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Percutaneous permeation of the *meta*, *ortho* and *para* isomers of *N*,*N*-diethyltoluamide

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Abstract

Ortho- and *para*-DEET occur as minor by-products in the synthesis of *N*,*N*-diethyl-*m*-toluamide (*meta*-DEET). *Meta*-DEET, however, is formulated into many commercial mosquito repellents without further purification. Therefore, commercial mosquito repellents may have small amounts of the *ortho* and *para* isomers of *meta*-DEET as impurities. A review of toxicological studies performed with *N*,*N*-diethyltoluamide show that the *ortho* isomer is twice as toxic as both the *meta* and *para* isomers when comparing the oral lethal dose 50% (LD_{50}) values. Reports of individuals exhibiting adverse reactions after topical use of DEET-containing products warrant further investigation of the dermal permeation of *meta*-, *ortho*- and *para*-DEET to determine the extent of permeation of each isomer and determine if the *ortho* isomer may be responsible for the observed toxicity. Therefore, the objective of the present study was to evaluate the permeation of *meta*-, *ortho*- and *para*-DEET across human skin from an aqueous vehicle, solutions in 45 and 90% ethanol, and as the neat isomer. Studies were conducted using the infinite dose technique with a Franz diffusion cell, and parameters such as steady-state flux (J_{ss}), permeability (P), and skin/vehicle partition coefficient (K), were obtained from the permeation profiles. No real significant difference ($p > 0.05$) in the flux was observed between the neat isomers, or the isomers when applied as an aqueous solution, and when applied as a solution in 90% ethanol. At a level of 45% ethanol, however, the J_{ss}, P and K values for the *ortho* and *para* isomers were significantly higher ($p < 0.05$) than the J_{ss}, P and K for *meta*-DEET. Although the flux values were significantly higher, it appears that the toxic effects seen after topical use of DEET-containing products cannot be directly attributed to the presence of the *ortho* isomer in small quantities in the commercial repellents, and further purification of *meta*-DEET prior to formulation is not suggested. © 1998 Elsevier Science B.V.

Keywords: Ethanol; In vitro percutaneous permeation; Isomers; *N*,*N*-Diethyltoluamide; Skin

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1. Introduction

N,*N*-diethyltoluamide was found to be quite an effective repellent for many insects with the *meta* isomer being the most effective (Gilbert, 1966). In the synthesis of *m*-DEET, both the *ortho* and *para* isomers of DEET occur as by-products of the reaction, and the isomers may occur either alone or as a mixture (Smith, 1958). Ambrose et al. (1959) and Ambrose and Yost (1965) performed toxicological studies on the three DEET isomers to evaluate the potential health hazards that might result from exposure, accidental or otherwise, to the compounds. The studies reported physiological effects following various routes of administration, oral lethal dose 50% (LD_{50}) values, and changes due to dermal application. The oral LD_{50} values showed that the *ortho* isomer (1.21 ± 0.042 g/kg) was almost twice as toxic as both the *meta* isomer (2 g/kg) and the *para* isomer (2.3 ± 0.19 g/kg), but the study suggested that, overall, the isomers probably do not pose a serious health problem (Ambrose et al., 1959; Ambrose and Yost, 1965). However, the absorption of the three isomers of DEET through skin and the resultant toxicity has not been evaluated.

An 1980 EPA Registration Standard Documentation stated, however, that there had never been an adequate animal study for oncogenicity or an adequate study on chronic toxicity as a result of dermal application of DEET (US Environmental Protection Agency, 1980). There have also been several reported cases of individuals exhibiting adverse reactions and even death following the use of DEET-containing products (Gryboski et al., 1961; Heick et al., 1980; Miller, 1982; deGarbino and Laborde, 1983; Roland et al., 1985; Edwards and Johnson, 1987; Lipscomb et al., 1992; Veltri et al., 1994). Robbins and Cherniack (1986) reviewed the biodistribution and toxicity of DEET and felt that there were several areas of toxicity that needed more complete investigation. Abou-Donia et al. (1996) attributed some of the neurotoxicity of Gulf War Syndrome to coexposure to DEET, pyridostigmine, and permethrin. In light of the incomplete toxicological assessment and the recent reports of reactions after human exposure to DEET, the dermal permeation of each of the isomers needs to be further evaluated to determine the extent of permeation of each isomer and the potential for toxicity due to each isomer.

