

In Vitro Human Skin Penetration of Geraniol and Citronellol

Sarah Gilpin, Xiaoying Hui, and Howard Maibach

Background: Geraniol and citronellol are commonly used fragrance components in consumer products. Both are listed as alleged fragrance allergens that should be declared in the European Union when used in cosmetics and consumer products. Such allergenic potential is determined largely by effects on the skin once these materials penetrate and elicit an immune response. Few data demonstrate their penetration abilities or their effects on percutaneous absorption. We wanted to determine the effects of these materials on skin absorption. Skin penetration characterization via flow-through diffusion study serves as a reasonable model for determining dermal dosing for fragrance materials. Such characterization can be used for more accurate safety exposure calculations and regulatory determinations. Extensive comparisons to in vivo data in humans or closely related animals will be required before accepting flow-through diffusion methods as in vivo alternatives by industry and regulatory bodies.

Objective: To evaluate the penetration abilities of geraniol and citronellol when they are used in a typical vehicle in consumer products.

Methods: In vitro skin penetration of radiolabeled geraniol and citronellol was studied under occlusion in human cadaver skin, using flow-through diffusion cells for scintillation counting to determine the percentage of dose absorbed. For comparison, two doses of each material were used: 2% and 5% in 3:1 diethyl phthalate/ethanol.

Results: After 24 hours, geraniol and citronellol had relatively low skin absorption rates; $3.8\% \pm 2.1\%$ of 2% citronellol, $4.7\% \pm 1.9\%$ of 5% citronellol, $3.5\% \pm 1.9\%$ of 2% geraniol, and $7.3\% \pm 1.1\%$ of 5% geraniol were recovered from skin and receptor fluid compartments. These materials showed good mass-balance recovery. The majority of the dose was recovered in the skin washes (a minimum of $64.7\% \pm 4.6\%$ recovered for 2% citronellol and a maximum of $79.3\% \pm 3.9\%$ recovered for 5% geraniol). Receptor fluid collection points over time showed a linear increase in the amounts of citronellol and geraniol that penetrated the skin, although overall absorption values were quite small.

Conclusion: In vitro results indicate that geraniol and citronellol have low potentials for skin penetration, which has implications for their ability to induce allergenicity and for more predictive toxicologic profiling of these materials. In vivo studies should be done to correlate the in vitro results.

GERANIOL AND CITRONELLOL are commonly used fragrance components in consumer products, including fine fragrances, shampoos, and creams. Geraniol has a pleasant roselike odor and is approximately 20% rose oil and approximately 80% palmarosa oil.¹ Worldwide usage exceeds 1,000 metric tons yearly² and includes use in 76% of deodorants in Europe, 41% of domestic personal care and household products, and 33% of naturally based cosmetics.³ Citronellol, which also has a roselike odor, is a major constituent of geranium oil ($\approx 30\%$) and rose oil

($\approx 25\%$).¹ Citronellol also has a worldwide usage exceeding 1,000 metric tons.⁴

Both geraniol and citronellol are listed as alleged fragrance allergens that should be declared in the European Union when used in cosmetics and consumer products.⁵ Such allergenic potential is determined largely by effects on the skin once the materials penetrate the skin and elicit an immune response. Few data show their penetration abilities or their effects on percutaneous absorption. Other data, such as those found by local lymph node assay (LLNA), indicate little risk of allergenicity. The LLNA classified geraniol as a weak contact allergen, and citronellol as an extremely weak contact allergen.¹

For a better understanding of the risk of allergy, dermal penetration determinations provide part of a complex biologic equation.⁶⁻¹⁴ Exposure assessment for fragrances has conservatively assumed 100% absorption.⁶ Such

From the University of Minnesota, Minneapolis, MN, and the University of California San Francisco, San Francisco, CA.

Address reprint requests to Sarah Gilpin, Aveda Corporation R&D, 4000 Pheasant Ridge Dr., Blaine, MN 55449. E-mail: sgilpin@aveda.com

DOI 10.2310/6620.2010.09056

© 2010 American Contact Dermatitis Society. All Rights Reserved.

DECKER



default estimates may lead to an overestimation of risk for fragrances as volatile compounds.⁷

To estimate dermal penetration, a widely used method is *in vitro* skin penetration via the use of flow-through diffusion cells.^{15,16} The method has been evaluated to account for occlusion, material volatility, temperature, vehicle effects, concentration, skin integrity, and many other variables.^{17,18} For the best study of fragrance materials, their physiochemical properties and anticipated exposure conditions upon use by consumers should be considered in the design of interpretable experiments. The volatile natures of geraniol and citronellol as well as their chemical structures and low molecular weights affect the way in which these substances should be studied *in vitro* to best mimic true dermal penetration and absorption.

