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# Absorption and evaporation of *N*, *N*-diethyl-*m*-toluamide from human skin in vitro

Arjun Santhanam, Matthew A. Miller, Gerald B. Kasting*

*College of Pharmacy, The University of Cincinnati Medical Center, Cincinnati, OH 45267-0004, USA*

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**Abstract**

The penetration of DEET through split-thickness cadaver skin was measured in non-occluded Franz cells placed either in a fume hood or on a laboratory workbench. DEET, dissolved in a small volume of ethanol and spiked with $^{14}C$ radiolabel was applied to skin at doses from 0.02 to 11 000 μg/cm$^2$. DEET penetration was greater for cells placed on the workbench, and the percentage of radioactivity penetrated after 72 h increased gradually with dose, for doses up to 680 μg/cm$^2$. At higher doses, it declined. Percent penetration ranged from 11.5 ± 3.2% for a dose of 0.021 μg/cm$^2$ in the fume hood to 71.9 ± 5.5% for a dose of 260 μg/cm$^2$ on the workbench. Results were interpreted in terms of a diffusion/evaporation model having three parameters—a solubility value for the chemical in the upper stratum corneum, $M_{sat}$; a mass transfer coefficient for evaporation, $k_{evap}$; and a characteristic time for diffusion, $h^2/D$. The parameters obtained from fitting the model to the data (normalized to the fume hood environment) were $M_{sat}$ = 18 μg/cm$^2$ and $k_{evap} = 2.6 \times 10^{-5}$ cm/h. The value of $h^2/D$ decreased from 16 h at a DEET dose of 25 μg/cm$^2$ to 10 h at 1480 μg/cm$^2$, consistent with an increase in skin permeability of about 1.5-fold over this dose range. This effect was confirmed by means of an additional study in which skin samples pretreated with increasing amounts of unlabeled DEET were washed and redosed with $^{14}C$-benzyl alcohol. A small (1.7-fold), but significant, increase in benzyl alcohol penetration with increasing amount of DEET was obtained. Thus, DEET enhanced its own skin permeation rate as well as that of another compound, but the effect was modest and not likely to be a major concern for compounds coadministered with DEET.
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*Keywords:* DEET; Evaporation; Percutaneous absorption; Mathematical model; Skin

## Introduction

*N*, *N*-diethyl-3-methylbenzamide (DEET, Fig. 1) is a widely used insect repellent whose percutaneous absorption and evaporation in humans has been extensively studied in vitro (Baynes et al., 1997; Moody and Martineau, 1990; Moody and Nadeau, 1993; Reifenrath and Robinson, 1982; Reifenrath et al., 1989; Riviere et al., 2003; Spencer et al., 1979) and in vivo (Feldmann and Maibach, 1970; Selim et al., 1995; Smith et al., 1963; Spencer et al., 1979; Taylor et al., 1994). This work and related animal absorption and toxicology studies have been summarized in two excellent reviews (Qui et al., 1998; Robbins and Cherniak, 1986). DEET is generally considered to be safe for topical use if applied as recommended (Qui et al., 1998). DEET is known to modulate the absorption of other compounds when coadministered topically (Baynes et al., 1997). In turn, its own absorption may be affected by other ingredients in a topical formulation (Qui et al., 1998; Reifenrath et al., 1989; Riviere et al., 2003) or by dose, occlusion or airflow over the skin surface (Qui et al., 1998; Reifenrath et al., 1989; Selim et al., 1995). Despite this extensive body of data, there is no accepted methodology by which one can relate the topical disposition of DEET or other pesticides to physicochemical properties, formulation and exposure variables in a way that can be extrapolated to new compounds, formulations or exposure conditions. This

* Corresponding author. The University of Cincinnati Medical Center, PO Box 670004, Cincinnati, OH 45267-0004. Fax: +1 513 558 0978.
*E-mail address:* Gerald.Kasting@uc.edu (G.B. Kasting).

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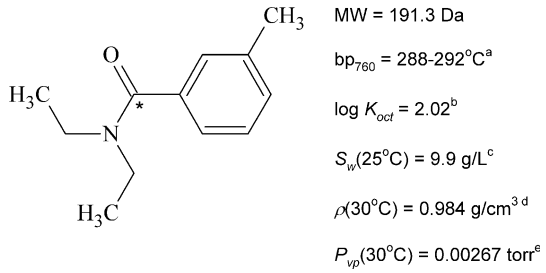


Fig. 1. Structure and physical properties of DEET. The asterisk denotes the location of the radiolabel. [a] Meylan (1999); [b] Hansch and Leo (1999); [c] Qui et al. (1998); [d] Calculated from reported value of 0.996 at 20 °C (Budavari, 1996) using an estimated thermal expansivity of 0.0012 $K^{-1}$; [e] Estimated from reported value of 0.00167 torr at 25 °C (Qui et al., 1998) according to modified Grange method (Meylan, 1999).

report represents a step toward this objective. DEET disposition on skin is described in terms of a diffusion/evaporation model having parameters that can be related to molecular weight, volatility, and lipid solubility. Model parameters were developed by measuring penetration of radiolabeled DEET from ethanol through human skin in vitro over a wide dose range under two laboratory airflow conditions. A related study to evaluate a more complete airflow dependence for DEET disposition is in progress (Bhatt and Kasting, 2003), and the application of the model to other low molecular weight permeants with varying volatility and lipophilicity is described in an MS thesis (Santhanam, 2004). Both of these studies will be reported separately.

The approach taken here combines a model for describing the absorption of a nonvolatile compound from skin over a large dose range (Kasting, 2001) with one used to describe volatiles evaporation and absorption at low doses (Kasting and Saiyasombati, 2001; Saiyasombati and Kasting, 2003a, 2003b). Both features are necessary to provide a useful framework for DEET pharmacokinetics on skin. The present model does not explicitly consider solvent or formulation effects, although their influence on depositing compounds into the upper stratum corneum is implicit in the initial condition. Stepwise expansion to consider binary or multicomponent diffusion may foreseeably allow this development, which has proven difficult to implement in compartmental kinetic models (Saiyasombati and Kasting, 2003b, 2004b).

