Epidermal Bioavailability of Volatile Compounds

Application to fragrance disposition and skin sensitization risk assessment

Gerald Kasting and Sara Farahmand, James L. Winkle College of Pharmacy, University of Cincinnati; Johannes Nitsche, State University of New York at Buffalo; Petra Kern, The Procter & Gamble Company (Belgium); and G. Frank Gerberick, The Procter & Gamble Company (Cincinnati)

By their very nature as small, lipophilic chemicals capable of stimulating olfactory receptors, fragrance ingredients have an innate ability to penetrate the skin. Depending on their chemical reactivity, some of these ingredients have the potential to sensitize individuals on repeated application; whether they do so or not depends primarily on dose and exposure conditions. These factors have been extensively reviewed as discussed recently by Kimber et al.[1] These researchers make the case, based on earlier work by Kligman, Friedmann and others, that dose per unit area is the relevant metric for assessing the risk of skin sensitization under most conditions.[2-5] This thought process forms the basis for established risk assessment and management methods for fragrances and fragranced products—a process that is coordinated for the industry by the International Fragrance Association (IFRA) and its research arm, the Research Institute for Fragrance Materials (RIFM).[1,6-9] Current risk assessment strategies for new fragrances or fragrance ingredients generally employ a tiered approach involving computer models (e.g., DEREK, TOPKAT, CASE) for initial hazard identification, "read across" comparisons with familiar ingredients having similar chemical structures, and a mouse assay (Local Lymph Node Assay or LLNA) to confirm hazard, and (if a sensitizer is identified) estimate allergenic potency.[10,11] This information is then combined with exposure estimates to evaluate risk, and appropriate safety factors are incorporated to allow risk management. Established ingredients are evaluated primarily on the basis of prior experience in humans.[9] Guidance for these processes is available on the IFRA Web site (*www.ifraorg.org*).

The necessary steps for acquisition of skin sensitization are well known and include: penetration of the sensitizing ingredient into the viable skin layers; incorporation of the hapten (the original ingredient or a reaction product thereof) into the native protein associated with Langerhans cells or other dendritic cells in the skin; migration of these cells through the afferent lymphatics to the draining lymph node; and activation and clonal expansion of T-lymphocytes located within these nodes. The activated T-lymphocytes are released into the bloodstream and migrate back into the skin, where they are able to mount an inflammatory attack against subsequent appearances of the hapten. The LLNA incorporates all of these steps except the dissemination of T-cells required for elicitation of a skin allergy response. Mice are dosed topically on the ears for three days, leading to skin penetration and (for sensitizers) reactions with dendritic cell surface proteins and migration of these cells to the draining lymph node. T-cell activation and expansion is inferred from the uptake of ^{3}H-thymidine by the node approximately five days after the initial dose of the test compound.[11] This methodology has been widely used in the cosmetic industry for the past 15 years.

In today's environment, the methodology for assessing consumer product safety is undergoing rapid change. Public opinion, backed by regulatory action, has forced a sharp turn toward alternative testing strategies that do not employ vertebrate animals. In the case of skin sensitization, legislation passed by the European Union in 2006 (the 7th Amendment to the EU Cosmetics Directive) dictates that new cosmetic ingredients marketed in the EU after 2013 shall not have been tested on animals. The legislation has spurred a great deal of research. Much of this work is coordinated by the Skin Sensitization Task Force of COLIPA, the European Cosmetics Association,

At a Glance

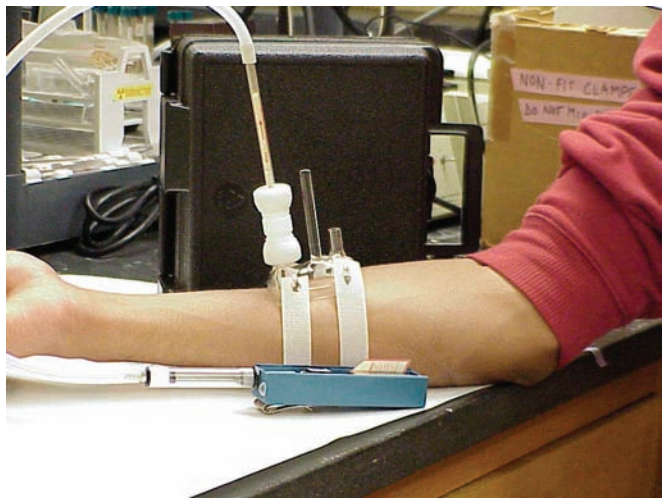
Fragrances and preservatives have long been recognized as problematic ingredients by the cosmetic industry due to their potential for causing allergic reactions on skin. This risk is carefully managed by the industry through product safety groups and trade organizations such as the International Fragrance Association (IFRA). New regulations limiting the use of animals in cosmetic ingredient safety testing are forcing changes to the risk assessment process for skin sensitization, which relies heavily on a mouse model for hazard identification. This article describes the development of a computer program to mimic the first step in the skin sensitization process—permeation of the ingredient across the stratum corneum and into the viable skin layers. As a byproduct of the calculation, the time profiles for release of volatile components into the air can be estimated. The method is illustrated by analysis and interpretation of a published study involving the evaporation of two model fragrance vectors from a human forearm.

a Brussels-based organization responsible for safety and regulatory strategy in the cosmetic, toiletry and perfumery industries.

This article reports on the status of a project carried out under COLIPA sponsorship to simulate the first stage of the skin sensitization process—skin penetration—using a sophisticated computer model to describe the absorption and evaporation of cosmetic ingredients applied topically to skin and their subsequent disposition once absorbed. Concentrations of the test compounds within the skin, as well as the freely diffusing and bound fractions thereof, can be estimated as a function of time after single or multiple applications. An estimate of the "epidermal bioavailability" (explained below) of the compound can be obtained. The objective of the sponsor is to combine this information with data from *in vitro* laboratory assays, e.g., peptide reactivity and dendritic cell activation, in such a way as to provide an alternative test battery to replace the LLNA in cosmetic ingredient testing.[12-14]

The epidermal bioavailability model is exemplified herein by calculations applied to two test fragrance mixtures studied by Firmenich in the mid 1990s.[15] The ingredients and their calculated properties are listed in **T-1**. The study involved application of two closely related mixtures of fragrance ingredients (formally called perfume raw materials or PRMs), identified as Vector A and Vector B, to the ventral forearm of a single female subject. Volatiles were collected over a 7.25 hr period following dosing by means of a glass trap placed over the 0.6 cm^2 dose site. The trap was similar in principle to the one shown in **F-1**, which was used to study