Geraniol and citronellol underwent a thorough toxicologic and dermatologic review by the Research Institute for Fragrance Materials (RIFM) in 2008; only one 4-hour study of skin absorption was cited for citronellol, and none was presented for geraniol.^{2,4} Without such data, is it hard to evaluate this aspect of skin sensitization and dose effects because sensitization potential is a result of the material's potency and the flux of the material through the dermal membrane.^{6,18–20}

This research evaluates the penetration ability of these materials when they are used in a typical vehicle, namely, diethyl phthalate (DEP) as recommended by the RIFM for predictive sensitization studies.²¹ Once the skin penetration abilities of these materials are known, more accurate estimates of contact allergy risk as related to dose can be made.

Materials and Methods

Model Compounds

An aqueous solution of citronellol ($1\text{-}^{14}\text{C}$) (molecular weight, 156.27; 0.05 mCi/mL; purity, 99%; specific activity, 52 mCi/mmol) and powdered radiolabeled geraniol ($1\text{-}^{14}\text{C}$) (molecular weight, 153.25; 0.05 mCi/mL; purity, 99%; specific activity, 55 mCi/mmol) were purchased from American Radiolabeled Chemicals, Inc. (St. Louis, MO). DEP (100% purity) was obtained from Univar USA (Redmond, WA). Ethanol (190 proof) was supplied by Brenntag Great Lakes, LLC (Wauwatosa, WI).

Stock Solution

Calculations were completed to ensure ample stock solution for the required dosing schedule to approximate

a dose volume of 20 μL per application ($\approx 200\text{--}1,000\text{ }\mu\text{g}/\text{cm}^2$ per percent dilution and compound), containing approximately 0.5 μCi radioactivity. Two concentrations of each material were prepared at 2% and 5% weight per volume by adding radiolabeled material to a prepared 3:1 solution of DEP and ethanol. Dose equivalent calculations were done to determine application dose amounts in micrograms per square centimeter.

Human Cadaver Skin

Human cadaver skin without obvious signs of skin disease was obtained within 24 hours of donor death from the Department of Pathology at the University of California, San Francisco. Skin was obtained from four donors (aged 71 ± 4 yr) and dermatomed with a Padgett Electro-Dermatome (Padgett Instruments, Inc., Kansas City, MO) to a target thickness of approximately 400 μm . The skin was immediately placed in aluminum foil and amply sealed and stored at -20°C until used. At the start of the experiment, skin was placed in saline at room temperature for a few minutes and was then cut into small circular sections to fit the diffusion cells.

Evaluation of Skin Integrity

Skin integrity was checked visually to eliminate those samples with surface damage or holes. Thickness was measured with an electronic digital caliper (Fisher Scientific, Fair Lawn, NJ). To ensure skin integrity, transepidermal water loss (TEWL) was assessed before sample application.²² After a 1-hour equilibrium period, each skin sample was measured with an evaporimeter (VapoMeter, Delfin Technologies Ltd., Kuopio, Finland) at least three times. TEWL values of less than 15 g/m²h were considered acceptable.^{23,24}

Experimental Procedures

Dermatomed human cadaver skin was clamped onto a continuous-flow diffusion cell (item LG-1084, Laboratory Glass Apparatus, Inc., Berkeley, CA) in a flow-through diffusion cell system. The dermal chamber below the skin was filled to capacity with receptor fluid (6% polyethylene glycol and sodium phosphate-buffered saline [pH, ≈ 7.4]). The receptor fluid was pumped to the diffusion cell at 1.4 rpm or 0.0190 mL/min by a PumpPro MPL pump (Watson-Marlow, Inc., Wilmington, MA). Receptor fluid was collected with a Retriever IV Fraction Collector (ISCO,

Inc., Lincoln, NE) at 1 hour, 2 hours, 4 hours, and every 4 hours thereafter up to a total duration of 24 hours.

Diffusion cells were placed in a diffusion apparatus (Crown Glass Laboratory, Inc., Sommerville, NJ), and the receptor fluid was stirred magnetically at approximately 600 rpm. A Lauda Ecoline 019 Heating Circulator (Lauda Dr. R. Wobser GmbH & Co. KG, Lauda-Koenigshofen, Germany) maintained the glass cell temperature at approximately 32°C (the skin surface temperature).

A 20 µL dose solution of approximately 0.5 µCi radioactivity was applied to the surface of the skin with a syringe. Immediately on dose application, the top of the cell was covered with a plastic chamber (Hill Top Chamber, Hill Top Research, Miami, OH) with a 2.2 cm² cotton pad (Hill Top Research), then sealed with Parafilm M Laboratory Wrapping Film (Pechiney Plastic Packaging, Chicago, IL) to block and absorb the volatile fragrance. A layer of adhesive tape was added as an additional occlusive membrane and to keep the Parafilm and chamber in place. Each fragrance concentration was run three times on four cadaver skin donors. At the end of the 24 hours, samples were collected and prepared for assay. Additional details are found in articles by Bronaugh and Stewart,²⁵ and Bronaugh.²⁶

Sample Collection and Assay

The skin surface was washed two times with small cotton balls wetted with 50% liquid dish soap (Trader Joe's Next to Godliness Dish Soap, Trader Joe's, San Francisco, CA).