The objective of the present study was to evaluate and compare the permeation of the *meta*, *ortho*, and *para* isomers of DEET across human skin. Since in a previous study, ethanol, the vehicle commonly used in mosquito repellent formulations of DEET was shown to enhance the permeation of *meta*-DEET at 30–45% ethanol, the effect of ethanol on the permeation of three isomers also needed to be evaluated (Stinecipher and Shah, 1997). In order to achieve these objectives, the *ortho* and *para* isomers, which are not commercially available, were synthesized from the appropriate toluic acid and were characterized by nuclear magnetic resonance (NMR) and gas chromatography/mass spectrometry (GC–MS). In order to investigate the permeation of the individual isomers, studies were conducted across full thickness human skin using an infinite dose technique with a Franz diffusion cell. The isomers were studied for permeation in the following manner: when applied as the neat isomer, when applied as a solution in an aqueous vehicle, and when applied as solutions in ethanol. The permeation profiles obtained in the in vitro percutaneous permeation experiments were used to calculate permeation parameters such as steady-state flux, permeability, and skin/vehicle partition coefficient, and the values for each isomer were compared. These studies show whether the *ortho* isomer of DEET permeates significantly more than *meta*-DEET and may, therefore, be responsible for the toxic side effects, and if *meta*-DEET should be further purified prior to its formulation into commercial mosquito repellents.

2. Materials and methods

2.1. Materials

m-DEET (97%) and *p*-toluic acid were purchased from Aldrich Chemical Company (Milwaukee, WI). *o*-Toluic acid was obtained from Stauffer Chemical Company (New York, NY).

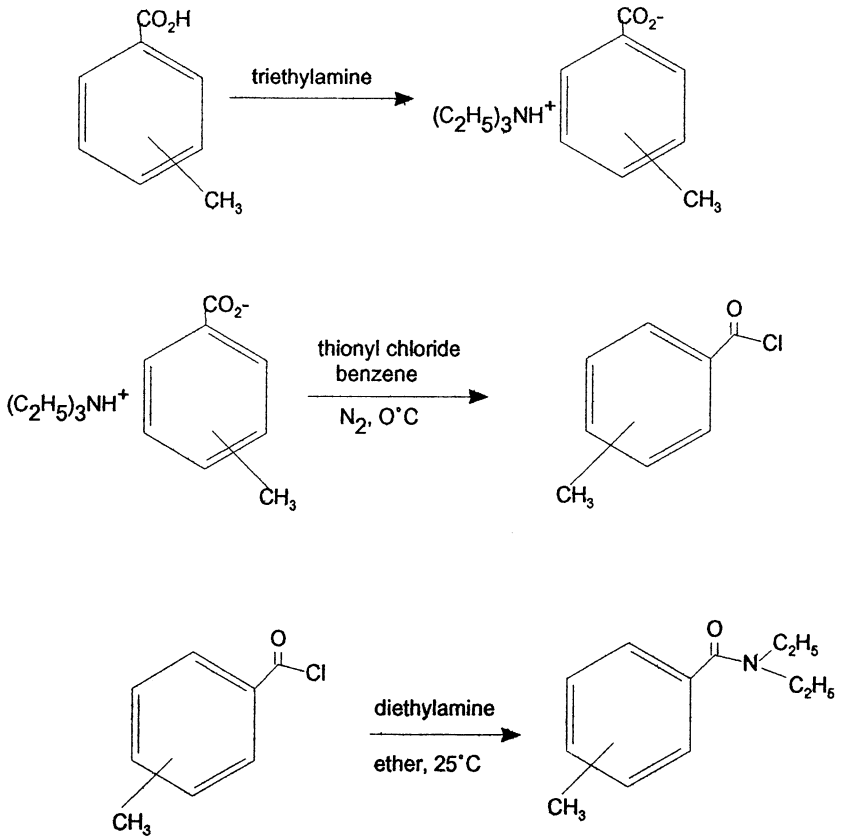

Fig. 1. Reaction scheme for synthesis of *o*- and *p*-DEET.

Thionyl chloride, benzene, triethylamine, diethylamine, ethyl ether, methanol (high-performance liquid chromatography (HPLC) Grade), ethyl alcohol, and octanol were purchased from Fisher (Fair Lawn, NJ). Phosphate buffer solution (0.1 M, pH 7.4) was prepared with sodium phosphate monobasic monohydrate (Mallinckrodt, St. Louis, MO) and sodium phosphate dibasic anhydrous (Curtin Matheson Scientific, Houston, TX).