## Theory

Consider the problem depicted in Fig. 2. A composition consisting of DEET in a volatile solvent, e.g., ethanol, is applied to the surface of the skin. The solvent carries the DEET into the relatively permeable upper 1–3 cell layers (Monteiro-Riviere et al., 1994) of the stratum corneum (SC) prior to dissipating by penetration and evaporation. We will assume the deposited DEET to have a constant concentration $C_0$ in the upper 10% of the SC ($h$/10), limited by its solubility $C_{sat}$. Any DEET in excess of the saturation dose, $M_{sat} = C_{sat} \times h/10$, remains as a uniform layer on the surface of the SC. The DEET dissipates from the deposited layer(s) by two mechanisms—permeation into the skin and evaporation into the surrounding atmosphere.

Since DEET is a moderately lipophilic permeant (log $K_{oct}$ = 2.02), we take as a model for the SC a simple lipid membrane of thickness $h$ (Potts and Guy, 1992). Within this membrane, DEET has diffusivity $D$, resulting in a diffusive time constant $h^2/D$ (Crank, 1975). Sink conditions are assumed to prevail at the lower or proximal boundary of the SC, a valid approximation for compounds of low-to-moderate lipophilicity (Cleek and Bunge, 1993). On the upper or apical boundary, DEET is lost by evaporation. For small doses (Case 1), DEET evaporates from the skin surface at a rate proportional to its local concentration in the skin. For large doses (Case 2), DEET evaporates from the deposited liquid film at a constant rate. It also partitions into the upper SC, maintaining saturation conditions in the permeable upper layers until the surface film is depleted, after which the situation reverts to Case 1. The equations describing DEET transport in the two cases are as follows:

*Case 1. Dose < $M_{sat}$*

Within the SC, DEET transport is governed by the diffusion equation

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} \qquad 0 \le x \le h \tag{1}$$

where $C(x,t)$ is the concentration and diffusivity $D$ is assumed to be constant. The initial condition is

$$C(x,0) = \begin{cases} Dose \div h/10 & 0 \le x \le h/10 \\ 0 & h/10 < x \le h \end{cases} \tag{2}$$

and the boundary conditions for $t > 0$ are

$$D\frac{\partial C}{\partial x}\Big|_{x=0} = k_{evap} \cdot \frac{\rho}{C_{sat}} \cdot C(0,t) \quad x = 0 \tag{3}$$

and

$$C(h,t) = 0 \qquad x = h \tag{4}$$

The proportionality factor $\rho/C_{sat}$ in Eq. (3) is a vehicle/membrane partition coefficient that adjusts for thermody-

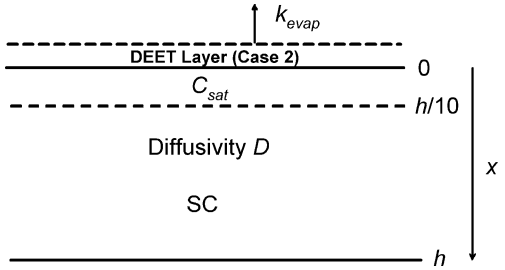


Fig. 2. Diffusion model for DEET disposition on skin.

namic activity differences between the two phases. It leads to a consistent value of the mass transfer coefficient, $k_{evap}$, for Case 1 and Case 2 and a continuous transition in evaporative flux between these regimes. The DEET density $\rho$ must be expressed in same units as $C_{sat}$ and has a value very nearly equal to $10^6$ μg/cm$^3$ (Fig. 1).

*Case 2. Dose ≥ $M_{sat}$*

In this case, a DEET dose corresponding to $M_{sat}$ is deposited into the top 10% of the SC. Any remaining DEET (mass $M$) forms a pool on the top of the skin. This pool acts as a reservoir replenishing the top layers of the membrane as the chemical permeates into the lower layers. DEET also evaporates from the pool. In the top 10% of the membrane, $C = C_{sat}$ until the pool is depleted. DEET transport within the membrane is governed by Eq. (1) with the initial condition

$$C(x,0) = \left\{ \begin{array}{ll} C_{sat} & 0 \le x \le h/10 \\ 0 & h/10 < x \le h \end{array} \right\}. \quad (2')$$

The boundary condition at the upper SC surface (Eq. (3)) is replaced by the mass balance relationship

$$-\frac{dM}{dt} = k_{evap} \cdot \rho + D\frac{\partial C}{\partial x}|_{x=h/10} \quad (3')$$

whereas Eq. (4) still applies at the base of the membrane. When the DEET film is depleted, i.e., as $M \rightarrow 0$, the situation reverts to Case 1.

The diffusion model as described above has several key approximations that make it tractable, but realistic for the case of a binary system in which the solvent is much more volatile than the solute. These are (1) the SC is considered to be a simple lipid membrane; (2) the solvent is not explicitly considered; (3) evaporative cooling is assumed to be negligible, once the solvent has dissipated. Thus, mass transport in the system can be described by the dilute solution formulation of the one-dimensional diffusion equation (Cussler, 1997). By making these approximations, one gives up the possibility of explicitly including solute–solvent interactions or solvent effects on skin permeability. The model has only three disposable parameters—an evaporation mass transfer coefficient $k_{evap}$, a diffusive time constant $h^2/D$, and a saturation dose $M_{sat}$. The meaning of these parameters will become evident in the analysis.

## Materials and methods

*Chemicals.* [Carboxyl-$^{14}$C]DEET at specific activity 52 mCi/mmol was custom synthesized by Vitrax (Placentia, CA). [Carboxyl-$^{14}$C]benzyl alcohol at specific activity 55 mCi/mmol was purchased from Moravek Biochemicals (Brea, CA). Radiochemical purities as stated by the suppliers were 99% and 98.3%, respectively. Unlabeled DEET and benzyl alcohol were purchased from Sigma-Aldrich (St. Louis, MO). All other chemicals used were reagent grade.