For each sample, the following were collected: dose standard (collected during dose application); receptor fluid (6% polyethylene glycol and sodium phosphate-buffered saline, collected after the experiment as dose penetrating through skin membrane); cover (cotton pad and tape-sealed Parafilm); surface wash (collected with the liquid soap wash as residue dose); inner washing (samples from the inside walls of the epidermal chambers wiped with a cotton swab); the first and second tape strips as residue dose; stratum corneum (the next 3 to 10 tape strips removed from the skin as dose penetrating the stratum corneum); separated epidermis; separated dermis; and edge skin (the nondosed area around dosed skin).

To determine the amount of material absorbed, the values for the material absorbed into the skin (the stratum corneum, epidermis, dermis, and surrounding edge skin) were added to the amount of material that penetrated through the skin (receptor fluid values).

All collected samples were measured for radioactivity with a PerkinElmer Tri-Carb 2900TR liquid scintillation

spectrometer (PerkinElmer Inc., Waltham, MA). Epidermal, dermal, and edge skin samples were first digested in Soluene-350 (PerkinElmer) for 48 hours at 37°C and were then mixed with the scintillation cocktail to assay. Scintillation cocktail (Universol, MP Biomedicals, Irvine, CA) was added to each vial of the duplicated aliquots of the receptor fluid and prepared for counting.

Results

After 24 hours, geraniol and citronellol had relatively low skin absorption rates ranging from 3.5% ± 1.9% for 2% geraniol to 7.3% ± 1.1% for 5% geraniol (Table 1). Increasing concentration increased absorption; geraniol's absorption percentage at 5% concentration was double that of its 2% concentration. Citronellol also showed an increase in skin absorption, increasing from 3.8% ± 2.1% at 2% concentration to 4.7% ± 1.9% at 5% concentration (see Table 1). Recovery for fragrance materials was good; minimum recovery values were 78.2% ± 5.2% for 2% geraniol and 86.3% ± 5.2% for 2% citronellol (see Table 1). Most of the dose was recovered in the skin washes; a minimum of 64.7% ± 4.6% was recovered for 2% citronellol, and a maximum of 79.3% ± 3.9% was recovered for 5% geraniol (Fig 1). Recovery was much less for all other compartments; the cover compartments for 2% and 5% citronellol had the next highest dose recoveries (15.5% ± 3.9% and 10.7% ± 0.9%, respectively), followed by the tape strips (6.1% ± 4.3% and 4.3% ± 0.3%, respectively). Geraniol 5% and citronellol 5% skin compartments had the next highest dose recoveries for those materials (6.4% ± 1.8% and 4.2% ± 1.5%, respectively) (see Fig 1). Examination of the time variable in box plots for the receptor fluid collection points showed a linear increase in the amounts of citronellol and geraniol that penetrated the skin even though the overall values of absorption were quite small (Fig 2).

Table 1. Mass Balance and Percutaneous Absorption of Geraniol and Citronellol by Human Skin in Vitro

Compound	Removed Dose after 24 Hours*	Penetrated Dose Absorbed after 24 Hours* (%)
Citronellol 2%	86.3 ± 5.2	3.8 ± 2.1
Citronellol 5%	81.6 ± 3.8	4.7 ± 1.9
Geraniol 2%	78.2 ± 5.2	3.5 ± 1.9
Geraniol 5%	81.9 ± 2.7	7.3 ± 1.1

*Values are means ± SD for the number of determinations shown in parentheses. Number of determinations = 12.