Apparatus for trapping volatiles evaporating from human forearm;[16] the portable pump draws room air at a programmable rate into the trap and through an adsorbent cartridge; volatiles are later thermally desorbed from the cartridge and analyzed by GC.; design courtesy of Pete Rodriguez, The Procter & Gamble Co. **F-1**



Perfume raw materials analyzed in this study **T-1**

ID	Compound	MW (Da)	P_{vp} [a] (torr)	log K_{oct}	S_w [b] (g L^{-1})	χ [c] (model - calculated)	C_{free}^{peak} [d] (μM)		C_{total}^{peak} [e] (μM)	
							Vector A	Vector B	Vector A	Vector B
I	Linalool	154	0.13	2.55	2.3	23.0	26	24	140	130
II	Dihydromyrcenol	156	0.19	3.03	0.76	43.6	1.6	1.5	14	12
III	10-Undecanal	170	0.093	4.05	0.072	48.9	0.5	0.4	15	13
IV	Citronellol	156	0.028	3.25	0.46	7.1	48	43	520	470
V	2-Phenyl-1-ethanol	122	0.039	1.36	35	1.8	236	214	600	540
VI	(E)-Cinnamic alcohol	134	0.0050	1.95	8.5	0.43	62	56	210	190
VII	α-Damascone	192	0.032	3.62	0.19	22.5	5.0	4.6	87	80
VIII	*cis*-7-*p*-Menthanol	156	0.019	3.33	0.38	5.0	30	27	360	320
IX	2,2,2- Trichloro-1-phenyl-ethylacetate	268	0.0029	4.05	0.072	9.7	1.0	0.9	34	31
X	M.P.C.C.[f]	192	0.010	3.87	0.11	7.7	3.3	3.0	82	75
XI	(E)-2-Benzylideneoctanal	216	0.00088	4.85	0.011	1.8	1.5	1.3	210	180
XII	15-Pentadecanolide	240	0.00010	5.35	0.0034	0.43	NA [g]	0.1	NA [g]	41

[a] For sources of physical properties, see Ref. 17.
[b] Estimated value taken from Ref. 18.
[c] Volatility parameter (Equation 9).
[d] Freely diffusing (unbound) mid-epidermal concentration (skin depth: 63.1 μm); the value has been calculated using a binding factor, which accounts for solute binding to albumin and partitioning into viable tissue's lipids.[19]
[e] Peak mid-epidermal concentration.
[f] 3-(4-methyl-3-pentenyl)-3-cyclohexene-1-carbaldehyde + 4-(4-methyl-3-pentenyl)-3-cyclohexene-1-carbaldehyde.
[g] This ingredient (a musk fixative) was included in Vector B, but not in Vector A.[15]

evaporation of a single PRM from the forearm in our laboratory.[16] An airflow rate of 5 Lh^{-1} was maintained during the study. Analysis was done by capillary GC. This research group (Kasting) had analyzed these data before using simpler, compartmental models to describe absorption and evaporation.[17,18] This article compares the two methods and highlights the new features available from the more complex model, as well as the areas in which further development is needed to make accurate estimates of PRM concentrations in skin.

Compartmental Model of Fragrance Disposition on Skin

The overall disposition of fragrance compounds on skin can be approximately understood on the basis of pharmacokinetic models in which the skin and the applied formulation are described in terms of well-stirred compartments. This research group looked at the simple one- and two-compartment models shown in **F-2** to determine what could be learned regarding single ingredients deposited from a volatile solvent or fragrance mixtures.[16-18,20,21] It found, as may be expected, that more details of the evaporation rate profiles could be explained in terms of two-compartment models than a one-compartment model; however, the overall balance between evaporation and absorption of the various ingredients was not significantly impacted by the added complexity.[18,20] It is, of course, possible to divide the skin into more compartments to explain additional features of dermal absorption profiles, as was extensively studied by Guy, Hadgraft and others in the early 1980s and reviewed more recently by McCarly and Bunge.[22-25] Comparisons of these models with diffusion models such as the one described later have shown that the first-order rate constants in compartmental models can be approximately estimated from physicochemical properties and transport parameters of the permeants.[17,23,25] However, progression of compartmental models into true predictive tools has proven to be difficult. Hence these authors illustrate the concept using the simplest possible model (a one-compartment model) originally described in reference 17.

Consider the drawing labeled Model 1 in **F-2**. A small amount A_0 of a single PRM is deposited onto the skin at time zero, possibly from a solvent that is much more volatile and rapidly evaporates. Any such solvent is not explicitly considered, i.e., the calculation begins at the moment (very shortly after application) when the solvent is gone and the PRM has been incorporated into the upper layers of the stratum corneum. From there it gradually evaporates and absorbs at a rate dependent upon its physicochemical properties. The key assumption is that the deposited amount is small enough to readily dissolve in the upper layers of the skin, which then serves as the matrix from which the compound dissipates. In this limit a first-order approximation for evaporation and absorption rates is plausible.[26] The analysis can readily be extended to mixtures in which the total amount of deposited material is low enough so that each ingredient can be considered to be dissolved independently in the skin.

Let the amount of PRM remaining in the skin at time t be $A(t)$. The evaporation rate is $k_1A(t)$ and the absorption rate is $k_2A(t)$. Under these assumptions, $A(t)$ is readily shown to have the form