2.2. Synthesis of *o*- and *p*-DEET

In the laboratory, *o*- and *p*-DEET were synthesized from *o*- and *p*-toluic acid, respectively, according to the reaction scheme presented in Fig. 1. The isomers were purified and subsequently characterized by ^{1}H-NMR, GC/MS, and UV spectra. ^{1}H-NMR spectra were run on a Varian Gemini 300 instrument at 300 MHz. Spectra were referenced to the solvent in which the isomers were dissolved (7.24 ppm for $CDCl_3$). Mass spectra were performed on a Finnigan Mat Model 5100 GC/MS–EI system. UV spectra were obtained on a Hewlett Packard Model 8452A diode array spectrophotometer.

2.3. Solubility and PC determination

The solubility in water was determined by placing excess (30 mg) of each isomer in a vial containing 1.5 ml water. The contents of the vials were agitated at 100 oscillations/min for 24 h. The solutions were then filtered with a 0.22 μm Millex®–GV syringe lure-lock filter (Millipore) and analyzed for DEET content by HPLC. The octanol/water partition coefficient was determined in a similar manner. Each isomer (15 mg) was placed in a vial containing 1.5 ml each of octanol

and water. The vial was then shaken at 100 oscillations/min for 24 h. A sample was taken from each layer and analyzed for DEET by the HPLC assay described below.

2.4. Permeation experiments

2.4.1. Skin preparation

Human skin samples were obtained from elective plastic surgery patients. The fat and other visceral debris were removed from the underside of the freshly excised skin. The skin was then washed with 0.1 M, pH 7.4 phosphate buffer solution before freezing at -20°C. The full thickness skin samples were cut into 2 cm^2 pieces and allowed to thaw overnight at room temperature in phosphate buffer solution prior to the in vitro percutaneous permeation experiments.

2.4.2. In vitro percutaneous permeation experiments

The permeation studies were conducted with vertical Franz diffusion cells (Crown Glass Company, Somerville, NJ) through full thickness human skin. The receptor compartment was filled with 0.1 M, pH 7.4 phosphate buffer solution and was constantly stirred to ensure uniform distribution of DEET and maintain sink conditions. The temperature of the entire diffusion cell assembly was maintained at 37°C using a recirculating water jacket. Complete details of the setup are described in a previous publication (Stinecipher and Shah, 1997).

The permeation studies with the isomers were conducted using the infinite dose technique in which a large excess of the permeant is applied to the skin in comparison to the amount permeating so that an approximately constant concentration of the permeant is maintained in the donor compartment during the entire course of the experiment. The permeation of the neat isomers and the isomers from the solutions in water and ethanol across human skin was studied to investigate the effects of water and ethanol on the permeation of each isomer. Permeation studies were conducted for 36 h since in earlier experiments run for 72 h, steady state was achieved before 36 h. The effect of ethanol was studied at 45 and 90%, since maximum enhancement in permeation of *meta*-DEET was observed at 45% ethanol, and a reduction in permeation at 90% ethanol (Stinecipher and Shah, 1997). Each isomer was applied to the skin in the donor compartment as either the neat isomer (78.13 mg/cm^2), a 5 mg/ml solution in water (7.81 mg/cm^2), 10% isomer in 90% ethyl alcohol (46.88 mg/cm^2), or 10% isomer in 45% ethyl alcohol (46.88 mg/cm^2) in order to maximize the thermodynamic activity of the isomers in each vehicle. For application of the neat isomer, 50 mg of each in the pure form was applied to the skin in the donor compartment. *m*-DEET, which is a liquid, was applied as a 50 μl aliquot. Both the *o*-DEET and *p*-DEET, which are solids at room temperature, were melted prior to application. The molten isomers were then pipetted (50 μl) onto the surface of the skin where they resolidified as they cooled to room temperature, coating the skin surface. Aliquots of 1 ml and 300 μl of the isomers in the aqueous solution and in the ethanolic solutions, respectively, were applied to the surface of the skin. In each case, the donor compartment was covered with a glass slip to prevent evaporation.

Aliquots of 300 μl of the receptor fluid were withdrawn and replaced periodically with fresh phosphate buffer for 36 h. All samples were stored at 4°C prior to analysis for DEET by HPLC.

2.5. HPLC analysis

The HPLC analytical method for *m*-DEET (Stinecipher and Shah, 1997) was found to be also suitable for the analysis of *o*- and *p*-DEET. System and method precision validation for analysis of each isomer was performed. The DEET isomers were analyzed using reverse-phase HPLC on an Alltech C8 column (5 μm, 25 cm $\times$ 4.6 mm) and eluted with the mobile phase at a flow rate of 0.7 ml/min. The mobile phase consisted of methanol:water (80:20) and DEET was detected using UV absorbance at 240 nm. The minimum detectable level (MDL) for the isomers of DEET was 24 ng/injection. The HPLC system consisted of a Waters Model 712 WISP autosampler equipped with a Waters Model 481 variable wave-

length detector, and a Shimadzu C-R3A Chromatopac.