*In vitro penetration studies.* Tissue preparation and handling were as previously described (Kasting and Bowman, 1990). Human cadaver skin, dermatomed to a thickness of 0.25 mm and stored frozen was obtained from the Ohio Valley Tissue and Skin Center. The samples were obtained from either the back, abdomen, or thigh. The skin was mounted in Franz diffusion cells, the tops of which were open to the atmosphere and extended 4 mm above the skin surface. The integrity of skin samples was assessed by $^3H_2O$ penetration prior to each study (Franz and Lehman, 1990; Kasting et al., 1994), after which the skin was allowed to equilibrate overnight. Median $^3H_2O$ penetration was 0.48 μl/cm$^2$ (range 0.2–1.8 μl/cm$^2$), and 94% of the samples yielded values less than Franz and Lehman's recommended limit of 1.2 μl/cm$^2$. The remaining 6% of the samples yielded DEET permeation rates comparable to the first 94%; hence, they were retained in the analysis. The receptor solution was Dulbecco's phosphate-buffered saline containing 0.02% sodium azide, magnetically stirred and maintained at 37 °C in a thermostatted block. Receptor solutions were exchanged at 1, 2, 4, 8, 12, 24, 48, and 72 h post-dose and analyzed for radioactivity by liquid scintillation counting (LSC). After the last sample was collected, the skin was dissolved in 2 ml of Soluene-350 (Packard Bioscience) and analyzed by LSC.

*DEET studies.* Eighteen dose solutions of $^{14}$C-DEET in ethanol ranging in concentration from 0.0004% to 50% and in specific activity from 0.001 to 0.05 μCi/mg were prepared. The specific dose of DEET on skin was varied in approximately half log units over the range 0.02–11 100 μg/cm$^2$. Application volume was 5 μl per 0.79 cm$^2$ cell, except for the largest doses in which either 10 or 20 μl were used.

*Air-flow correction factor.* Six trials were conducted—three on a laboratory work bench, with ambient room conditions, and three in a Hamilton Safeaire fume hood with a sash height of 25 in. The face velocity of the hood was 127 ft$^3$/min at a sash height of 21 in. The air flow rate was significantly higher in the hood, resulting in more evaporation and less penetration (Bhatt and Kasting, 2003). To normalize the data, a separate calibration study was carried out with 30 cells from one skin donor randomly distributed between the hood and the lab workbench. The dose solution used was $^{14}$C-DEET in ethanol at a specific dose of 117 μg/cm$^2$ and a dose volume of 5 μl.

*Benzyl alcohol study.* This study was carried out in the fume hood. The skin samples were pretreated for 16 h with formulations consisting of unlabelled DEET in ethanol at

concentrations and dose volumes comparable to those used with $^{14}$C-DEET. They were then washed with a dilute soap solution prepared from commercially available Dial® hand wash (1 part) and distilled water (9 parts). A 300-μl aliquot of this soap solution was pipetted onto the skin, and the skin was gently brushed with a cotton swab for 10–15 s. The soap solution was removed and the skin was rinsed twice in the same manner using distilled water. The cells were then dosed with 10 μl of a 1% solution of $^{14}$C-benzyl alcohol in ethanol (0.068 μCi/cell), yielding a benzyl alcohol dose of 125 μg/cm$^2$. The receptor solution was exchanged at 1, 2, 4, 8, and 24 h post-dose and analyzed by LSC. The data were normalized by dividing by the average percent penetration from the three lowest doses of DEET, which was equal to 8.5% at 24 h post-dose.

*Data analysis.* Eqs. (1–4) were solved using a one-dimensional finite difference scheme with an equal spaced grid ($n$ = 100 layers in the SC). An implicit time stepping algorithm that switched from Crank Nicholson to reverse Euler methodologies at long times was employed (Dahlquist and Bjork, 1974). The solution was checked against analytical solutions at high (Crank, 1975) and low (Carslaw and Jaeger, 1959) dose limits. The parameters in the theoretical model—$M_{sat}$, $k_{evap}$, and $h^2/D$—were fit to the data using a nonlinear least squares fitting routine adapted from Bevington (1969). The code was implemented as a Visual Basic add-in for Microsoft Excel to provide an easy user interface. Quality of fit was assessed by comparing the sum of squared residuals, normalized by degrees of freedom [or $\chi^2_v$—see Bevington, 1969]. Thus, the quantity minimized was

$$\chi^2_v = \frac{1}{n-p}\sum_{i=1}^{n}\left[y_i(\text{obs}) - y_i(\text{fit})\right]^2 \quad (5)$$

where $n$ was the number of observations, $p$ the number of adjustable parameters, and the $y_i$ values were cumulative percents of dose penetrated at the various sampling times. The estimated evaporation at 72 h was determined by a mass balance involving amounts penetrated and residual amounts in skin at 72 h and included as an experimental observation. Statistical comparisons of absorption and skin retention were made using the Student's $t$-test with a significance level (two-sided) of $\alpha = 0.05$. For the benzyl alcohol study, a linear regression was conducted on the normalized 24-h penetration data versus the logarithm of the DEET pretreatment dose for DEET doses ≥30 μg/cm$^2$. The slope and standard error of the regression line $m \pm se_m$ were tested by a $z$ test against the null hypothesis $m = 0$.