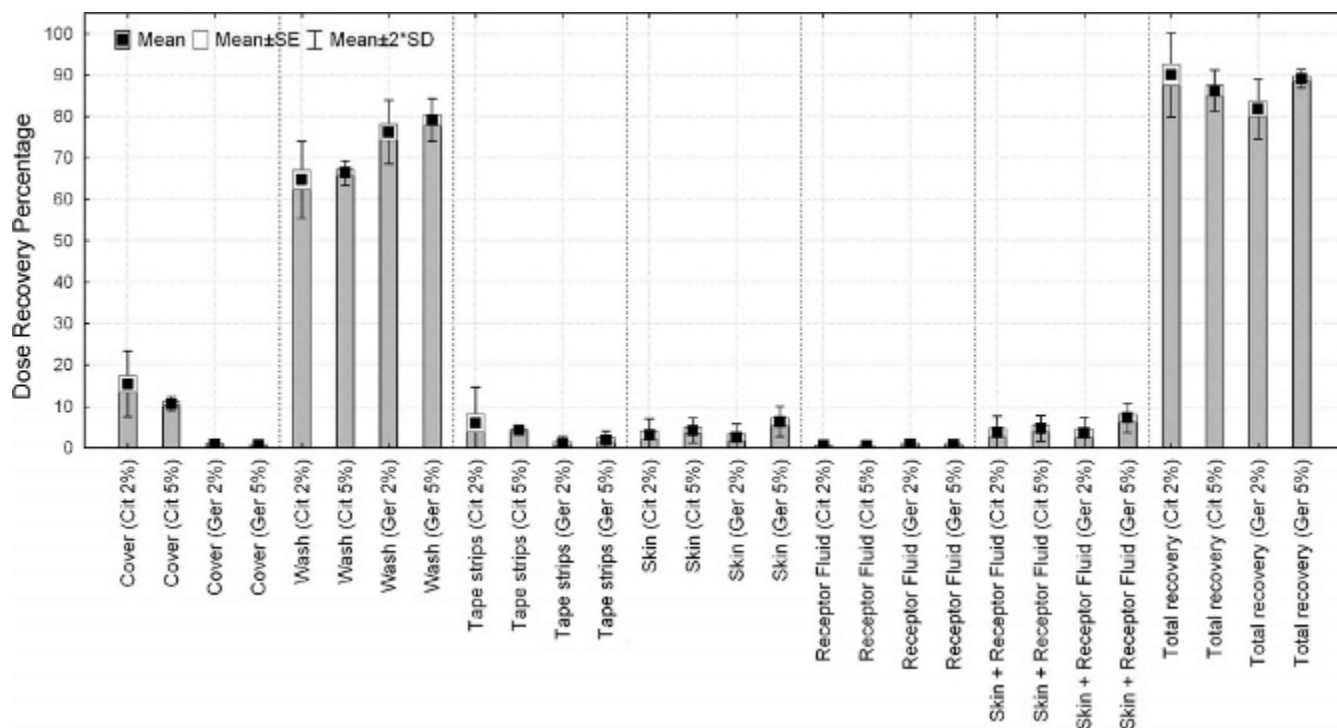


Figure 1. Total dose recovery of 2% and 5% citronellol and geraniol and mean dose percentage absorption per sampling compartment. (Cit = citronellol; Ger = geraniol.)

These data suggest that the evaporation potential and physiochemical properties of each material may lead to low human skin penetration in this model. A low skin penetration value implies that little compound is getting through the skin barrier, having little effect on underlying tissues. This is especially important to consider when reviewing the sensitization potential of these two fragrances.

Dose equivalents for each material, shown in Table 2, were also calculated to allow further review of threshold and saturation questions. Dose equivalents were calculated by determining the dose applied by weighing a syringe before and after dosing and multiplying the difference of the two values (net dose) by the dose volume and concentration of each compound. Dose equivalents are necessary to allow comparison with typical consumer use application; such comparison aids in assessing safety and determining exposure scenarios. Cosmetic product fragrance levels and acute dose equivalents were taken from consumer use surveys.²⁷ These data show that typical percentages of fragrance (0.4–8.0%) in consumer products lead to mostly lower acute doses as compared to the doses of geraniol and citronellol we studied. Leave-on toilet waters have the highest level of fragrance and therefore the highest acute dose per use.

Discussion

The in vitro skin penetration of geraniol and citronellol was investigated to provide data on human exposure potential. The compounds at low (2%) and higher (5%) concentrations demonstrated low skin penetration as shown by the absorbed dose percentages administered in vitro (see Table 2 and Fig 1). Such low absorption even under occlusion does not support the conservative assumption of 100% absorption currently used to make regulatory and safety determinations.⁵ For a substance to be an allergen, absorption is requisite. The degree of absorption is a key determinant in the level of exposure. The current model for determining the allergenic potential of materials without experimental dose absorption data assumes 100% absorption.^{6,7} The data call into question this assumption in regard to geraniol and citronellol; we found considerably lower absorption rates under occlusion and very little of each compound reaching or penetrating through the viable epidermis.