2.6. Data analysis

The cumulative amount in $\mu g/cm^2$ (A) of DEET permeating into the receptor compartment was plotted against time (t) to obtain the permeation profile. The steady-state flux (J_{ss}) was estimated from the slope of the linear portion of the permeation profile as described by the following equation:

$$A = J_{ss}(t - t_L) \quad (1)$$

The lag time (t_L) was estimated from the x-intercept of the linear portion of the profile, and was used to calculate the apparent diffusion coefficient (D) of DEET using the following equation:

$$D = \frac{h^2}{6t_L} \quad (2)$$

where h is the thickness of stratum corneum, the rate-limiting barrier, and assumed to be 0.001 cm (Scheuplein, 1978). From the values of J_{ss}, D, h and C_d, the concentration in the donor compartment, the permeability (P) and skin/vehicle partition coefficient (K) of DEET were calculated using the following equations:

$$P = \frac{J_{ss}}{C_d} \quad (3)$$

$$K = \frac{Ph}{D} \quad (4)$$

The estimated parameters are presented as mean $\pm$ standard deviation (S.D.) and were evaluated for differences using an analysis of variance (ANOVA) test ($p < 0.05$).

3. Results

Average permeation profiles for the neat isomers across human skin at 37°C are shown in Fig. 2. Each curve represents the average of three experiments. Each isomer of DEET was found to continuously permeate for the full 36 h. The typical J-shape for the permeation profiles is seen in each case up to 10 h. However, the profiles for both the *meta* and *para* isomers start to plateau after 10 h. The cumulative amount of isomer permeated at 36 h follows the order of *ortho* > *meta* > *para*, but there is no statistically significant difference in permeation between the three isomers.

The physicochemical properties, aqueous solubility and octanol/water partition coefficient, as well as the steady-state flux (J_{ss}), lag time (t_L), and % permeated at 36 h of the neat isomers are compared in Table 1. The *para* isomer of DEET had a much lower aqueous solubility than both the *meta*- and *ortho*-DEET. Therefore, as expected, the partition coefficient of *para*-DEET (71.18 ± 11.60), was higher as compared to the partition coefficient of *meta*-DEET (27.49 ± 10.13) and *ortho*-DEET (30.71 ± 17.26), indicating the slightly more lipophilic character of *para*-DEET. The flux values, lag time values, and % permeated at 36 h obtained were similar for all three isomers, in spite of the slight differences in the solubility and partition coefficients.

Table 2 presents the estimated permeation parameters of DEET obtained from the perme-

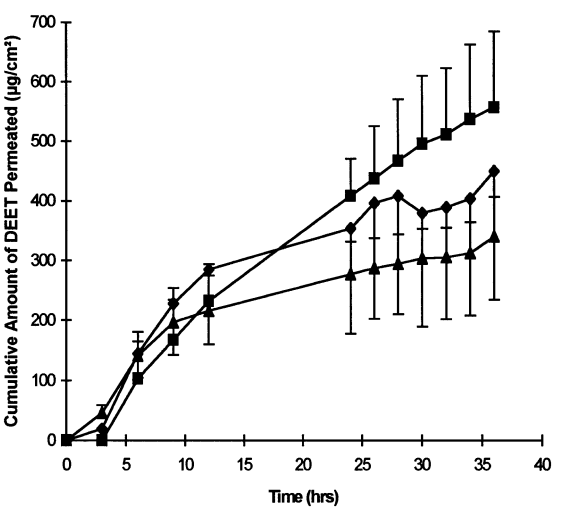

Fig. 2. Average permeation profiles ($n = 3$) for neat *meta*- (◆), *ortho*- (■), and *para*- (▲) DEET across human skin at 37°C. The cumulative amount of DEET permeated is plotted as a function of time. In each case, 78.13 mg/cm^2 of the neat isomer were applied to the surface of the skin in the donor compartment.