## Results

### *DEET skin penetration*

Radioactivity associated with $^{14}$C-DEET was absorbed slowly through the skin over the 72-h test period (Table 1). Representative plots of absorption rates over a range of doses are shown in Figs. 3 and 4, and the disposition of

Table 1
Absorption of radiolabel for $^{14}$C-DEET skin penetration studies (mean of three donors, $n$ = 3–4 cells per donor)

| Dose (μg/cm$^2$) | Percentage of applied dose in receptor compartment | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0–1 h | 1–2 h | 2–4 h | 4–8 h | 8–12 h | 12–24 h | 24–48 h | 48–72 h |
| *Laboratory workbench* | | | | | | | | |
| 1.2 | 5.0 | 8.9 | 17.1 | 16.3 | 4.7 | 2.9 | 1.0 | 0.4 |
| 2.4 | 5.1 | 10.2 | 15.7 | 12.3 | 2.9 | 1.7 | 0.7 | 0.3 |
| 3.4 | 4.3 | 8.7 | 15.0 | 13.7 | 3.6 | 2.4 | 1.3 | 0.6 |
| 15.6 | 6.1 | 10.2 | 16.5 | 13.7 | 3.6 | 2.5 | 1.2 | 0.5 |
| 25.5 | 4.5 | 10.0 | 18.8 | 17.1 | 3.8 | 2.4 | 1.1 | 0.5 |
| 150 | 4.4 | 9.1 | 18.1 | 18.6 | 6.4 | 4.4 | 1.3 | 0.4 |
| 260 | 4.5 | 8.7 | 18.0 | 24.4 | 9.4 | 5.1 | 1.4 | 0.5 |
| 3160[a] | 0.3 | 0.7 | 2.0 | 7.1 | 4.8 | 12.6 | 20.5 | 13.4 |
| 11,100[b] | 0.1 | 0.2 | 0.5 | 1.3 | 1.3 | 3.7 | 7.1 | 6.8 |
| *Fume hood* | | | | | | | | |
| 0.021 | 0.9 | 1.7 | 2.2 | 3.0 | 1.5 | 1.3 | 0.6 | 0.3 |
| 0.057 | 0.8 | 1.9 | 2.3 | 2.9 | 1.7 | 1.8 | 1.1 | 0.5 |
| 118 | 0.5 | 2.3 | 4.3 | 8.5 | 5.0 | 3.6 | 1.9 | 0.6 |
| 370 | 0.2 | 1.5 | 3.0 | 5.4 | 5.4 | 9.0 | 5.5 | 2.2 |
| 680 | 0.2 | 0.9 | 2.0 | 5.0 | 4.8 | 8.3 | 8.8 | 3.8 |
| 1480[a] | 0.0 | 0.4 | 1.0 | 2.7 | 2.9 | 6.8 | 9.7 | 5.6 |
| 2320[a] | 0.0 | 0.3 | 0.7 | 1.9 | 2.1 | 5.7 | 8.8 | 5.5 |
| 4770[a] | 0.1 | 0.3 | 0.6 | 1.5 | 1.7 | 4.7 | 7.2 | 4.8 |
| 8300[b] | 0.1 | 0.2 | 0.4 | 1.0 | 1.1 | 3.2 | 5.8 | 4.6 |

The dose was 5 μl/cell unless otherwise indicated.
[a] 10-μl dose.
[b] 20-μl dose.

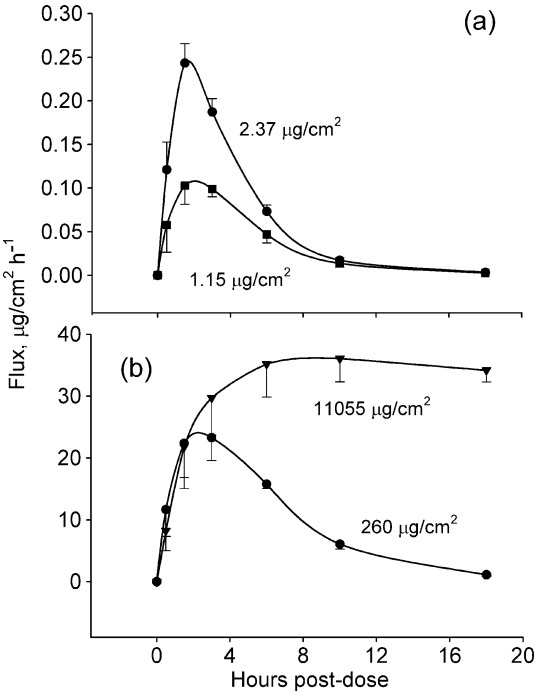


Fig. 3. Representative absorption rates of $^{14}$C-DEET from ethanolic solution through human skin mounted in Franz cells placed on a laboratory workbench (data from Table 1).

radioactivity after 72 h is shown in Table 2. Absorption rates were maximal between 1 and 2 h for small doses (Figs. 3a and 4a) and between 8 and 12 h for large doses (Figs. 3b and 4b). For doses less than 1000 μg/cm$^2$, the disposition of radioactivity was nearly complete by 72 h, with 49–72% of

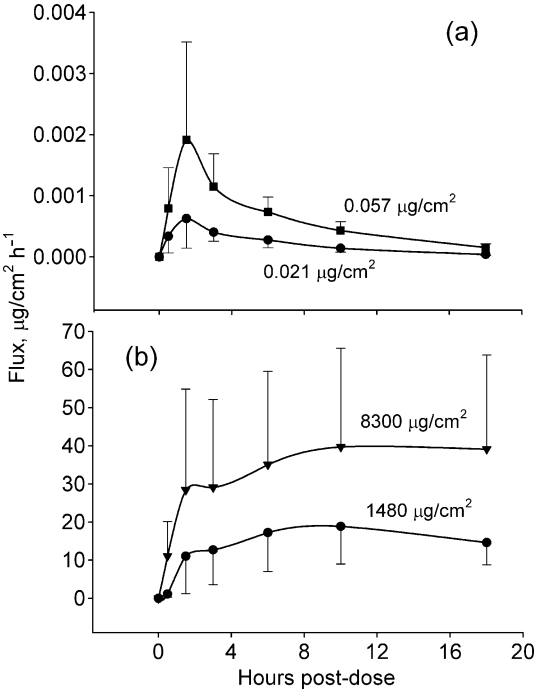


Fig. 4. Representative absorption rates of $^{14}$C-DEET from ethanolic solution through human skin mounted in Franz cells placed in fume hood (data from Table 1).