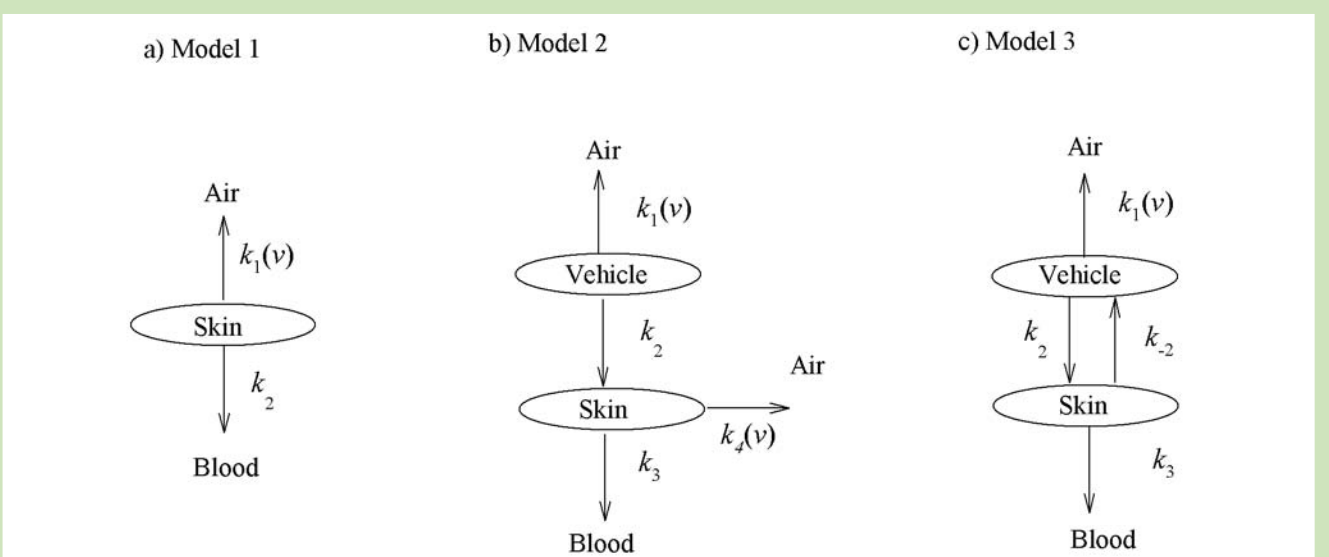
$$A(t) = A_0 e^{-(k_1+k_2)t} \qquad (1)$$

The percentages of the PRM evaporated and absorbed at time t can be calculated from the equations

$$\%\text{evap}(t) = 100\left(\frac{k_1}{k_1+k_2}\right)\left[1 - e^{-(k_1+k_2)t}\right] \qquad (2)$$

Schematic diagrams for compartmental models of skin disposition[18,20]
a) One-compartment (Model 1); b) Two-compartments with evaporation from vehicle and skin (Model 2); c) Two-compartments with evaporation from vehicle only (Model 3)

F-2



$$\%\text{abs}(t) = 100\left(\frac{k_2}{k_1 + k_2}\right)\left[1 - e^{-(k_1+k_2)t}\right] \quad (3)$$

Based on skin absorption theory available at the time, it is argued in reference 17 that the evaporation and absorption rate constants k_1 and k_2 can be estimated from the following expressions:

$$k_1 = k_1^{\text{v}} * P_{\text{vp}r} / (K_{\text{oct}} S_{\text{w}})_r \quad (4)$$

and

$$k_2 = k_2^{\text{T}} * \text{MW}_r^{-2.7} \quad (5)$$

Here P_{vp}= vapor pressure in torr, K_{oct} = octanol/water partition coefficient, S_{w} = water solubility in gL^{-1}, and MW = molecular weight. The subscript 'r' indicates a reduced or dimensionless form of each parameter. The properties $P_{\text{vp}r} = P_{\text{vp}}$/1 torr, $(K_{\text{oct}}S_{\text{w}})_r = (K_{\text{oct}}S_{\text{w}})/1000\text{gL}^{-1}$, and MW_r = MW/100 Da were chosen for computational convenience. Both k_1^{v} and k_2^{T} are constants, which were determined by analysis of the experimental results in the Firmenich study.[15] The product $K_{\text{oct}}S_{\text{w}}$ was used, for convenience, to represent octanol solubility, which is a measure of solubility in stratum corneum lipids. The assumptions made in the derivation of equations 4 and 5 were: small doses are applied that do not saturate the skin and for which each ingredient can be considered independently; Henry's Law applies at the skin air interface; the chemical environment of the PRM in the skin is equivalent to *n*-octanol; and diffusivity of permeants in the SC falls with rising molecular weight according to $\text{MW}^{-2.7}$.

Substitution of Equations 4 and 5 into Equation 2 and simplification of the resulting expression shows that the ratio of the rate constants $k_1/(k_1 + k_2)$ can be written as $x_r/(k + x_r)$, where $k = k_2^{\text{T}} / k_1^{\text{v}}$ is a parameter depending on temperature T and airflow velocity v, but having the same value for all permeants. The parameter x_r is a ratio of the dimensionless physicochemical parameters appearing in Equations 4 and 5,

$$x_r = \frac{P_{\text{vp}r}\,\text{MW}_r^{2.7}}{(K_{\text{oct}}S_{\text{w}})_r} \quad (6)$$

At times, long relative to the time constant $1/(k_1 + k_2)$, the percentage of PRM evaporated and absorbed can be expressed as:

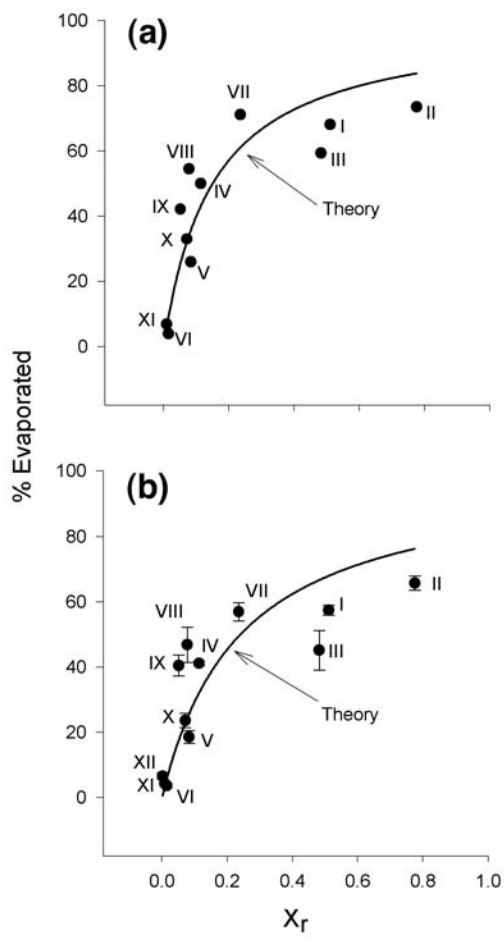
$$\%\text{evap} = 100\, x_r (k + x_r) \quad (7)$$

$$\%\text{abs} = 100 - \%\text{evap} \quad (8)$$

F-3 shows a graph of the cumulative evaporation data 7.25 hr post-dose in the Firmenich study plotted versus the dimensionless parameter x_r for each ingredient. The solid lines show the theory represented by Equations 6 and 7. The squared correlation coefficients (r^2) for this analysis were 0.74 for Vector A and 0.52 for Vector B, and the root mean square deviations of the observed values of evaporation percentage from the fitted ones were 12% and 14%, respectively. It is noteworthy that the evaporation of the high notes linalool (I), dihydromyrcenol (II) and 10-undecananal (III) was overpredicted, a feature that results from the independent evaporation assumption made in the analysis. Further analysis showed that Model 1 fails to accurately represent the details of the evaporation rate curves, a feature that was improved but not eliminated by the two-compartment models, Models 2 and 3 in **F-2**.[18]