Table 1
Comparison of physicochemical properties and permeation parameters of neat DEET isomers (78.13 mg/cm^2) across human skin at 37°C

	meta	*ortho*	*para*
Aqueous solubility (mg/ml)	10.73 ± 0.51[a]	16.04 ± 2.48[b]	4.86 ± 0.35[b]
PC (O/W)	27.49 ± 10.13[c]	30.71 ± 17.26[b]	71.18 ± 11.60[b]
J_{ss} (μg/cm$^2\cdot$h)[c,d]	25.26 ± 8.31	21.68 ± 9.53	25.08 ± 8.24
t_L (h)[c,d]	1.57 ± 0.86	1.33 ± 1.05	0.79 ± 0.63
% Permeated at 36 h[c,d]	0.57 ± 0.28	0.71 ± 0.16	0.43 ± 0.085

[a] $n = 5$.
[b] $n = 2$.
[c] $n = 3$.
[d] No significant difference in J_{ss}, t_L, and % permeated at 36 h between the isomers ($p > 0.05$).

ation profiles of the isomers when applied as an aqueous solution. Flux values range from 15.46 ± 1.25 μg/cm$^2\cdot$h for *ortho*-DEET to 34.99 ± 4.25 μg/cm$^2\cdot$h for *para*-DEET. The J_{ss}, P, and % permeated at 36 h for the *ortho*-DEET were significantly lower ($p < 0.05$) than the corresponding values obtained for *meta*- and *para*-DEET. The flux values, however, were all similar to the values obtained for the pure isomers (Table 1). The skin/vehicle partition coefficients were all significantly different ($p < 0.05$) and may be ranked as *meta* < *ortho* < *para*. The % permeated at 36 h was significantly higher ($p < 0.05$) for the three isomers from the aqueous solution when compared to the neat isomers (Table 1), as would be expected due to the increased hydration level of the skin.

The permeation parameters, J_{ss}, P, t_L, K, and % permeated at 36 h for the isomers from 90% ethanol are shown in Table 3. There is no significant difference in the J_{ss}, P, t_L, and % permeated at 36 h between the *meta* and *ortho* isomers. The isomers have a much longer lag time from the ethanol vehicle compared to the lag times of the neat isomers or the isomers in water. The isomers exhibit a 10-fold decrease in permeability from the 90% ethanol solution when compared to the aqueous solution (Table 2). The skin/vehicle partition coefficient for *ortho*-DEET is significantly higher than the K-values for both the *meta* and *para* isomers ($p < 0.05$).

Table 2
Permeation parameters of DEET isomers (7.81 mg/cm^2) from an aqueous solution (5 mg/ml) ACROSS human skin at 37°C ($n = 3$)

	meta	*ortho*	*para*
J_{ss} (μg/cm$^2\cdot$h)[a]	29.82 ± 4.12	15.46 ± 1.25	34.99 ± 4.25
t_L (h)[c]	0.90 ± 0.43	7.29 ± 0.29	5.45 ± 1.31
$P \times 10^3$ (cm/h)[a]	5.96 ± 0.82	3.09 ± 0.25	7.00 ± 0.85
K[b]	32.50 ± 16.74	135.00 $\pm$ 16.14	228.00 $\pm$ 58.81
% Permeated at 36 h[a]	13.27 ± 1.86	5.64 ± 0.40	13.73 ± 1.95

[a] J_{ss}, P, and % permeated at 36 h for the *ortho* isomer are significantly lower ($p < 0.05$) than J_{ss}, P, and % permeated at 36 h for the *meta* and *para* isomers.
[b] K for *meta* < K for *ortho* < K for *para* ($p < 0.05$).
[c] The value of t_L for the *meta* isomer is significantly lower ($p < 0.05$) than for the *ortho* or *para* isomer.

Table 3
Permeation parameters of DEET isomers (46.88 mg/cm^2) from a 90% ethanol solution across human skin at 37°C, where $n = 3$ for *m*- and *o*-DEET and $n = 2$ for *p*-DEET

	meta	*ortho*	*para*
J_{ss} (μg/cm$^2\cdot$h)[a]	28.21 ± 5.71	85.73 ± 49.95	19.87
t_L (h)[a]	17.44 ± 5.87	22.67 ± 3.77	19.38
$P \times 10^4$ (cm/h)[a]	2.82 ± 0.57	8.58 ± 5.01	1.99
K[b]	30.90 ± 14.73	112.00 ± 51.71	23.1
% Permeated at 36 h[a]	1.33 ± 0.46	2.30 ± 1.57	0.81

[a] No significant difference in J_{ss}, P, t_L, and % permeated at 36 h between the *meta* and *ortho* isomers ($p > 0.05$).
[b] The value of K for the *ortho* isomer is significantly greater ($p < 0.05$) than for the *meta* isomer.

