Journal of Toxicology and Environmental Health, Part A, 66:133–151, 2003

1528-7394/03 $12.00 + .00
DOI: 10.1080/15287390390155796

Taylor&Francis
healthsciences

PERCUTANEOUS ABSORPTION OF TOPICAL *N,N*-DIETHYL-*m*-TOLUAMIDE (DEET): EFFECTS OF EXPOSURE VARIABLES AND COADMINISTERED TOXICANTS

Jim E. Riviere, Ronald E. Baynes, James D. Brooks, James L. Yeatts, Nancy A. Monteiro-Riviere

Center for Chemical Toxicology Research and Pharmacokinetics, North Carolina State University, Raleigh, North Carolina, USA

Exposure to N,N-diethyl-m-toluamide (DEET) commonly occurs in the general population and has been implicated as a contributory factor to the Gulf War Illness. The focus of the present studies was to determine the effect of coexposure factors, potentially encountered in a military environment, that could modulate transdermal flux of topically applied DEET. Factors investigated were vehicle, dose, coexposure to permethrin, low-level sulfur mustard, occlusion, and simultaneous systemic exposure to pyridostigmine bromide and the nerve agent simulant diisopropylfluorophosphate (DFP). Studies were conducted using the isolated perfused porcine skin flap (IPPSF), with a few mechanistically oriented studies conducted using in vitro porcine skin and silastic membrane diffusion cells. DEET was quantitated using high-performance liquid chromatography. The vehicle-control transdermal DEET flux in the IPPSF was approximately 2 μg/cm²/h for both 7.5 and 75% DEET concentrations, a value similar to that reported in humans. DEET absorption was enhanced by coinfusion of pyridostigmine bromide and DFP, by the presence of sulfur mustard, or by dosing under complete occlusion. The greatest increase in baseline flux was fivefold. In vitro diffusion cell studies indicated that silastic membranes had two orders of magnitude greater permeability than porcine skin, and showed vehicle effects on flux that were not detected in the IPPSF. These results suggest that coexposure to a number of chemicals that potentially could be encountered in a military environment may modulate the percutaneous absorption of topically applied DEET beyond that seen for normal vehicles at typically applied concentrations.

The focus of research into the pathogenesis of the Gulf War Illness has focused on chemical and nonchemical factors that could result in the observed syndrome. Potential factors include repeated vaccinations, oil-well fires, sand, depleted uranium, stress, and exposure to chemicals; the primary candidates are the nerve agent sarin, the nerve agent prophylactic drug pyridostigmine bromide, the insecticide permethrin, and the insect repellent *N,N*-diethyl-*m*-toluamide (DEET) (Fulco et al., 2000; Jagannathan et al., 2000; Wessely, 2001). A great deal of research has focused on the potential interactions of pyridostigmine

Received 11 March 2002; accepted 27 May 2002.

This study was supported by USAMRMC grant DAMD-17-99C-9047. We thank all staff members of the Center for Chemical Toxicology Research and Pharmacokinetics for their technical contributions to this work.

Address correspondence to Jim E. Riviere, Center for Chemical Toxicology Research and Pharmacokinetics, College of Veterinary Medicine, North Carolina State University, 4700 Hillsborough Street, Raleigh, NC 27606, USA. E-mail:Jim_Riviere@ncsu.edu

bromide, permethrin, and DEET, which could result in symptoms consistent with the Gulf War Illness (Abou-Donia et al., 1996, 2001; Abu-Qare & Abou-Donia, 2001a, 2001b; McCain et al., 1997; Hoy et al., 2000).

In order for these chemicals to exert a toxicologic effect, they must be present in the systemic circulation. Pyridostigmine bromide is formulated as an oral drug (Mestinol) and achieves effective concentrations in blood (Marino et al., 1998). However, permethrin and DEET must be absorbed across the skin after topical exposure to exert a toxicologic effect. Research efforts in this context have focused on the dermal absorption of permethrin, which would have to show significantly enhanced absorption to play a role in the pathogenesis of the Gulf War Illness (Baynes et al., 1997; NRC, 1994). Our previous studies have demonstrated that systemic pyridostigmine may enhance the absorption of [^{14}C]permethrin activity after topical exposure (Baynes et al., 2002). However, very little effort has been spent on addressing the dermal absorption of DEET and how coadministered agents could modulate its absorption.