Cumulative evaporation of perfume ingredients in Firmenich study, plotted versus the ratio of physicochemical properties defined in Equation 6;[15] each point represents the mean ± SE of two trials; the absence of an error bar indicates the SE was smaller than the size of the symbol; the theoretical curve is the result of fitting Equation 6 and 7 to these data; (a) Vector A, k = 0.15; (b) Vector B, k = 0.24

F-3



Diffusion Model for Volatile Compounds on Skin

Now consider the scenario depicted in **F-4**. Rather than representing the skin as a well-stirred compartment or a series of such compartments, one takes the more fundamental approach of studying the skin microstructure and

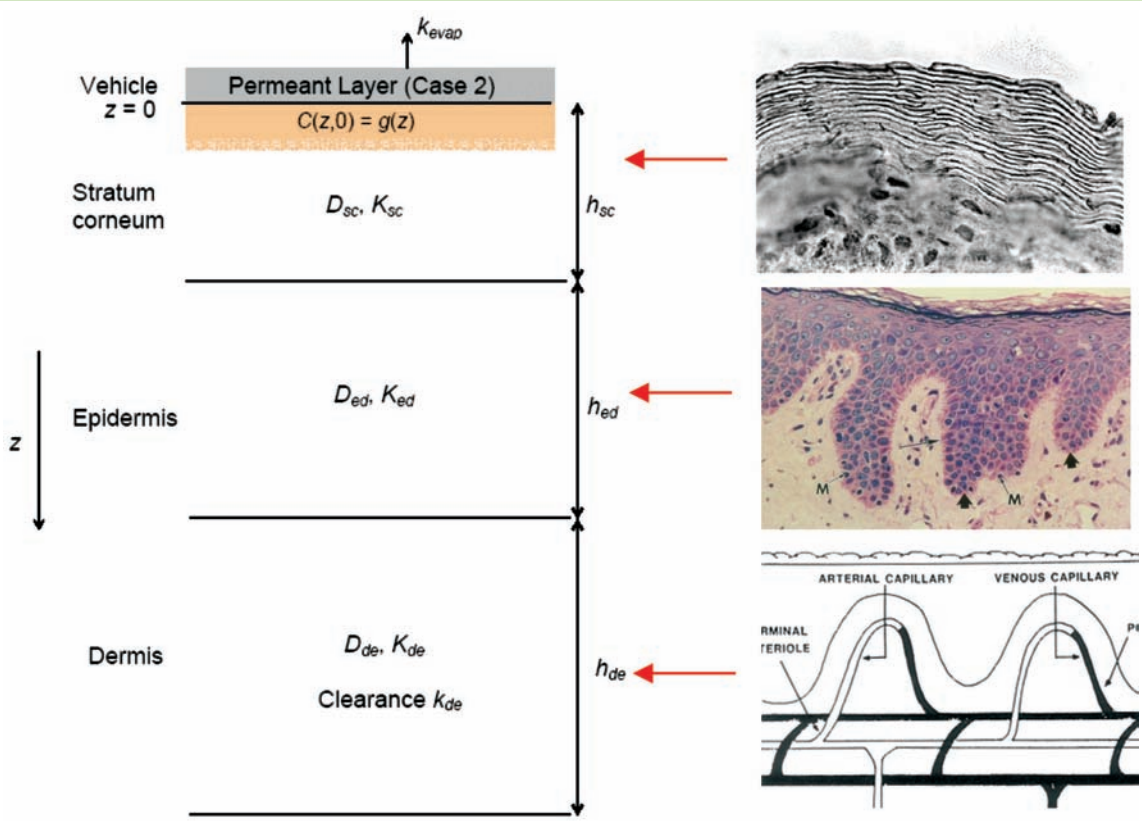
attempts to incorporate the most important aspects of this microstructure into a mass transport model that tracks the motion and local concentrations of permeants as they diffuse across the tissue. It seems that the result would be pretty complex. But would it be too complex to be useful? The answer is, not necessarily. Important work in mathematical physics, dating back to pioneering analyses by Maxwell and Rayleigh, has shown that in many cases a complex microsystem can be represented as a much simpler "effective medium" characterized by average transport coefficients in terms of its overall macroscopically observable behavior.[27,28] Such coarse-graining theory has been extended by numerous authors, as developed in a very general form and summarized by Brenner and Edwards.[29] If the premises of effective medium theory are satisfied, the skin architecture can, in principle, be represented by a stack of homogeneous layers such as those depicted in the left segment of **F-4**.[29] A considerable effort is involved in establishing the relationship between the microscopic transport and partition properties and those of the homogenized model. But once this relationship is established, the much simpler calculations associated (for skin) with the slablike geometry depicted in **F-4** can be conducted. This is an area in which the research groups (Kasting and Nitsche) have been involved for the past 10 years.

An outcome of this work has been the development of effective medium models for stratum corneum and dermis.[19,30-34] At the present time, viable epidermis is treated as unperfused dermis; however, a microscopic transport model for this skin layer is currently under development (Nitsche). These layer-specific models have been combined with a mass transport model for deposition and volatilization of ingredients from the upper SC into a single calculation implemented on an Excel spreadsheet and associated add-in.[26,35] At this stage of development it is reasonably user-friendly and has been tested by several skin toxicology groups. The spreadsheet is available from the authors (Kasting) at no charge.[38]

It is not the intent of this article to describe exactly how the spreadsheet calculation for disposition on skin works. Rather, it focuses on the inputs and outputs associated with the model calculations. For more information the reader is referred to the original papers and several recent summaries thereof.[35-38] The key paper for understanding the SC component of the model is reference 32, and the corresponding paper for dermis is reference 19. The skin deposition and volatilization model originally described in reference 26 has been tested on a number of volatile agents including solvents, fragrance ingredients and pesticides.[35,37,39-41]

Effective transport model for skin; microscopic transport models representing the various layers of the skin in considerable detail are homogenized into a one-dimensional array with effective diffusivities D_i and partition coefficients K_i in each layer; the dermis has also a first-order loss constant k_{de} representing capillary clearance

F-4



The spreadsheet calculation draws on the physicochemical properties of the permeant (**T-2**) and a number of exposure and environmental variables (**T-3**). These values are entered into the spreadsheet, which has the capability of temperature-correcting certain inputs and filling in missing information. For example, if water solubility information is lacking, this value is estimated using the method of Jain and Yalkowski.[42] The USEPA's *EpiSuite* package, available for download from the EPA Web site (*www.epa.gov/oppt/exposure/pubs/episuite*) at no cost, is very helpful for locating or estimating permeant physical properties.[43] Once the required information is entered, a simulation button is pressed to execute the calculation, which is conducted within the add-in. Results are available within a few seconds and include tabular data and plots of evaporation rate, absorption rate and skin concentration depth profile as a function of time.