To correctly measure the absorption of a volatile material, one must consider the evaporation potential.²⁸ For example, when absorption is increased by increasing the dose or via occlusion, evaporation is lessened.^{29,30} Because of this, we examined geraniol, citronellol, and

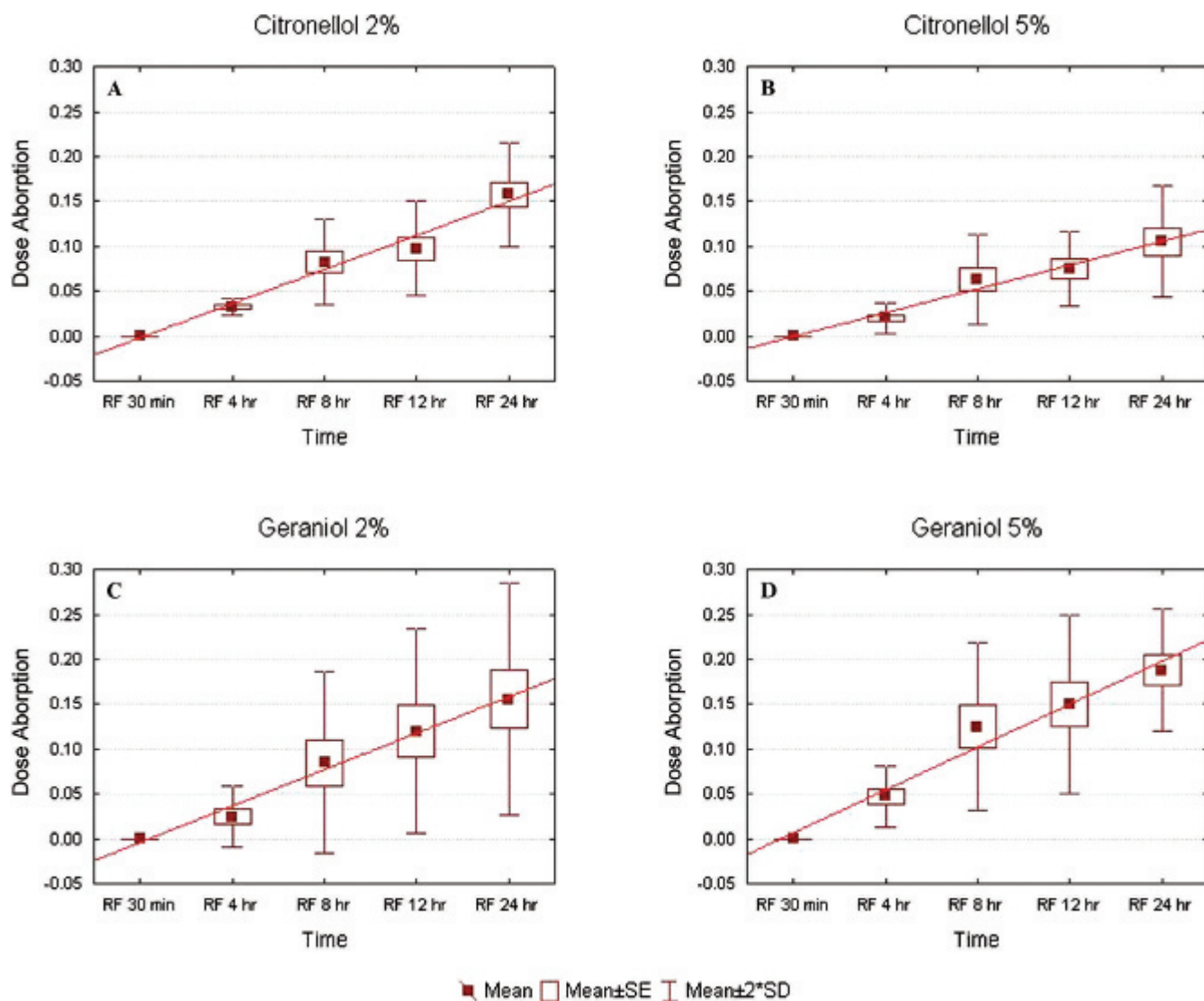


Figure 2. Mean dose absorption percentages in receptor fluid over time for citronellol and geraniol. (Cit = citronellol; Ger = geraniol; RF = receptor fluid.)

DEP to evaluate the evaporation rates for both radiolabeled and nonradiolabeled compounds. The evaporation rates for nonradiolabeled materials were low; after 24 hours, geraniol lost 8.9%, citronellol lost 27.0%, and DEP lost 14.5%. The volatility data for radiolabeled materials demonstrated that geraniol loses up to 39% of dose, citronellol loses up to 26%, and DEP loses up to 14% within 40 minutes. We concluded that the tendency of fragrance materials to evaporate can greatly affect the dose applied when predictive patch testing is performed,³¹ in turn affecting the determination of allergenicity and ultimately the regulatory and assessment process in regard to the fragrance material's safety.