Table 2
Distribution of radiolabel after 72 h for $^{14}$C-DEET skin penetration studies (mean ± SE of three donors, $n$ = 3–4 cells per donor)

| Dose (μg/cm$^2$) | Percent absorbed (%) | Percent in skin (%) | Percent evaporated (est)[a] (%) |
|---|---|---|---|
| *Laboratory workbench* | | | |
| 1.2 | 56.4 ± 4.7 | 3.4 ± 1.2 | 39.6 ± 7.8 |
| 2.4 | 49.0 ± 2.0 | 5.4 ± 0.3 | 43.6 ± 0.4 |
| 3.4 | 49.8 ± 3.9 | 9.8 ± 3.9 | 44.2 ± 6.0 |
| 15.6 | 54.3 ± 1.8 | 6.9 ± 1.6 | 39.7 ± 3.7 |
| 25.5 | 58.2 ± 4.1 | 4.2 ± 1.4 | 40.2 ± 6.0 |
| 150 | 62.6 ± 1.7 | 1.5 ± 0.0 | 35.0 ± 2.2 |
| 260 | 71.9 ± 5.5 | 1.7 ± 0.3 | 31.1 ± 3.7 |
| 3160 | 61.2 ± 8.7 | 23.0 ± 2.8 | 24.4 ± 3.6 |
| 11,100 | 21.0 ± 1.5 | 42.2 ± 2.2 | 36.7 ± 12.8 |
| *Fume hood* | | | |
| 0.021[b] | 11.5 ± 3.2 | 8.2 ± 0.4 | 80.4 ± 3.6 |
| 0.057[b] | 13.1 ± 2.9 | 9.3 ± 1.6 | 77.6 ± 4.5 |
| 118 | 26.8 ± 9.4 | 5.9 ± 4.0 | 67.3 ± 6.1 |
| 370 | 32.2 ± 2.9 | 7.8 ± 3.0 | 59.9 ± 4.0 |
| 680 | 33.8 ± 4.1 | 10.9 ± 6.8 | 55.3 ± 10.9 |
| 1480 | 29.1 ± 8.4 | 21.0 ± 2.8 | 49.8 ± 10.0 |
| 2320 | 24.9 ± 11.4 | 24.3 ± 5.2 | 50.7 ± 6.3 |
| 4770 | 21.0 ± 12.9 | 40.8 ± 8.8 | 38.1 ± 5.0 |
| 8300 | 16.7 ± 10.1 | 40.1 ± 6.8 | 43.4 ± 3.3 |

[a] Estimated for individual samples as 100 − % penetrated − % in skin.
[b] Radiotracer only (no unlabeled DEET).

dose penetrated in the lab bench studies and 11–34% of dose penetrated in the fume hood studies (Table 2). A small amount of radiolabel, ranging from 1% to 11% of dose, remained in the skin even at the lowest doses. At doses above 1000 μg/cm$^2$, residual radiolabel levels in skin after 72 h were higher, reflecting the incomplete absorption and evaporation processes. This can be clearly seen in Table 2 and Figs. 3b and 4b.

It was evident from the data in Tables 1 and 2 that $^{14}$C-DEET absorption through the skin was consistently higher in the lab bench studies than in the fume hood studies. This is to be expected for volatile compounds whose evaporation rates are dependent on airflow (Bhatt and Kasting, 2003; Selim et al., 1995). As the doses and the skin donors were different for these two sets of experiments, we conducted an additional study using a single dose and skin donor in order to quantify the effect of location. The results of this study are shown in Table 3. Absorption of radioactivity in the lab bench environment was found to be significantly higher than that in the fume hood ($P < 0.001$), with a ratio of approximately 7/4. The small difference between the

Table 3
Results of location comparison study with $^{14}$C-DEET applied to skin at a dose of 125 μg/cm$^2$

| Location | Dose distribution after 72 h (mean ± SE, $n$ = 15) | | |
|---|---|---|---|
| | Percent absorbed (%) | Percent in skin (%) | Percent evaporated (est) (%) |
| Lab bench | 72.2 ± 4.0 | 1.9 ± 0.3 | 25.9 ± 4.3 |
| Fume hood | 40.9 ± 5.2[a] | 1.3 ± 0.2 | 57.7 ± 5.3[a] |

[a] Significantly different from Lab Bench result.

amounts of radioactivity retained in the skin was not significant ($P > 0.1$).

In order to examine the trends in DEET absorption versus dose, we chose to combine the data sets in Table 1 after normalizing the penetration rates based on the above correction factor. To do this, we multiplied the amount penetrated during each time interval in the laboratory bench experiments by 4/7, then recalculated cumulative penetration based on these "fume hood-normalized" values. That this is a reasonable procedure is supported by other studies in our laboratory on DEET (Bhatt and Kasting, 2003) and benzyl alcohol (Saiyasombati and Kasting, 2003a) showing that evaporation rates are proportional to airflow over the skin surface. The normalized data (Fig. 5) were then compared in a variety of ways, as detailed below. They were also subjected to quantitative modeling using the diffusion/evaporation model represented by Fig. 2 and Eqs. (1)–(4).

Numerous trials of the fitting program in different modes, including fitting individual doses with three variable parameters and fitting all doses together, were used to arrive at an optimum value of 18 μg/cm$^2$ for $M_{sat}$. This value was fixed for the remaining analysis. Fitting the doses individually with two variable parameters, $h^2/D$ and $k_{evap}$, yielded excellent fits with an overall $r^2$ of 0.998 and a $\chi^2_v$ value of 1.3. Representative fits for low and high doses are shown in Fig. 6. However, this procedure yielded systematic variations with dose in the value of $k_{evap}$ at the highest doses that were hard to reconcile with the model assumption of a constant mass transfer coefficient at the air–skin interface. Consequently, we refit the model to the full data set with the requirement of a single value for $k_{evap}$. This procedure yielded $k_{evap} = 2.6 \times 10^{-5}$ cm/h and the values of $h^2/D$ shown in Fig. 7. The value of $h^2/D$ decreased 1.5-fold over the dose range 30–1500 μg/cm$^2$ and was constant at lower and higher doses. The results of the final fitting procedure are shown in Table 4. The absorption data for the two tracer level DEET experiments (0.021 and 0.057 μg/cm$^2$) were not well described using the model parameters in Table 4. Absorption at these doses was much smaller than that calculated from the model. This phenomenon is discussed below.