DEET is rapidly and efficiently ($\leq$25% of applied dose) absorbed across the skin after topical application (Baynes et al., 1997; Ross & Shah, 2000; Selim et al., 1995, Moody et al., 1995; Reifenrath & Robinson, 1982), thus allowing it to potentially be a toxicologic factor in the Gulf War Illness. It is widely used in over-the-counter civilian preparations and is generally considered to possess a relatively moderate toxicity index if used appropriately, although acute massive overdoses can occur (Osimitz & Grothaus, 1995; Qiu et al., 1998; Young & Evans, 1998). What remains unclear is how other exposure variables and simultaneously administered drugs and chemicals affect percutaneous absorption and dermal penetration of DEET. These include coexposure to permethrin, pyridostigmine bromide, and low-level chemical warfare agents, as well as a number of other formulation variables that could modulate DEET absorption. These factors are important to investigate since they could significantly modulate exposure and facilitate systemic toxicosis.

The focus of the present article is to assess the effect of these exposure factors on the percutaneous absorption of topically applied DEET in two recognized experimental models of skin absorption: in vitro flow-through diffusion cells (Bronaugh et al., 1999) and the isolated perfused porcine skin flap (IPPSF) (Wester et al., 1998; Riviere et al., 1992). Porcine skin is widely accepted to be an appropriate animal model for assessing chemical absorption in humans (Monteiro-Riviere, 1991; Wester & Maibach, 1993). Use of both avascular (diffusion cells) and vascular (IPPSF) models allows for obtaining mechanistic insight into the nature of the observed interactions. Second, the IPPSF allows simultaneous systemic exposure to a drug or toxicant to be simulated by infusing the compound into the arterial cannula. Finally, diffusion-cell studies were also conducted using an inert silastic membrane to directly assess the vehicle effects on DEET absorption. The exposure factors investigated in IPPSF in the present study include topical permethrin, infused pyridostigmine bromide, infused nerve agent simulant diisopropylfluorophosphate (DFP), topical vesicant agent sulfur mustard (HD), and fabric occlusion, as well as altering concentrations of vehicle

components (ethanol/water) and concentration of DEET (7.5/75%). These studies were conducted to illustrate the complexity of these interactions that can occur when topical exposure to an individual chemical such as DEET is confounded with simultaneous exposure to topical and systemic agents.

MATERIAL AND METHODS

Chemicals and Doses

N,N-Diethyl-*m*-toluamide (DEET) (98% pure) was purchased from Chem Service Company, West Chester, PA. DEET was generally applied at a surface concentration of 75% to mimic that used by Gulf War veterans. One exposure was at 7.5% to assess low-dose effects. Permethrin was obtained from Sigma Chemical (St. Louis, MO) and used at a surface concentration of 40 μg/cm^2 in all studies. Pyridostigmine bromide (Mestinol) was obtained from ICN Biomedicals Inc., Costa Mesa, CA, and was added to the IPPSF perfusion media at a concentration (50 ng/ml) to simulate the highest pyridostigmine concentrations seen in soldiers taking this drug as a prophylactic treatment for nerve agent exposure (Marino et al., 1998).

Diisopropylfluorophosphate (DFP), employed as a chemical warfare nerve agent simulant in these studies, was obtained from Sigma Chemical (St. Louis, MO) and was added to the perfusion media (30 ng/ml) to mimic previously modeled DFP concentrations in the IPPSF (Carver et al., 1989). Surety dilute sulfur mustard in ethanol was obtained from the U.S. Army Medical Research and Development Command, and was dosed at a surface concentration of 40 μg/cm^2, an amount previously determined to be a subvesicating dose in the IPPSF (Monteiro-Riviere & Inman, 1995, 1997). A subvesicating dose was selected since such exposure would go undetected and thus could potentially modify DEET absorption. In contrast, a blister-producing dose of sulfur mustard would be detected and appropriate medical attention secured, removing this factor as a potential modulator of DEET absorption. Vehicles consisted of ethanol or ethanol/water (3:2 fixed v/v ratio), with the latter to assess effects of hydration conditions as would be seen with perspiration. All water was purified with an ultra-high-purity water filtration system (Dracor Water Systems, Durham, NC). High-performance liquid chromatography (HPLC) grade acetonitrile, ammonium acetate, and reagent-grade glacial acetic acid was purchased from Fisher Scientific (Fair Lawn, NJ). All other chemicals used in these experiments were HPLC grade and obtained from Sigma Chemicals or Fisher Scientific.