Bronaugh and Stewart documented the percutaneous absorption of fragrances and also found an absorption rate

of less than 100% for several materials. They studied several volatile fragrances in vitro and in vivo with varying application techniques and models, including occlusion and non-occlusion and in human and monkey skin. Initially, they evaluated cinnamyl anthranilate, safrole, cinnamic alcohol, and cinnamic acid, demonstrating that the more highly volatile the compound, the less it was absorbed. This was hypothesized to be due to the competition between the material's penetration of the skin and evaporation from the skin.³² Bronaugh and colleagues then studied diethyl maleate, benzyl acetate, and five other benzyl derivatives in vivo, using monkey and human skin. These materials showed a variability in absorption between compounds, which was hypothesized as being due to variations in evaporation from the application sites.²⁹

Table 2. Mean Dose Equivalent Values for Citronellol and Geraniol Compared to Various Common Consumer Cosmetic Products

<i>Test Compound or Cosmetic Product Type and Fragrance Level</i>	<i>Test Compound Dose Equivalent or Cosmetic Product Acute Dose ($\mu\text{g}/\text{cm}^2$)</i>
Citronellol 2%	418
Citronellol 5%	1,068
Geraniol 2%	207
Geraniol 5%	520
Toilet water 8%	300
Fragranced cream 4%	57
Toilet soap 1.5%	0.4
Body lotion 0.4%	28

Along with experimentally determining fragrance absorption, researchers have estimated fragrance absorption by using evaporation data from pharmacokinetic models. Saiyasombati and Kasting demonstrated that, by using their model of deposition for volatiles on skin, the measured evaporation rate of benzyl alcohol could accurately model the absorption rate constant in vitro and in vivo.³³

To limit the loss of material by evaporation in this study, occlusion of the diffusion cells was used. Although the tendency would be to surmise that skin penetration would be increased, the data show that this is not necessarily the case, especially for lipophilic molecules such as those in fragrance compounds.^{34–36} A review of occlusion, barrier function, and percutaneous absorption by Zhai and Maibach details the existing research on this topic.³⁷ In most cases, skin occlusion is recommended to lessen loss from evaporation.³⁸ Bronaugh and colleagues and Bronaugh and Stewart found significant increases in percutaneous absorption of the chemicals they studied in vitro under occluded versus non-occluded conditions,^{29,32} and this was the case for linalool. The RIFM found a threefold increase in linalool absorption under occluded conditions as opposed to non-occluded conditions.³⁹ Geraniol and citronellol showed low values of

penetration even under occlusion (see Table 1). We did not test them non-occluded, and we cannot surmise what the results would have been. Likely, there would have been even less skin absorption.

Examining and comparing the physiochemical properties of the two compounds in regard to these results was informative. Fragrance chemicals can be characterized by structural and functional attributes that point to their likely behaviors. Geraniol and citronellol were chosen for study because their chemical structures suggest the likelihood of their evaporating from the skin if the application site is left open to the environment. Factors such as octanol/water partition coefficients, low solubility in water, moderate vapor pressures, and low molecular weights (Table 3) led to this premise.^{8,40–42}

Dosage and maximum use by consumers also must be factored into the analysis and discussion of fragrance safety. The dose levels of geraniol and citronellol (calculated in micrograms per square centimeter) are many times higher than those of even the worst-case scenarios of high-level use exposure as determined by a review by Cadby and colleagues. As determined by the short-term single-use exposure calculation in which a material used at $P\%$ in fragrances is $3P \mu\text{g}/\text{cm}^2$, the dose levels of citronellol and geraniol used in this study were much higher than any that would likely be found in a consumer cosmetic product—even a fine fragrance with the highest exposure level.²⁷ The single-use exposure would be $6 \mu\text{g}/\text{cm}^2$ for a 2% dilution and $15 \mu\text{g}/\text{cm}^2$ for a 5% dilution. Compare these to the values listed in Table 2, and notice that the levels were at least 35 times this worst-case consumer use rate.

It has recently been shown that fragrance metabolism can occur in the skin^{3,43}; this was not evaluated here.

Conclusion

To provide a more complete model of skin penetration for fragrances, future studies should be undertaken to consider

Table 3. Regulatory Identifiers and Physicochemical Values for Geraniol, Citronellol, and Diethyl Phthalate

	<i>CAS No.</i>	<i>EINECS No.</i>	<i>Molecular Weight*</i>	<i>Solubility in Water* (mg/L at 25°C)</i>	<i>Log P* (oct/water)</i>	<i>Vapor Pressure (mm Hg at 20°C)</i>
Geraniol ($\text{C}_{10}\text{H}_{18}\text{O}$)	106-24-1	203-377-1	154.25	100	3.56	0.02 [†]
Citronellol ($\text{C}_{10}\text{H}_{20}\text{O}$)	106-22-9	231-415-7	156.27	212	3.91	$\approx 0.009^{\ddagger}$
DEP ($\text{C}_{12}\text{H}_{14}\text{O}_4$)	84-66-2	201-550-6	222.24	1,080	2.42	0.0021*

CAS = Chemical Abstracts Service; DEP = diethyl phthalate; EINECS = European INventory of Existing Commercial chemical Substances; oct = octanol.