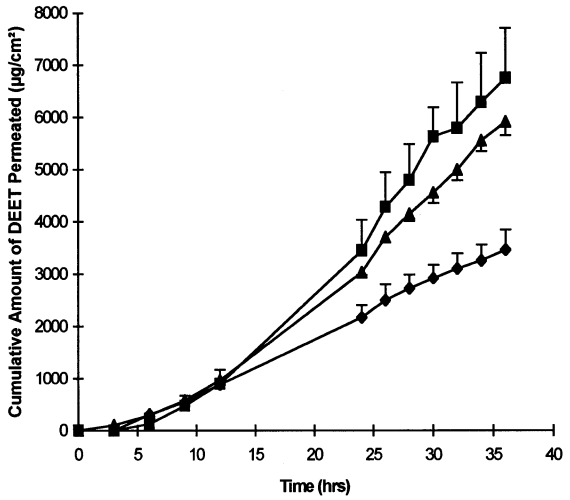

Fig. 3. Average permeation profiles ($n = 3$) for *meta*- (◆), *ortho*- (■), and *para*- (▲) DEET from a 45% ethanolic solution across human skin at 37°C. The cumulative amount of DEET permeated is plotted as a function of time. In each case, 46.88 mg/cm^2 of the isomer were applied to the surface of the skin as an ethanolic solution in the donor compartment.

The permeation of the DEET isomers was also studied from a 45% ethanol solution, and the permeation profiles are compared in Fig. 3. Previous results (Stinecipher and Shah, 1997) showed maximum enhancement of the permeation of *m*-DEET from a 45% ethanolic solution. Therefore, the results from this study also should show the highest cumulative amount of DEET isomers permeating across human skin. The permeation profiles of DEET isomers from the 45% ethanolic solution are averages of three experiments and indeed show significantly higher amounts of each isomer permeated at the end of 36 h as compared to the neat isomers (Figs. 2 and 3). The cumulative amount of the isomers permeated may be ranked as follows: *ortho* (6.9 mg/cm^2) > *para* (5.9 mg/cm^2) > *meta* (3.5 mg/cm^2). These values are much higher than those obtained when the isomers were applied as neat isomers (Fig. 2), as a solution in water, or as a solution in 90% ethanol. A much shorter lag time is also exhibited by the DEET isomers in the 45% ethanol compared to that from the 90% ethanol.

The permeation parameters obtained from the profiles for the isomers in 45% ethanol are listed in Table 4. Steady-state flux values range from 107.31 ± 13.09 μg/cm$^2\cdot$h for *meta*-DEET to 266.10 ± 45.02 μg/cm$^2\cdot$h for *ortho*-DEET, which are significantly higher ($p < 0.05$) than J_{ss} for the isomers when applied as the neat isomer, as a solution in water, and as a solution in 90% ethanol. The J_{ss}, P, t_L, K, and % permeated at 36 h for *meta*-DEET are significantly lower ($p < 0.05$) than those values for the *ortho* and *para* isomers. This difference in J_{ss} for the isomers suggests that not only does 45% ethanol enhance the permeation of each isomer but the magnitude of enhancement is different for each isomer. The permeation of the *para* isomer is enhanced to a significantly greater extent than that of the *ortho* or *meta* isomer.

4. Discussion

Commercial mosquito repellents contain *meta*-DEET, the isomer found to have the higher insect repellency, in concentrations ranging from 5 to 95% (Gilbert, 1966; Robbins and Cherniack, 1986). Trace amounts of the *ortho* and *para* isomers, which are the impurities in the synthesis of *meta*-DEET, are also present in the commercial formulations (Smith, 1958). The results of the present study show that the *meta*, *ortho*, and *para* isomers of DEET do permeate through human

Table 4
Permeation parameters of DEET isomers (46.88 mg/cm^2) from a 45% ethanol solution across human skin at 37°C ($n = 3$)

	meta	*ortho*	*para*
J_{ss} (μg/cm$^2\cdot$h)[a]	107.31 ± 13.09	266.10 ± 45.02	231.85 ± 9.35
t_L (h)[a]	3.31 ± 1.95	10.22 ± 3.48	10.35 ± 0.28
$P \times 10^3$ (cm/h)[a]	1.08 ± 0.13	2.66 ± 0.45	2.32 ± 0.10
K [a]	21.90 ± 13.57	168.00 ± 75.22	144.00 ± 7.09
% Permeated at 36 h	7.37 ± 0.83	14.27 ± 2.02	12.50 ± 0.56