Of particular interest for the present analysis are the cumulative evaporation profile (the equivalent of Equation 2 in the compartmental model) and the permeant concentrations in the viable epidermis and dermis, which were not estimated within the compartmental model, yet have potential relevance to skin sensitization. It is not yet known which features of the skin concentration versus time profile will best correlate with skin sensitization thresholds. However, in the absence of such information, one can take the peak value of the permeant concentration at the mid-epidermis, which is the primary location of the sentinel Langerhans cells, as a representative output. Within the diffusion model this concentration has two components—a freely diffusing concentration C_{free}, and a component that is reversibly bound to soluble protein, C_{bound}; thus the total concentration C_{total} is the sum of C_{free} and C_{bound}. In general, for lipophilic compounds C_{total} is much greater than C_{free}, because they are highly bound to albumin and other proteins found in skin.[19,45] The model yields the full time courses of C_{free} and C_{total}. We select the peak (highest) values over time, C_{free}^{peak} and C_{total}^{peak}, as our pharmacokinetic indicators.

Within the diffusion model there is a dimensionless parameter χ that closely parallels the volatility parameter x_r in the compartmental model (Equation 6). χ is defined by the relationship[26]

$$\chi = \frac{hk_{evap}\rho}{DC_{sat}} \qquad (9)$$

Required permeant physical properties for skin transport calculations; temperature-dependent properties are determined at skin temperature, normally taken to be 32°C [26] **T-2**

Parameter	Units	Definition
MW	Da	Molecular weight
log K_{oct}	-	Octanol/water partition coefficient
S_w	g/cm^3	Water solubility of unionized form
mp	°C	Melting point
bp	°C	Normal boiling point (760 torr)
P_{vp}	torr	Vapor pressure
pK_a	-	Ionization constant(s)[a]
f_u	-	Fraction unbound in a 2% albumin solution[b]
ρ	g/cm^3	Density

[a]The fraction nonionized (f_{non}) in the stratum corneum (pH 5) and viable skin tissues (pH 7.4) must be estimated. All pK_a values relevant to this calculation should be included.

[b]In the absence of experimental data, the method of Yamazaki and Kanaoka[44] is employed to obtain this value.[19]

Exposure and environmental inputs for skin transport calculations **T-3**

Parameter	Units	Definition	Default value
M_0	µg/cm^2	Specific dose of applied chemical	-
Veh	-	Vehicle (solvent) in which chemical is applied	-[a]
M_v	mg/cm^2	Amount of vehicle	-
Species	-	Skin type (human or mouse)	Human
Environ	-	Simulation environment (in vivo or in vitro)	In vivo[b]
Hyd	-	Hydration state of stratum corneum (partial or full)	Partial[c]
T	°C	Temperature of skin surface	32
u	m/s	Wind velocity	0.17 (indoors)[d] 0.70 (outdoors)[d]

[a]Current choices are water, olive oil, volatile solvent and none.

[b]In vivo environment activates capillary clearance k_{de}.

[c]Corresponds to air-exposed skin with 30% average water content.

[d]Calculation employs turbulent flow model with evaporation rate proportional to $u^{0.78}$.

In Equation 9, h is the thickness of the stratum corneum, k_{evap} is the skin-phase permeant evaporation mass transfer coefficient, ρ is the permeant density, D is its diffusion coefficient in the stratum corneum and C_{sat} is its solubility in the stratum corneum. χ is the ratio of the maximum evaporation rate $k_{evap}\rho$ of the permeant to its maximum flux through skin DC_{sat}/h. In engineering parlance, χ is a Biot number.[46] This ratio is used in the next section to compare the results of the diffusion model with those of the compartmental model for fragrance disposition on skin.

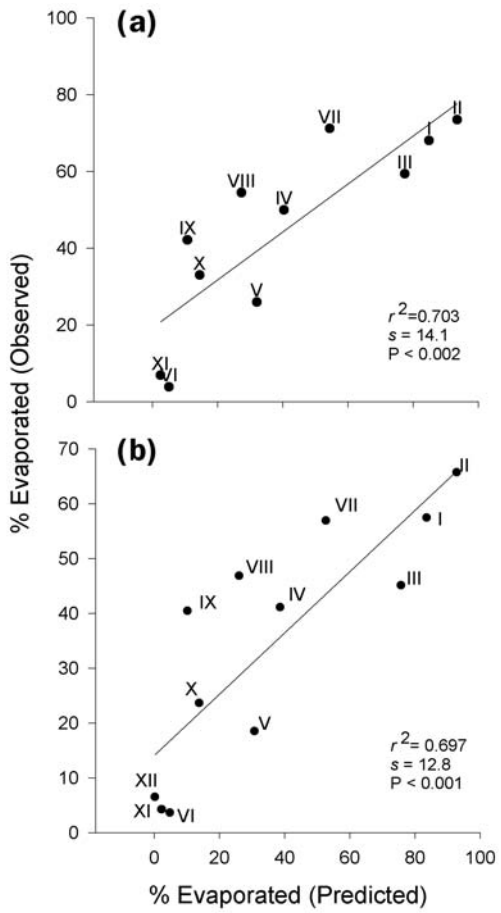
Application of the Diffusion Model to Fragrance Evaporation from Skin

The spreadsheet diffusion model is not quite ready to accurately describe the kinetics of the fragrance mixture studied by Firmenich (**T-1**), as it assumes each ingredient evaporates and penetrates independently, and cannot yet describe the drydown of complex mixtures. Overcoming this limitation is one of the current objectives of the authors' research groups. However, the model can be applied to the problem in an approximate manner, by assuming the ingredients evaporate and absorb independently from an appropriate volume of olive oil. The authors have conducted model calculations under this assumption to illustrate the capabilities of the as-yet-unfinished diffusion model. Wind velocity was assumed to be u = 0.17 m/s, the default value for indoor exposures. This choice can be defended on the basis of comparisons between *in vivo* and *in vitro* volatiles trapping studies conducted in the authors' laboratory.[16] Results are shown in **T-1, F-5, F-6** and **F- 7**.