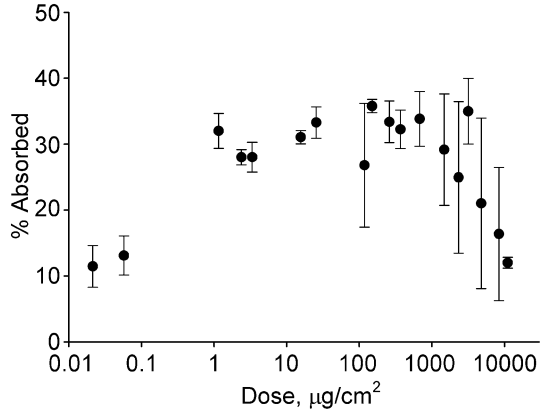


Fig. 5. Dose dependence of DEET absorption after 72 h. The data have been normalized to the fume hood environment as described in the text.

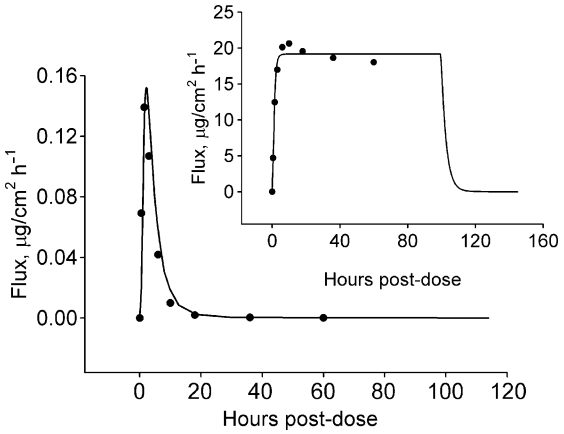


Fig. 6. Fits of Eqs. (1–4) to representative DEET absorption data, normalized to the fume hood environment; dose = 2.3 μg/cm$^2$ (Inset dose = 11,100 μg/cm$^2$).

### *Effect of DEET on benzyl alcohol penetration*

The effect of DEET pretreatment on the skin penetration of radiolabeled benzyl alcohol is shown in Fig. 8. A modest increase in benzyl alcohol penetration with increasing DEET dose was found. The slope of the regression line of relative benzyl alcohol penetration versus log dose, for doses ≥30 μg/cm$^2$, was $m = 0.185 \pm 0.043$. This value was significantly different from zero ($P = 0.005$), supporting the contention that the trend seen in Fig. 8 was statistically significant.

## Discussion

### *DEET absorption rate*

As for other volatile compounds, absorption of DEET through the skin may be expected to depend on dose and airflow over the skin surface, as well as a variety of other factors. The results in Tables 1 and 2 show this to be true for the case of DEET dissolved in ethanol. In these studies,

Table 4
Results of fitting diffusion/evaporation model parameters in Eqs. (1)–(4) to the fume hood-normalized DEET absorption data

| Parameter | Units | Value |
|---|---|---|
| $M_{sat}$ | μg/cm$^2$ | 18 |
| $k_{evap}$ | cm/h | $2.6 \times 10^{-5}$ |
| $h^2/D$ | h | 10–16 (dose dependent—see Fig. 7) |
| $n$ | – | 180 |
| $s$ | Percent of dose | 4.95 |
| $r^2$ | – | 0.96 |
| $X^2_v$ | (Percent of dose)$^2$ | 24.5 |

The two tracer doses (0.021 and 0.057 μg/cm$^2$) were not included in this analysis.

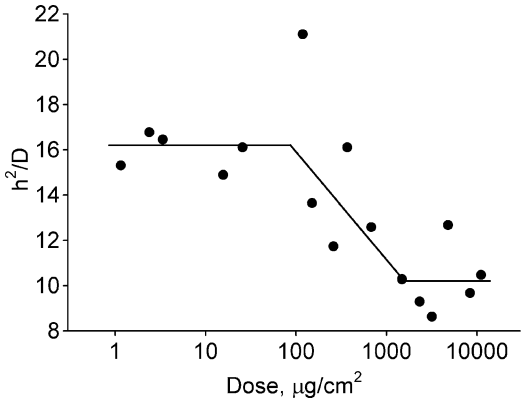


Fig. 7. Variation of time constant $h^2/D$ with DEET dose for the values of $M_{sat}$ and $k_{evap}$ shown in Table 4. The solid line indicates the proposed variation discussed in the text. The two tracer level doses (data not shown) yielded large values of $h^2/D$ that were not consistent with this model.

the DEET solutions were applied to skin in small volumes (6–25 μl/cm$^2$), and the components were free to evaporate following the application. These aspects of the study mimic the most common exposure scenario for an insect repellent; other aspects of this in vitro study do not. In vivo exposures to DEET invariably involve various combinations of ruboff, washoff, and skin sloughing that were not simulated; furthermore, in occupational exposures, the subject is usually outdoors and either in motion or subject to winds of varying speeds. These factors combine to yield in vivo absorption values that are generally lower than those reported in Tables 1 and 2. Thus, Selim et al. (1995) reported that less than 10% of radioactivity from $^{14}$C-DEET was absorbed from human volar forearm sites that were swabbed 8 h post-dose with isopropyl alcohol, then sequentially tape-stripped. In this study, the washes and stripping removed 55–68% of the applied radioactivity before it could be absorbed. Feldmann and Maibach (1970) reported an average absorption after 4 days of only 16.7% of $^{14}$C-DEET applied to human volar forearm. However, the dose site was unprotected and the subjects were allowed to wash the dosed area after 24 h. Other estimates of human in vivo skin penetration of DEET based solely on evaporation measurements (Smith et al., 1963; Spencer et al., 1979) have lead to values comparable to those in the present study.