[a] J_{ss}, P, t_L, K, and % permeated at 36 h for the *meta* isomer are significantly lower ($p < 0.05$) than for the *ortho* and *para* isomers.

skin. This is not surprising since DEET is a small, low-molecular-weight, lipophilic compound and, thus, is an ideal permeant of skin (Moody et al., 1987). Several investigators have previously shown that *m*-DEET is readily absorbed through the skin in both animal and human models following dermal application (Feldmann and Maibach, 1970; Snodgrass et al., 1982; Selim et al., 1995; Schoenig et al., 1996). It follows that the *ortho* and *para* isomers might permeate the skin as well, since they are only positional isomers on the benzene ring. Therefore, the molecule does not change radically in structure or in size.

Isomers, however, can be very different in both their physical and chemical properties although they have the same molecular formula. In fact, the *ortho* and *para* isomers are solids at room temperature, while the *meta* isomer is a liquid. The permeation profiles of the three neat isomers, however, show little difference in the permeation characteristics over a 36 h time period. The profiles are all typically shaped with fairly short lag times. Both the *meta*- and *para*-DEET profiles begin to plateau off at 10 h, while the *ortho*-DEET profile continues steadily upward. Because of this plateau, data only up to 12 h were used to calculate flux and lag times. The plateau suggests that the concentrations of the isomer in the receptor compartment may have exceeded their solubility. However, the concentrations in the receptor were always lower than their experimentally determined solubilities (Table 1, Fig. 2), and the amount of isomer in the receptor does continue to increase with time (Anderson, 1993). Although the plateau suggests donor depletion, donor depletion was not observed. However, the saturation of the stratum corneum with the isomers may have produced a donor depletion-like effect, resulting in a plateau in the permeation profile. The *ortho* isomer does permeate to a greater extent (0.57 μg/cm^2) than the *meta* and *para* isomers at 36 h; however, there is not a significant difference ($p > 0.05$) in the amount permeated between the three isomers.

The lipid bilayer of the stratum corneum has been equated with octanol in terms of lipophilicity, and a general guideline for permeation of compounds has been developed which suggests that the higher the O/W PC, the greater will be the permeation (Scheuplein, 1978). The partitioning into the skin alone, however, is not the only controlling factor for the permeation of a compound across a membrane (Scheuplein, 1978). Not only must a compound partition from the donor phase into the skin, but it must also partition from the skin into the receptor phase, which may be controlled by a compound's solubility in the receptor compartment (Guy and Hadgraft, 1989). This phenomenon is well illustrated by *para*-DEET which has a similar steady-state flux value compared to both *meta*- and *ortho*-DEET although it has a much higher O/W PC ($p < 0.05$).

The permeation of each DEET isomer across human skin from an aqueous solution was also tested, and the profiles of each isomer were typical with the *meta* and *para* isomer being almost identical. The cumulative amount of each isomer permeated at 36 h was higher than that found from the neat isomers. This is not surprising, since water hydrates the skin and, therefore, decreases its overall barrier function (Walters, 1989). Water has been shown previously to enhance the permeation of several compounds including corticosteroids, phenols, and salicylate esters (Wurster and Kramer, 1961; McKenzie and Stoughton, 1962; Roberts, 1991). The steady-state flux (J_{ss}) and permeability (P) of *ortho*-DEET in water is significantly lower ($p < 0.05$) than the J_{ss} and P of *meta*- and *para*-DEET in water. This may be attributed to *ortho*-DEET's higher aqueous solubility (Table 1). In other words, *o*-DEET has a greater affinity for the aqueous vehicle as opposed to the lipid-rich skin and will tend to stay within the vehicle to a greater extent than *m*- and *p*-DEET, thereby resulting in an overall lower flux and permeability (Scheuplein, 1978). The flux values are similar to those of the neat isomers and the skin/vehicle partition coefficients follow a similar pattern to the O/W PC values of the neat isomers (Table 1). Water appears to act as a natural permeation enhancer of the three isomers by increasing the hydration level of the skin. Differences in the permeation parameters between the isomers from the aqueous solution as compared to the neat isomers show the effect of vehicle on enhancing permeation by altering the

skin and influencing the partitioning of the isomer from the vehicle into the skin.