*From U.S. National Library of Medicine.⁴⁴

[†]From Lapczynski A et al.²

[‡]From Lapczynski A et al.⁴

the added step in examining material that remains in the skin or that has penetrated through the skin as a metabolized compound.³ In vitro flux indicates only part of the complexity of penetration.¹¹ Additional comparisons in vivo in humans and in closely related animals should clarify the reliability of in vitro studies for fragrance toxicology.

Acknowledgments

The authors are grateful to Dr. Peter Cadby and Mr. Ben Smith for their assistance in reviewing the manuscript.

Financial disclosures of author(s) and reviewer(s): Financial support was provided by Aveda Corp.

References

- Vuilleumier C, van de Waal M, Fontannaz H, et al. Multi-dimensional visualization of physical and perceptual data leading to a creative approach in fragrance development. *Perfumer & Flavorist* 2008;33:54–61.
- Lapczynski A, Bhatia SP, Letizia CS, Api AM. Fragrance material review on geraniol. *Food Chem Toxicol* 2008;46 Suppl 1:S160–70.
- Hagvall L, Malte Baron J, Borje A, et al. Cytochrome P450-mediated activation of the fragrance compound geraniol forms potent contact allergens. *Toxicol Appl Pharmacol* 2008;233:308–13.
- Lapczynski A, Bhatia SP, Letizia CS, Api AM. Fragrance material review on dl-citronellol. *Food Chem Toxicol* 2008;46 Suppl 1:S103–9.
- Scientific Committee on Consumer Products 2006. The SCCP's notes of guidance for the testing of cosmetic ingredients and their safety evaluation_6th Revision (adopted by the SCCP during the 10th plenary meeting of 19 December 2006). Available at http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_03j.pdf.
- Kasting GB, Saiyasombati P. A physico-chemical properties based model for estimating evaporation and absorption rates of perfumes from skin. *Int J Cosmet Sci* 2001;23:49–58.
- Saiyasombati P, Kasting GB. Disposition of benzyl alcohol after topical application to human skin in vitro. *J Pharm Sci* 2003;92:2128–39.
- Sartorelli P, Aprea C, Cenni A, et al. Prediction of percutaneous absorption from physicochemical data: a model based on data of in vitro experiments. *Ann Occup Hyg* 1998;42:267–76.
- McDougal JN, Jurgens-Whitehead JL. Short-term dermal absorption and penetration of chemicals from aqueous solutions: theory and experiment. *Risk Anal* 2001;21:719–26.
- Chaudhuri SR, Gajjar RM, Krantz WB, Kasting GB. Percutaneous absorption of volatile solvents following transient liquid exposures II. Ethanol. *Chem Eng Sci* 2009; 64:1665–72.
- Wester RC, Maibach HI. Cutaneous pharmacokinetics: 10 steps to percutaneous absorption. *Drug Metab Rev* 1983;14:169–205.
- Poet TS, McDougal JN. Skin absorption and risk assessment. *Chem Biol Interact* 2002;140:19–24.
- Saiyasombati P, Kasting GB. Two-stage kinetic analysis of fragrance evaporation and absorption from skin. *Int J Cosmet Sci* 2003;25: 235–43.
- Degim IT. Understanding skin penetration: computer aided modeling and data interpretation. *Curr Comput Aided Drug Des* 2005;1:11–9.
- Bronaugh RL, Steward RF. Methods for in vitro percutaneous absorption studies IV: the flow-through diffusion cells. *J Pharm Sci* 1985;74:64–7.
- Addicks WJ, Glynn GL, Weiner N. Validation of a flow-through diffusion cell for use in transdermal research. *J Pharm Res* 1987;4: 337–41.
- European Commission guidance document on dermal absorption. Sanco/222/2000 rev. 7. European Commission, Health and Consumer Protection Directorate-General; 2004 Mar 19. Available at http://ec.europa.eu/food/plant/protection/evaluation/guidance/wrkd0c20_rev_en.pdf.
- Dutkiewicz T, Baranowska-Dutkiewicz B, Konczalik J. Percutaneous absorption studies after forty years. *Int J Occup Environ Health* 2000;6:111–3.
- Guy RH, Guy AH, Maibach HI, Shah V. The bioavailability of dermatological and other topically administered drugs. *Pharm Res* 1986;3:253–62.
- de Silva O, Basketter BA, Barratt MD, et al. Alternative methods for skin sensitization testing. The report and recommendations of ECVAM Workshop 19. *ATLA* 1996;24:683–705.
- Api AM. Toxicological profile of diethyl phthalate: a vehicle for fragrance and cosmetic ingredients. *Food Chem Toxicol* 2001;39: 97–108.
- Levin J, Maibach H. The correlation between transepidermal water loss and percutaneous absorption: an overview. *J Control Release* 2005;103:291–9.
- De Paepe K, Houben E, Adam R, et al. Validation of the VapoMeter, a closed unventilated chamber system to assess the transepidermal water loss vs. the open chamber Tewameter. *Skin Res Technol* 2005;11:61–9.
- Rougier A, Lotte C, Maibach HI. In vivo relationship between percutaneous absorption and transepidermal water loss. In: Bronaugh RL, Maibach HI, editors. *Topical absorption of dermatological products*. New York: Marcel Dekker, Inc.; 2002. p. 115–28.
- Bronaugh RL, Stewart RF. Methods for in vitro percutaneous absorption studies VI: preparation of the barrier layer. *J Pharm Sci* 1986;75:487–91.
- Bronaugh RL. A flow-through diffusion cell. In: Bronaugh RL, Maibach HI, editors. *In vitro percutaneous absorption: principles, fundamentals, and applications*. Boca Raton (FL): CRC Press; 1991. p. 17–23.
- Cadby PA, Troy WR, Vey MGH. Consumer exposure to fragrance ingredients: providing estimates for safety evaluation. *Regul Pharmacol Toxicol* 2002;36:246–52.
- Kasting GB, Miller MA. Kinetics of finite dose absorption through skin 2: volatile compounds. *J Pharm Sci* 2006;95:268–80.
- Bronaugh RL, Wester RC, Bucks D, et al. In vivo percutaneous absorption of fragrance ingredients in rhesus monkeys and humans. *Food Chem Toxicol* 1990;28:369–73.
- Bronaugh RL, Maibach HI. Percutaneous absorption of nitroaromatic compounds: in vivo and in vitro studies in the human and monkey. *J Invest Dermatol* 1985;84:180–3.
- Gilpin SJ, Hui X, Maibach HI. Volatility of fragrance chemicals: predictive patch testing implications. *Dermatitis* 2009;20:200–7.