In vitro studies with DEET also show a large variation in absorption rates, many of which can be traced to the conditions of the test. Airflow is a significant factor, as is evident from Tables 1 and 2. In a separate study (Bhatt and Kasting, 2003), our laboratory has found that DEET absorption through cadaver skin samples mounted in a modified Franz cell (Saiyasombati and Kasting, 2003a) can be varied from 20% to 70% of an applied dose of 127 μg/cm$^2$ in 24 h by modulating the airflow over the cell. Evaporation rate varied inversely with absorption rate and proportionally to the airflow. The results support that DEET evaporation, which was not measured in the present study, can reasonably be described by Eqs. (3) and (3′) with a mass transfer coefficient ($k_{evap}$) proportional to airflow. This is the basis on which the two experiments in Tables 1 and 2 can be combined to produce a "fume hood-normalized" data set for further analysis. We chose to normalize to the fume hood conditions because this choice is more consistent with in vivo DEET absorption measurements and it can be more safely reproduced in the laboratory. The normalized results are consistent with Moody's extensive in vitro studies of DEET absorption through human skin (Moody and Martineau, 1990; Moody and Nadeau, 1993) in which the disposition of small doses of acetone-deposited $^{14}$C-DEET was measured. The range of absorption at 48 h was 24–48%. Reifenrath and colleagues also made extensive in vitro measurements of $^{14}$C-DEET absorption through human skin (Reifenrath and Robinson, 1982) and pig skin (Hawkins and Reifenrath, 1984) under controlled airflow conditions. Although considerably smaller fractions of radiolabel completely permeated the skin in these studies (4.2% at 12 h, Reifenrath and Robinson, 1982; and 14% at 50 h, Hawkins and Reifenrath, 1984), much larger amounts were found in the skin at the end of the studies. These studies employed either full thickness human skin or pig skin dermatomed to 1.9 mm—thicknesses that are now known to lead to low absorption estimates for lipophilic compounds when tested in vitro (Bronaugh and Stewart, 1984). When the amounts in skin in Reifenrath's studies are added to the amounts penetrated, the total percentages (33.4% for human skin, 52% for pig skin) are comparable to the 72 h absorption values in Table 2.

### *DEET skin penetration enhancement*

The results in Figs. 5, 7 and 8 support the contention that DEET enhances not only its own permeation through skin, but also that of other compounds. A number of earlier investigators have reached this same conclusion. Windheuser et al. (1982) found through in vivo experiments that DEET enhanced the transdermal delivery of topically applied hydrocortisone. Hussain and Ritschel (1988) studied the influence of DEET on the ex vivo permeation of

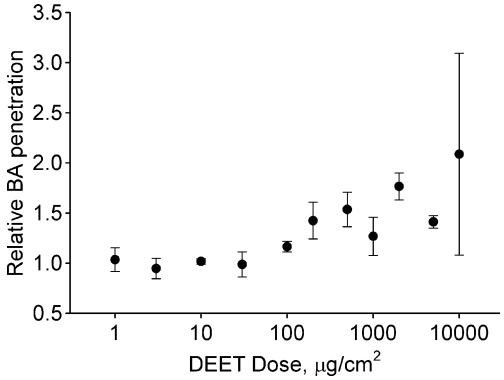


Fig. 8. Skin penetration of $^{14}$C-benzyl alcohol after 16 h pretreatment of skin with unlabeled DEET, normalized as described in the text.

phosphonoformic acid through rat skin. They reported that DEET doubled the amount of phosphonoformic acid found in skin and increased fourfold the amount permeating across skin. Kondo et al. (1988) reported much larger enhancement effects associated with DEET. These workers showed that the addition of a small amount of DEET or Azone to propylene glycol solutions of nifedipine produced a large increase in nifedipine penetration compared to that from propylene glycol alone. They also found that the permeation of nifedipine from the DEET/propylene glycol vehicle was much greater than that from a suspension of the drug in DEET. In light of the large body of work on penetration enhancement by binary solvents (e.g., Aungst, 1989; Cooper, 1984), we suggest that DEET may play the role of the "polar lipid" in binary combinations with short chain diols (Cooper, 1984), but be a relatively mild enhancer in their absence.

Baynes et al. (1997) found that the presence of DEET actually retarded, rather than enhanced, the absorption of two pesticides, permethrin and carbaryl. This seeming discrepancy with the above findings may be understood on the basis of solution thermodynamics. The dose solutions in the Baynes study consisted of the pesticide in a volatile solvent, with or without added DEET. The addition of DEET would be expected to greatly reduce the thermodynamic activity of the remaining components after the more volatile solvent evaporated, thus reducing the driving force for skin permeation. This phenomenon may also have influenced the somewhat mixed results of Moody et al. (1987) with fenitrothion applied from DEET or acetone.

To further analyze this penetration enhancing effect of DEET, we chose to study its effect on the skin penetration of benzyl alcohol. To ensure that the DEET did not affect the thermodynamic activity of the test compound, the skin was pretreated with increasing amounts of unlabeled DEET and then washed prior to dosing the radiolabeled benzyl alcohol. A modest increase in benzyl alcohol penetration with increasing DEET pretreatment dose was found (Fig. 8), in agreement with the earlier cited work suggesting that DEET is a permeation enhancer.