Ethanol, a commonly used vehicle in commercial mosquito repellents, was evaluated for its influence on the permeation of the three isomers. Ethanol is known for its ability to enhance the permeation of many different compounds such as estradiol, nitroglycerin and salicylates (Berner et al., 1989; Kurihara-Bergstrom et al., 1990; Liu et al., 1991). Previous results have shown an increased permeation of compounds at certain percentages of ethanol across human skin (Berner et al., 1989; Kurihara-Bergstrom et al., 1990; Mahjour et al., 1993; Wada et al., 1993), while showing a decrease in the permeation at higher concentrations of ethanol due to the extraction of skin lipids (Barry, 1983). A similar pattern of permeation enhancement was observed for *m*-DEET from ethanolic solutions as well (Stinecipher and Shah, 1997). In fact, the permeability (P) values for the isomers (Table 3) are 10-fold lower in 90% ethanol than the P-values in 45% ethanol (Table 4). The permeation profiles of the three isomers in a 90% ethanol solution were typical, with relatively long lag times as compared to the lag times of the neat isomers. The long lag times suggest a lower diffusion coefficient (D) of *o*-, *m*- and *p*-DEET from 90% ethanol; however, there is no significant difference in D between the neat isomers and the isomers in 90% ethanol. The skin/vehicle partition coefficient (K) for *ortho*-DEET is significantly higher ($p < 0.05$) than K for both *meta*- and *para*- DEET from 90% ethanol, and thus explains why the steady-state flux value for *ortho*-DEET is higher. This high affinity of *ortho* isomer may have resulted in an increase in steady-state flux; however, the value of K is not increased to such an extent that the *ortho* isomer may have difficulty in partitioning into the receptor phase. Overall, the partition coefficient values of the isomers in 90% ethanol are lower than those values found for the isomers in an aqueous vehicle. This decrease in partitioning into the skin may occur due to the high concentration (90%) of ethanol in the donor compartment. At high concentrations of ethanol, extensive extraction of the lipids and a decrease in the barrier function of the skin have been observed (Barry, 1983; Kurihara-Bergstrom et al., 1990). The addition of extracted lipids to the ethanol may enhance the affinity of the DEET isomers for the vehicle, as evidenced by the decrease in the partitioning (K); however, there is no significant difference in flux, permeability, and % permeated at 36 h between the isomers at the 90% ethanol level.

Previous results have shown an optimum enhancement in flux of *m*-DEET in the range 30–45% ethanol (Stinecipher and Shah, 1997). Therefore, the flux for the DEET isomers was evaluated from a 45% ethanol solution to depict the maximum flux that may occur or 'the worst case scenario' that might occur. The permeation profiles show that significant amounts of each isomer were found to permeate at 36 h; *ortho* (6.9 mg/cm^2) > *para* (5.9 mg/cm^2) > *meta* (3.5 mg/cm^2). The flux values were also quite high, ranging from 107.31 ± 13.09 $\mu g/cm^2 \cdot h$ for *m*-DEET to 266.10 ± 45.02 $\mu g/cm^2 \cdot h$ for *o*-DEET. It appears that at the lower concentration of ethanol (45%), the lipids may be partially extracted from the skin enough to alter the polar pathway, thereby resulting in an increase in permeation (Kurihara-Bergstrom et al., 1990). This increase in permeation may be also attributed to the partitioning of ethanol into the skin, and thereby the affinity of the isomers for the skin is increased as well. Evidence for this theory is shown by an increase in the skin/vehicle partition coefficient of the DEET isomers (Table 4) which in turn explains the increased flux and permeability. The steady-state flux (J_{ss}), permeability (P), and skin/vehicle partition coefficient (K) for *meta*-DEET are significantly lower than for both *ortho*- and *para*-DEET. This difference between isomers was not seen previously when the isomers were applied neat, as a solution in water, or a solution in 90% ethanol. Therefore, ethanol at the 45% level appears to have a differential effect on the permeation enhancement of the isomers. Thus, the slight differences in physicochemical properties of the isomers fail to show significant differences in permeation when applied neat. However, when applied in 45% ethanol, which has the maximum permeation-enhancing effect, significant differences in permeation properties of each isomer emerge due to differences in their physicochemical

properties. Although the flux for the *ortho* and *para* isomers is significantly higher (2–3-fold) than that for *meta*-DEET, since the *ortho* and *para* isomers are present in the commercial mosquito repellent formulations in relatively small quantities (5% of *m*-DEET), they may not produce high plasma levels which may elicit toxic side effects. Hence, the results of this study do not suggest that *meta*-DEET should be further purified to remove the isomers prior to its formulation. These results also provide additional information on the dermal permeation of *N*,*N*-diethyltoluamide in an effort to complete the toxicological assessment of the repellent.

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