F-5 shows the relationship between observed percentage evaporation at 7.25 hr and the values calculated from the diffusion model. The squared correlation coefficients are about 0.7 for both Vectors A and B, and the root mean square deviations (s) are 14% and 13%, for Vectors A and B, respectively. These values are comparable to those obtained with the compartmental model. The difference is that the diffusion model arrived at this result with no prior information regarding the evaporation rates of these PRMs, whereas the compartmental model result was a fit to the data. In other words, the diffusion model yielded an *a priori* prediction of comparable quality to the compartmental model fit to the data. **F-6** shows the cumulative evaporation data plotted versus the dimensionless parameter χ (Equation 9), a plot that may be directly compared with the compartmental analog, **F-3.**

In addition, the diffusion model yielded skin concentration profiles for each PRM during the 7.25 hr exposure. The peak mid-epidermal concentrations, C_{free}^{peak} and C_{total}^{peak}, estimated from the model are shown in **T-1**. Estimates for C_{free}^{peak} ranged from 0.1 to 240 µM, the highest value being associated with the solvent 2-phenylethanol (V), whereas those for C_{total}^{peak} ranged from 12 to 600 µM. It is of interest, but not yet known, whether peak skin concentrations calculated in this manner can be correlated with skin sensitization thresholds in either *in vivo* or *in vitro* assays.

Observed cumulative evaporation of perfume ingredients in Firmenich study,[15] plotted vs. the predicted values from the diffusion model: (a) Vector A; (b) Vector B **F-5**



So, what physical properties are most closely associated with the peak skin concentration values for fragrance ingredients? The answer is surprising and is shown in **F-7**. C_{free}^{peak} values for this dataset were inversely correlated with octanol/water partition coefficient, K_{oct}, with the slopes of log-log plots having values close to –0.7 for both Vectors A and B. This result seems counter-intuitive, as one tends to think of highly lipophilic molecules as better skin permeants. A comparable correlation with a positive slope can be demonstrated between C_{free}^{peak} and water solubility, S_w. This is not surprising because S_w for these permeants was estimated using a relationship in which S_w and K_{oct} for liquids are inversely related.[42] The explanation for this result appears to lie in the fact that many of the PRMs in the Firmenich dataset are highly lipophilic, with log K_{oct} values of three or greater. It can be shown from the diffusion model, in agreement with previous work on this subject, that such compounds experience high diffusive resistance in the viable skin layers, so that their skin permeation rates are no longer controlled by the stratum corneum.[47] It is likely that molecular weight would also show an inverse relationship with C_{free}^{peak}, were

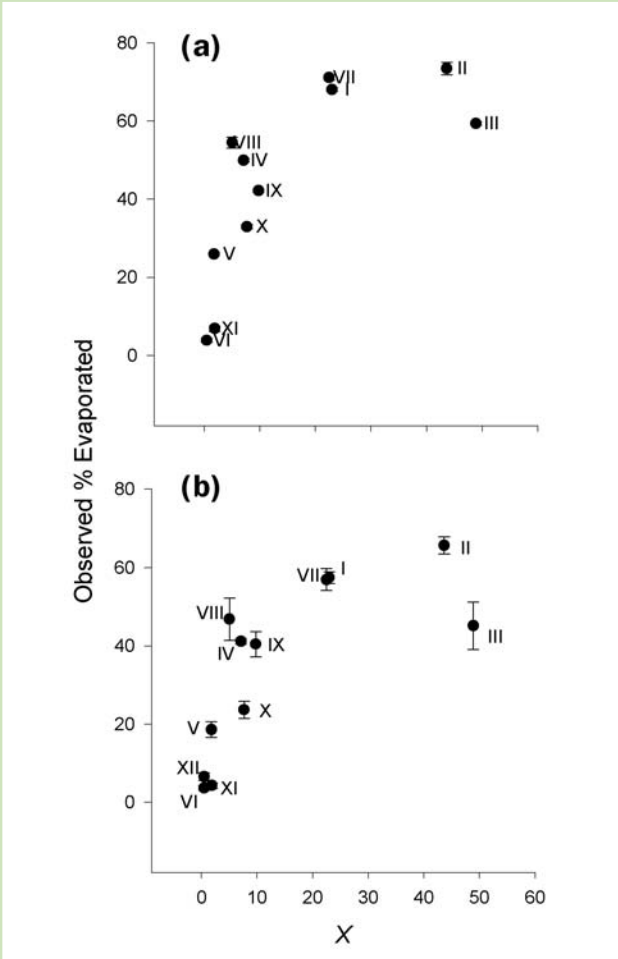
compounds having a wider range of sizes included in the study. But this is not a likely occurrence for fragrance ingredients.

Interestingly, no strong correlation was observed between peak *total* skin concentration, C_{total}^{peak}, and any of the physicochemical properties listed in **T-1**. Highly lipophilic compounds were predicted to bind more tightly to skin proteins, increasing the bound/free ratio in the epidermis and dermis. This result, if correct, means that experimental measurements of PRM concentrations in skin are not likely to confirm the relationship depicted in **F-7**, as most experimental techniques for analyzing permeants in tissue do not distinguish between free and reversibly bound material. The diffusion model therefore offers the opportunity to estimate a quantity, C_{free}^{peak}, that cannot be easily measured and may be the most important correlating factor between epidermal bioavailability and skin sensitization.

It might be considered disappointing to find that the diffusion model, with all the additional effort behind it, did not correlate the fragrance evaporation data from this particular forearm study any better than did the simple one-compartment model (cf. **F-3 and F-6**). However, the limitations in both analyses are evident—neither analysis fully considered the kinetics of the dry-down process on skin and the changing thermodynamic activities of the various fragrance components. In addition, both analyses relied on calculated values of the vapor pressures, partition coefficients and water solubility of each component, rather than employing measured values. While substantial error may incur from such approximations, it is interesting to note that two sets of experimental vapor pressure measurements for these ingredients were available to the authors when reference 17 was drafted, each supplied by a different fragrance manufacturer. There was substantial disagreement between the measurements. Consequently, the decision was made to use calculated vapor pressures that could at least be reproduced. The authors retained this choice in the present analysis. The dilemma points out that, as in all physical properties-based models, the results are no better than the data that are supplied. Caution is advised when drawing inferences from uncertain information.