32. Bronaugh RL, Stewart RF. Comparison of percutaneous absorption of fragrances by humans and monkeys. *Food Chem Toxicol* 1985; 23:111–4.
33. Saiyasombati P, Kasting GB. In vivo evaporation rate of benzyl alcohol from human skin. *J Pharm Sci* 2004;93:515–20.
34. Treffel P, Muret P, Muret-D'Aneillo, et al. Effect of occlusion on in vitro percutaneous absorption of two compounds with different physiochemical properties. *Skin Pharmacol* 1992;5: 108–13.
35. Bucks DAW, McMaster JR, Maibach HI, Guy RH. Bioavailability of topically administered steroids: a “mass balance” technique. *J Invest Dermatol* 1988;91:29–33.
36. Bucks DAW, Maibach HI. Occlusion does not uniformly enhance penetration in vivo. In: Bronaugh RL, Maibach HI, editors. *Topical absorption of dermatological products*. New York: Marcel Dekker, Inc.; 2002. p. 9–32.
37. Zhai H, Maibach HI. Occlusion and barrier function. In: Zhai H, Maibach HI, editors. *Dermatotoxicology*. 6th ed. Boca Raton (FL): CRC Press; 2004. p. 13–28.
38. Bronaugh RL, Collier SW. Protocol for in vitro percutaneous absorption studies. In: Bronaugh RL, Maibach HI, editors. *In vitro percutaneous absorption: principles, fundamentals, applications*. Boca Raton (FL): CRC Press; 1991. p. 237–41.
39. Lalko J, Brain KR, Green DM, Api AM. In vitro human skin penetration of the fragrance material linalool [Abstract P32]. In: *Proceedings of the 28th Annual Meeting of the American College of Toxicology*, 2007 Nov 7–10, Charlotte (NC); Bethesda (MD): International Journal of Toxicology.
40. Potts RO, Guy RH. Predicting skin permeability. *Pharm Res* 1992;9: 663–9.
41. Schafer H, Redelmeir TE. Relationship between the structure of compounds and their diffusion across membranes. In: *Skin barrier—principles of percutaneous absorption*. Basel, Switzerland: S. Karger AG; 1996. p. 87–100.
42. Basketter D, Pease C, Kasting G, et al. Skin sensitization and epidermal disposition: The relevance of epidermal disposition for sensitization hazard identification and risk assessment. *ATLA* 2007;35:137–54.
43. Buckley DA, Basketter DA, Smith CK, et al. Simultaneous sensitivity to fragrances. *Br J Dermatol* 2006;154:885–8.
44. U.S. National Library of Medicine. ChemIDplus Advanced database. Available at: <http://chem.sis.nlm.nih.gov/chemidplus> (accessed July 11, 2009).