthalate over 24 Hours (1,440 Minutes)

	Time (min)									
	5	15	40	60	180	240	360	1,260	1,440	
Geraniol										
Mean % loss ± SD	36.2 ± 6.3	39.4 ± 24.1	12.33 ± 20.3	15.3 ± 23.0	43.1 ± 30.5	62.0 ± 25.1	52.8 ± 17.4	87.9 ± 16.7	87.2 ± 7.9	
Citronellol										
Mean % loss ± SD	26.9 ± 13.3	13.7 ± 17.2	24.6 ± 7.2	12.7 ± 17.0	40.0 ± 52.0	83.8 ± 13.4	87.2 ± 5.2	99.2 ± 0.2	94.0 ± 8.4	
DEP										
Mean % loss ± SD	-1.1 ± 10.6	13.8 ± 24.5	-5.8 ± 1.9	-69.1 ± 6.6	-23.9 ± 15.2	69.0 ± 24.7	43.0 ± 15.3	55.5 ± 8.4	-0.9 ± 11.1	

DEP = diethyl phthalate.

and occlusion all influence the extent of a chemical's absorption.^{16–18} Other factors also noted include the hydration of the stratum corneum of the skin and the role of the vehicle in enhancement of penetration.¹¹

Because of the lack of time-course evaporation data on fragrance materials and the importance of this variable to risk assessment, volatility was assessed for the two commonly used aroma materials geraniol and citronellol. Geraniol makes up approximately 20% of rose oil and approximately 80% of palmarosa oil. It is included in the list of 26 alleged fragrance allergens that should be declared when used in cosmetics² and is classified as a weak contact allergen.¹⁹ Citronellol, a major constituent of geranium oil ($\approx 30\%$) and rose oil ($\approx 25\%$), is also included on the list of 26 alleged fragrance allergens. It has been classified as having an extremely weak allergenic potency.¹⁹ DEP was chosen to enable future studies as it is recommended as the vehicle of choice at 75% when mixed with 25% ethanol in the RIFM human repeated patch-test protocol.²⁰

Geraniol and citronellol have chemical structures that suggest they are likely to volatilize if left open to the environment. These include the combination of K_{ow} , low solubility in water, moderate vapor pressure, and low molecular weight (Table 2).

Vapor pressure is a key indicator of volatilization potential. The higher the vapor pressure, the more molecules will enter the vapor state. If there are more molecules in the vapor state, the evaporation rate is higher. As can be seen from the physical data in Table 2, geraniol has the greatest vapor pressure (0.02 mm Hg), followed by citronellol (0.009 mm Hg) and DEP (3.45×10^{-4} mm Hg). Our experimental evaporation values for each material correlate to documented vapor pressure values; high levels of evaporation over time for geraniol and citronellol, and a substantially lower loss for DEP. The vapor pressure was a good indicator of the evaporation potential of the materials studied.

Recently published work examined volatility and other physicochemical data on perfumery ingredients to create a database to help with the development of fragrances with enhanced performance for certain product applications.²¹ Also being studied are the vapor pressures of several aroma compounds to explain their phase equilibrium behaviors, to aid in the development of processes for food and chemical industries.^{22,23} None of these studies addresses the need to characterize volatility to aid in health risk assessment. However, one of the earliest studies to do this was a study by Feldmann and Maibach that suggested the loss of material from the skin surface is an important variable to consider when assessing the skin penetration characteristics. Although none of the materials studied were fragrances, the link with volatile organic materials would be assumed to be relevant as well.²⁴

The importance of volatility to exposure in fragrance materials was further investigated in studies by Bronaugh and colleagues. Several fragrance materials were studied for percutaneous absorption in vitro and in vivo with various application techniques and models; including occlusion and non-occlusion and human and monkey skin. In an initial study, Bronaugh and Stewart evaluated cinnamyl anthranilate, safrole, cinnamic alcohol, and cinnamic acid. The relative volatilities of all materials were tested to accurately determine their percutaneous absorption properties. It was demonstrated that the more highly volatile the compound, the less it was absorbed through skin. This was concluded to be due to the competition between the material's penetrating the skin and evaporating from it.²⁵ Bronaugh and colleagues subsequently studied a second group of fragrance materials in vivo, using monkey and human skin to look at percutaneous absorption. Diethyl maleate, benzyl acetate, and five other benzyl derivatives were studied. These materials showed variability in absorption because of variations in the rates of evaporation from the tested application sites.²⁶ These two studies gave

Table 2. Regulatory Identifiers and Physicochemical Values for Geraniol, Citronellol and Diethyl Phthalate

	CAS No.	EINECS No.	Molecular Weight	Solubility in Water (mg/L at 25°C)	K_{ow}	Vapor Pressure (mm Hg)*
Geraniol	106-24-1	203-377-1	154.25	Practically insoluble	3.47	0.02
Citronellol	106-22-9	203-375-0	156.27	Practically insoluble	3.56	≈ 0.009
DEP	84-66-2	201-550-6	222.23	1,080	1.4–3.3	3.45×10^{-4}

Adapted from Lapczynski et al^{37,38} and United States Department of Health Services, Agency for Toxic Substance and Disease Registry (ATSDR).³⁹ CAS = Chemical Abstracts Service; DEP = diethyl phthalate; EINECS = European Inventory of Existing Commercial Chemical Substances; K_{ow} = octanol/water partition coefficient.