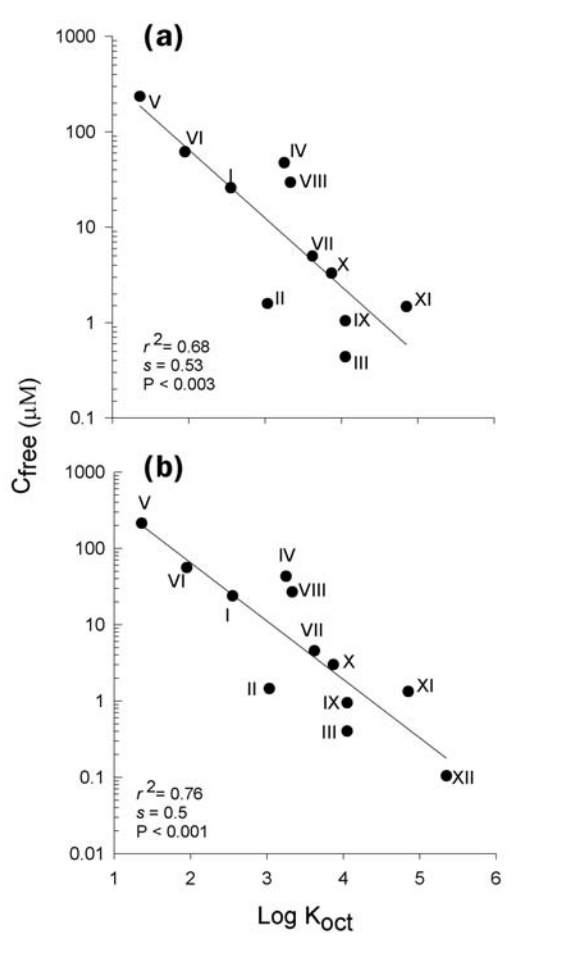
Cumulative evaporation of perfume ingredients in Firmenich study, plotted vs. the model-calculated volatility parameter (Equation 9);[15] each point represents the mean ± SE of two trials; he absence of an error bar indicates the SE was smaller than the size of the symbol: (a) Vector A; (b) Vector B.

F-6



Correlation of log-scaled free mid-epidermal concentrations of C_{free}^{peak} perfume ingredients with their lipophilicities; (a) Vector A; (b) Vector B

F-7



Conclusions

A diffusion model suitable for evaluating the disposition of fragrance components and other volatile compounds on and in the skin has been developed. It helps to obtain evaporation and absorption profiles and skin concentrations under a variety of exposure conditions. Presently, this work is envisioned as a component of an alternative test battery for skin sensitization involving a variety of *in silico* and *in vitro* assays; however, upon its maturity, this physical science may help perfumers understand the interplay of fragrance ingredients on skin in a quantitative way—one that complements the sensory approaches presently used in formulation development of fragranced products.

Acknowledgements

This work was supported by a grant from COLIPA, the European Cosmetics Association. The diffusion model and spreadsheet development have received additional support from NIOSH via research grant R01 OH007529. The results and opinions expressed herein are those of the authors and do not reflect endorsement by either of the sponsors.

Address correspondence to Gerald Kasting, The University of Cincinnati Academic Health Center, P.O. Box 670004, Cincinnati, Ohio 45267-0004; gerald.kasting@uc.edu.

References

1. I Kimber, RJ Dearman, DA Basketter, CA Ryan, GF Gerberick, PM McNamee, J Lalko and AM Api, Dose metrics in the acquisition of skin sensitization: Thresholds and importance of dose per unit area, *Reg Toxicol Pharmacol,* 52, 39–45 (2008)

2. AM Kligman, The identification of contact allergens by human assay. II. Factors influencing the induction and measurement of allergic contact dermatitis, *J Invest Dermatol,* 47, 375–392 (1966)

3. PS Friedmann, C Moss, S Shuster and JM Simpson, Quantitative relationships between sensitizing dose of DNCB and reactivity in normal subjects, *Clin Exp Immunol,* 53, 709–715 (1983)

4. PS Friedmann, The immunology of allergic contact dermatitis: the DNCB story, *Adv Dermatol,* 5, 175–196 (1990)

5. PS Friedmann, J Rees, SI White and JN Matthews, Low-dose exposure to antigen induces sub-clinical sensitization, *Clin Exp Immunol,* 81, 507–509 (1990)

6. MK Robinson, GF Gerberick, CA Ryan, P McNamee, I White and DA Basketter, The importance of exposure estimation in the assessment of skin sensitization risk, *Contact Derm* 42, 251–259 (2000)

7. DA Basketter, Skin sensitization: risk assessment, *Int J Cosmet Sci,* 20, 141–150 (1998)

8. DA Basketter, C Pease, GB Kasting, I Kimber, S Casati, MTD Cronin, W Diembeck, GF Gerberick, J Hadgraft, T Hartung, JP Marty, E Nikolaidis, GY Patlewicz, D Roberts, E Roggen, C Rovida and H van de Sandt, Skin sensitisation and epidermal disposition. The relevance of epidermal bioavailability for sensitisation hazard identification/risk assessment, *ATLA,* 35, 137–154 (2007)

9. GF Gerberick, MK Robinson, SP Felter, IR White and DA Basketter, Understanding fragrance allergy using an exposure-based risk assessment approach, *Contact Derm,* 45, 333–340 (2001)

10. JC Dearden, In silico prediction of drug toxicity, *J Comp-Aided Mol Des,* 17, 119–127 (2003)

11. A Cockshott, P Evans, CA Ryan, GF Gerberick, CJ Betts, RJ Dearman, I Kimber and DA Basketter, The local lymph node assay in practice: a current regulatory perspective, *Hum Exp Toxicol,* 25, 387–394 (2006)

12. GF Gerberick, JD Vassallo, RE Bailey, JG Chaney, SW Morrall and J-P Lepoittevin, Development of a peptide reactivity assay for screening contact allergens, *Tox Sci,* 81, 332–343 (2004)

13. GF Gerberick, JD Vassallo, LM Foertsch, BB Price, JG Chaney and J-P Lepoittevin, Quantification of chemical peptide reactivity for screening contact allergens: A classification tree model approach, *Tox Sci,* 97, 417–427 (2007)