### *Diffusion/evaporation model*

Previous work in our laboratory has led to the development of compartmental kinetic models to describe the disposition of volatile compounds on skin. These models have been found to provide reasonable descriptions of the skin disposition of benzyl alcohol (Saiyasombati and Kasting, 2003a, 2004a), fragrance compound mixtures (Kasting and Saiyasombati, 2001; Saiyasombati and Kasting, 2003b, 2004b), and DEET (Bhatt and Kasting, 2003), at doses less than or equal to about 100 μg/cm$^2$. The model parameters have a plausible relationship with permeant physicochemical properties—vapor pressure, lipid solubility, and molecular weight. However, the well-stirred compartment approach has several significant limitations, chief among them the limitation to low doses of non interacting ingredients. There is no natural way to implement the concept of a solubility limit or to provide a plausible time lag for diffusion across the skin. The latter can be simulated by adding additional compartments to the model (Guy and Hadgraft, 1983; Guy et al., 1982; McCarley and Bunge, 2001), but the unambiguous assignment of values for the rate constants becomes problematical as the complexity increases. In short, the advantages obtained from the simpler mathematics of compartmental models are outweighed by the difficulty associated with assigning values to a multitude of poorly defined parameters.

The present mathematical model stems from recent efforts from our group (Kasting, 2001) and others (Anissimov and Roberts, 2001, 2004) to understand the transient absorption of solvent-deposited compounds through skin. Analytical solutions to some limiting cases of this problem may be derived; in fact, an equivalent heat flow solution to the problem represented by Case 1 is available (Carslaw and Jaeger, 1959). However, these solutions are somewhat difficult to calculate, and they are not easily extended to include solubility limits or multiple component diffusion effects. Therefore, we have chosen a simple numerical framework that can be readily extended to accommodate the above phenomena. Using the principle of Occam's Razor (Ockham, 1320), the level of detail has been kept as simple as possible, consistent with the problem addressed: the skin is treated as a (nearly) homogeneous lipid membrane with a permeable upper region, the solvent is not explicitly considered, and the evaporative cooling associated with its loss is not treated. These are reasonable approximations for a small, lipophilic solute of low-to-moderate volatility applied to skin in a highly volatile solvent. As a one-component diffusion model, the present model does not allow for solvent effects on transport (beyond the initial deposition into the skin) or for thermodynamic interactions between multiple components. Development of these features is a subject of research in our laboratory.

According to the model, the dissipation process for DEET on skin can be described by three parameters—a saturation dose, $M_{sat}$; a mass transfer coefficient for evaporation, $k_{evap}$; and a time constant $h^2/D$. We found that the choice of a small value (18 μg/cm$^2$) for $M_{sat}$ lead to the best description of the absorption profiles over a range of doses. This value is comparable to the estimated lipid content of the upper 2 μm of the SC (~20 μg/cm$^2$), assuming the lipid content to be about 10% w/w. Thus, it is reasonable to consider $M_{sat}$ to be the dose required to saturate the surface-accessible SC lipids following topical application.

The optimum value of $k_{evap}$ was found to be $2.6 \times 10^{-5}$ cm/h when the data were normalized to the fume hood conditions. A comparable analysis (not shown) was conducted with data normalized to the lab bench conditions, resulting in a $k_{evap}$ value of $1.2 \times 10^{-5}$ cm/h for this

scenario. This difference reflects the direct relationship of $k_{evap}$ to airflow over the skin as has been seen in separate studies (Bhatt and Kasting, 2003; Saiyasombati and Kasting, 2003a). It is also anticipated that $k_{evap}$ is proportional to vapor pressure (Kasting and Saiyasombati, 2001); however, a variety of volatile permeants must be studied in order to establish this relationship. It is fair to say that the present model oversimplifies the evaporation process, as seen by systematic variations in calculated $k_{evap}$ values with dose when the data at each dose were analyzed separately. Having no compelling physical argument for explaining this variation, we have chosen to report only the optimum value of this parameter when averaged over all doses.

The time "constant" $h^2/D$ for DEET absorption, however, must be allowed to vary as shown in Fig. 7 in order to reasonably describe the absorption data in Table 1. Assuming no significant swelling of the SC by DEET, one is lead to the conclusion that DEET diffusivity in skin ($D$) increased with dose. In simpler terms, DEET enhanced its own permeation rate. This conclusion was supported by its effect on benzyl alcohol penetration (Fig. 8) and by the findings of other investigators (Hussain and Ritschel, 1988; Windheuser et al., 1982). It may be inferred from Fig. 7 that the magnitude of the increase in $D$ was about 1.5-fold and that it occurred in the range 30–1500 μg/cm$^2$. Absorption of tracer doses (<0.1 μg/cm$^2$) was lower than the model predictions using average parameter values, possibly reflecting a change in the system. Since the percentage of radioactivity retained in skin was only slightly increased at these doses (Table 2), ruling out tissue binding, we hypothesize that some $^{14}$C-DEET may have been lost through volatilization with the solvent. This finding reinforces a common observation that it is wise to conduct radioactivity experiments in the presence of realistic chemical concentrations in order to avoid artifacts.

There is an inherent inconsistency in analyzing DEET transport in skin using a model (Eqs. (1)–(4)) that assumes a constant diffusion coefficient to analyze experiments that support the contention that $D$ increases with dose. To the extent that the increase in $D$ is rapidly reversible as DEET levels subside, one would expect that a model in which $D = D(C)$ with $C$ being the local concentration in the SC could provide an improved interpretation of the results. It is noteworthy that the increase in $D$ (or decrease in $h^2/D$; Fig. 7) begins at a dose that is closely related to the saturation dose $M_{sat}$ identified by the curve fitting procedure (Table 4). If the deposition layer concept is valid, the corresponding SC concentration $C_{sat} = M_{sat} \div h/10$ should represent an upper limit to the concentration range over which $D(C)$ may vary. Alternatively, there may be a skin hydration component to the increase in $D$ brought about by partial occlusion of the skin by large doses of DEET. These alternatives are amenable to further testing.

The present model is relatively simple and may be considered a work in progress. In addition to the constant diffusivity approximation, we have neglected complications such as the persistence of a solvent layer, combined heat and mass transfer effects, and the heterogeneous nature of the skin. Accounting for these effects will possibly provide a better description of the problem, and is a difficult but promising direction for future study.

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