*At 20°C.

evidence of the importance of determining fragrance volatility to accurately measure risk for systemic skin absorption and exposure.

Others have studied benzyl alcohol because of its widespread use as a fragrance material. It was used to study the pharmacokinetics of a volatile material *in vitro* and *in vivo*.²⁷ This testing helped with later studies in which the author proposed a physical properties–based model for skin absorption and evaporation of volatile materials specifically to aid in improved dermal risk assessments.²⁸ Other studies of benzyl alcohol penetration were also conducted to evaluate dose and airflow dependence. Investigators commented on the need to correctly estimate absorbed dose, absorption rate, and skin concentrations in order to understand systemic risks and sensitization.²⁹ Volatility would be a key contributor to this understanding as well.

One model for study of volatility and its effects on a material's behavior and safety assessment is N,N-diethyl-m-toluamide (DEET). Although DEET is not a fragrance material and does not share the chemical structures often seen in fragrances, it is one of the few chemicals that has been studied extensively *in vitro* and *in vivo*, so comparison to our data is relevant. These studies characterize the volatility of DEET and have therefore led to the ability to predict and measure percutaneous absorption for safety and human health assessment.^{24,30–32} These volatility and skin permeation studies have led to the attempt to link cases of DEET toxicity to its dermal absorption profile.^{32,33} There are other similar types of stepwise skin penetration characterizations that are done on groups of pesticides. Van der Merwe and colleagues comprehensively examined organophosphates to characterize dermal absorption. One major factor that was reviewed was the effects of solvent evaporation rates on absorption.³⁴ Such work on solvent evaporation rates may have similar applications to the exploration of vehicle effects on the evaporation rates of fragrances. Whether petrolatum or other vehicles allow for less evaporation would be an interesting corollary to this solvent research and would have application to fragrance study.

A comprehensive review of models and methods on dermal absorption and its effects on the process of human health risk assessments was done by Poet and McDougal.⁴ They provided an overview of the then-current state of dermal risk assessment and noted the need to consider volatility when determining dose. They also emphasized the lack of an adequate model for dermal risk assessment. This lack of a comprehensive model raises the need to characterize materials via physical experiments such as those for volatility. To this end, there should be

established protocols for accurately evaluating volatile materials.

Accurate measuring of volatiles relies on how the testing is being conducted. We demonstrated that differences in how evaporation and volatility are measured, whether with radiolabeled or nonradiolabeled compounds, affects the degree of loss and the accurateness of laboratory measurements. Measuring evaporation loss by weight alone, as with nonradiolabeled materials, leaves greater room for inaccuracy than does targeting the compound of interest with radiolabeling. Tagging the volatile compound helps ensure accurate determination of the remaining material, not water taken up from the air or other laboratory contaminants. Radiolabeling allows a more accurate tracing of the fragrance compounds at a patch-test dosing amount ($\approx 2\%$ [w/v]) characteristic of that recommended by industry and used by clinicians in diagnostic patch testing. This is an important finding, especially for application to safety and health assessments, in which volatility data can have the most impact.

This impact can be demonstrated by evaluating the effects of fragrance chemicals on dermal dose for relevance on the clinical practice of predictive patch testing. Concerning its method for the predictive patch testing of fragrance materials (offered as the industry standard in August 2006), the RIFM states the following in regard to sample preparation and application: "Patches treated with test article will volatilize for 15 to 40 minutes prior to application."⁷ Our volatility data on the three materials studied demonstrate that, when in an aqueous vehicle, geraniol can lose up to 39% of its dose, citronellol can lose up to 26%, and DEP evaporates up to 14% within 40 minutes, the time given to patch-test a subject in the RIFM protocol.

As patch testing is the main method of testing for contact allergy, this would be an important factor in the evaluation of fragrance chemicals. In diagnostic testing, care is required when results are interpreted and linked to fragrance allergy.^{35,36} This linkage requires positive patch-test results to be consistent with the real doses that patch tests apply to the skin. For volatile chemicals such as fragrances, the loss of material in an aqueous vehicle may be indicative of the behaviors of fragrances in other vehicles used in practice, which would lessen the dose applied.

To aid in the modeling of fragrance chemicals, future research would evaluate skin penetration and absorption to determine the difference between applied and absorbed doses in skin models. Vehicle effects could also be investigated to determine if less fragrance material is lost in the DEP-ethanol vehicle prescribed by the RIFM than in water alone.

Additional studies comparing the reduction of sensitizing dose due to the loss of material by evaporation in the standard protocols of diagnostic patch testing, predictive human repeat-insult patch testing, and consumer product usage are also needed to accurately assess the risk of fragrance material use. From these studies, established protocols and models could be determined and used throughout industry to aid in the risk assessment of fragrances.

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