14. P Aeby, C Wyss, H Beck, P Griem, H Scheffler and C Goebel, Characterization of the sensitizing potential of chemicals by in vitro analysis of dendritic cell activation and skin penetration, *J Invest Dermatol,* 122, 1154–1164 (2004)

15. C Vuilleumier, I Flament and P Sauvegrain, Headspace analysis study of evaporation rate of perfume ingredients applied onto skin, *Int J Cosmet Sci,* 17, 61–76 (1995)

16. P Saiyasombati and GB Kasting, Evaporation of benzyl alcohol from human skin in vivo, *J Pharm Sci,* 93, 515–520 (2004)

17. GB Kasting and P Saiyasombati, A physicochemical properties based model for estimating evaporation and absorption rates of perfumes from skin, *Int J Cosmet Sci,* 23, 49–58 (2001)

18. P Saiyasombati and GB Kasting, Two-stage kinetic analysis of fragrance evaporation and absorption from skin, *Int J Cosmet Sci,* 25, 235–243 (2003)

19. K Kretsos, MA Miller, G Zamora-Estrada and GB Kasting, Partitioning, diffusivity and clearance of skin permeants in mammalian dermis, *Int J Pharm,* 346, 64–79 (2008)

20. P Saiyasombati and GB Kasting, Disposition of benzyl alcohol following topical application to human skin in vitro, *J Pharm Sci,* 92, 2128–2139 (2003)

21. P Saiyasombati and GB Kasting, Prediction of fragrance headspace concentrations from physicochemical properties, *Perfum Flavor,* 29, 38–47 (2004)

22. RA Guy, J Hadgraft and HI Maibach, A pharmacokinetic model for percutaneous absorption, *Int J Pharm* 11, 119–129 (1982)

23. RA Guy and J Hadgraft, Physicochemical interpretation of the pharmacokinetics of percutaneous absorption, *J Pharm Biopharm,* 11, 189–203 (1983)

24. RA Guy and J Hadgraft, Prediction of drug disposition kinetics in skin and plasma following topical administration, *J Pharm Sci* 73, 883–887 (1984)

25. KD McCarley and AL Bunge, Pharmacokinetic models of dermal absorption, *J Pharm Sci,* 90, 1699–1719 (2001)

26. GB Kasting and MA Miller, Kinetics of finite dose absorption through skin 2. Volatile compounds, *J Pharm Sci,* 95, 268–280 (2006)

27. JC Maxwell, *A Treatise on Electricity and Magnetism*. 3rd ed., Oxford: Clarendon Press. 440–441 (1892)

28. Lord Raleigh, On the influence of obstacles arranged in rectangular order upon properties of a medium, *Phil Mag (Fifth Ser)* 34, 481–502 (1892)

29. H Brenner and DA Edwards, *Macrotransport Processes*, Boston: Butterworth-Heinemann (1993)

30. JM Nitsche, T-F Wang and GB Kasting, A two-phase analysis of solute partitioning into the stratum corneum, *J Pharm Sci*, 95, 649–666 (2006)

31. T-F Wang, GB Kasting and JM Nitsche, A multiphase microscopic model for stratum corneum permeability. I. Formulation, solution and illustrative results for representative compounds, *J Pharm Sci,* 95, 620–648 (2006)

32. T-F Wang, GB Kasting and JM Nitsche, A multiphase microscopic model for stratum corneum permeability. II. Estimation of physicochemical parameters and application to a large permeability database, *J Pharm Sci,* 96, 3024–3051 (2007)

33. K Kretsos and GB Kasting, A geometrical model of dermal capillary clearance, *Math Biosci,* 208, 430–453 (2007)

34. K Kretsos, GB Kasting and JM Nitsche, Distributed diffusion-clearance model for transient drug distribution within the skin, *J Pharm Sci,* 93, 2820–2835 (2004)

35. GB Kasting, MA Miller and V Bhatt, A spreadsheet-based method for estimating the skin disposition of volatile compounds: application to N,N-diethyl-m-toluamide (DEET), *J Occup Environ Hyg* 10, 633–644 (2008)

36. GB Kasting, MA Miller and JM Nitsche, Absorption and evaporation of volatile compounds applied to skin. In: KA Walters, MS Roberts, editors. *Dermatologic, Cosmeceutic and Cosmetic Development*, New York: Informa Healthcare USA, 385–400, (2008)

37. VD Bhatt, RS Soman, MA Miller and GB Kasting, Permeation of tecnazene through human skin in vitro as assessed by HS-SPME and GC-MS, *Environ Sci Technol,* 42, 6587–6592 (2008)

38. JM Nitsche and GB Kasting, Biophysical models for skin transport and absorption. In: MS Roberts, KA Walters, editors. *Dermal Absorption and Toxicity Assessment*, 2nd ed., New York: Informa Healthcare USA, 249–267, (2008)

39. S Ray Chaudhuri, RM Gajjar, WB Krantz and GB Kasting, Percutaneous absorption of volatile solvents following transient liquid exposures. II. Ethanol, *Chem Eng Sci,* 64, 1665–1672 (2009)

40. MA Miller, V Bhatt and GB Kasting, Absorption and evaporation of benzyl alcohol from skin, *J Pharm Sci,* 95, 281–291 (2006)

41. MA Miller and GB Kasting, Towards a better understanding of pesticide dermal absorption: diffusion model analysis of parathion absorption in vitro and in vivo, *J Toxicol Environ Health,* in press (2009)

42. N Jain and SH Yalkowski, Estimation of aqueous solubility I. Application to Organic Non-Electrolytes, *J Pharm Sci,* 90, 234–252 (2000)

43. Estimation Programs Interface Suite for Microsoft Windows 4.0., Washington, DC, USA: United States Environmental Protection Agency, 2009

44. K Yamazaki and M Kanaoka, Computational prediction of the plasma protein-binding percent of diverse pharmaceutical compounds, *J Pharm Sci,* 93, 1480–1494 (2004)

45. HM Weiss, M Fresneau, T Moenius, A Stuetz and A Billich, Binding of pimecrolimus and tacrolimus to skin and plasma proteins: implications for systemic exposure after topical application, *Drug Metab Dispos,* 36, 1812–1818 (2008)

46. S Ray Chaudhuri, WB Krantz and GB Kasting, Percutaneous absorption of volatile solvents following transient liquid phase exposures I. Model development, *Chem Eng Sci* 64, 1027–1035 (2009)

47. RL Cleek and AL Bunge, A new method for estimating dermal absorption from chemical exposure. 1. General approach, *Pharmaceut Res,* 10, 497–506 (1993)