Isolated Perfused Porcine Skin Flaps

IPPSFs were prepared and perfused as described in detail elsewhere (Riviere et al., 1986; Monteiro-Riviere, 1990; Bowman et al., 1991; Riviere & Monteiro-Riviere, 1991). Skin flaps were perfused in a non-recirculating perfusion chamber maintained at 37 °C and 50–60% relative humidity, perfused

at 1 ml/min with an oxygenated (95% O_2/5% CO_2) Krebs–Ringer bicarbonate buffer solution, maintained between pH 7.4 and 7.5 and spiked with dextrose (0.12%) and bovine serum albumin (4.5%). In some treatments, pyridostigmine bromide and/or DFP were included in the perfusate to simulate systemic exposure conditions. After 1 h of equilibration, a flexible 1×5 cm dosing template (Stomahesive, ConvaTec-Squibb, Princeton, NJ) was affixed to the skin flap surface with Skin Bond (Pfizer Hospital Products, Largo, FL). Test solutions (100 µl), containing chemicals at the doses already stated, were applied to the 5-cm^2 dosing sites according to the experimental design protocols ($n = 4$ replicates) in Table 1. In 2 groups ($n = 4$ each), 100% cotton fabric or cellophane tape was applied over the dose site using a double Stomahesive patch to simulate conditions of a uniform versus complete occlusion. Glucose utilization and vascular resistance were monitored hourly to ensure viable skin flap preparations. Perfusate samples (3 ml) were collected at 0, 10, 20, 30, 45, 60, 75, and 90 min, and every $\frac{1}{2}$ h until termination at 8 h, and then were analyzed for

TABLE 1. Topical DEET Dosing Protocols

Isolated Perfused Porcine Skin Flaps ($n = 4$/Treatment)	
1	75% DEET:25% EtOH
2	75% DEET:25% EtOH:permethrin
3	75% DEET:25% EtOH:pyridostigmine-IA
4	75% DEET:25% EtOH:permethrin:pyridostigmine-IA
5	75% DEET:15% EtOH:10% water
6	75% DEET:15% EtOH:10% water:permethrin
7	75% DEET:15% EtOH:10% water:pyridostigmine-IA
8	75% DEET:15% EtOH:10% water:permethrin:pyridostigmine-IA
9	75% DEET:15% EtOH:10% water:permethrin:pyridostigmine + DFP-IA
10	7.5% DEET:55.5% EtOH:37% water:permethrin
11	75% DEET:25% EtOH:permethrin:HD
12	75% DEET:25% EtOH:permethrin:HD:pyridostigmine-IA
13	75% DEET:25% EtOH:permethrin:HD:DFP-IA
14	75% DEET:25% EtOH:permethrin:HD:pyridostigmine + DFP-IA
15	75% DEET:15% EtOH:10% water:permethrin:pyridostigmine + DFP-IA: cellophane-occluded
16	75% DEET:15% EtOH:10% water:permethrin:pyridostigmine + DFP-IA: fabric-occluded
Porcine Skin Flow-Through Diffusion Cells ($n = 5$/Treatment)	
1	75% DEET:25% EtOH:
2	75% DEET:25% EtOH:permethrin
3	75% DEET:15% EtOH:10% water
4	75% DEET:15% EtOH:10% water:permethrin
Silastic Membrane Flow-Through Diffusion Cells ($n = 5$/Treatment)	
1	75% DEET:25% EtOH
2	75% DEET:25% EtOH:permethrin
3	75% DEET:15% EtOH:10% water
4	75% DEET:15% EtOH:10% water:permethrin

absorbed DEET concentrations as described later. All nonsampled venous perfusate effluent was collected and sampled for DEET analysis.

Flow-Through Diffusion-Cell Experiments

Flow-through diffusion cells, as originally described by Bronaugh and Stewart (1985), were used in these studies. Perfusate (4 ml/h) and perfusion conditions (temperature, pH) were similar to that already described for IPPSFs. IPPSF perfusate was used in these experiments. Inert 250-μm silastic (polydimethylsiloxane) membranes (Dow Corning, Midland, MI) or dermatomed (500 μm) porcine skin, obtained from the dorsal area of female weanling Yorkshire-cross pigs and punched biopsied to obtain a dosing area of 0.64 cm^2, were used in these experiments. These skin sections were used fresh. All animal protocols were approved by the North Carolina State University Institutional Animal Care and Use Committee. Dosing solutions of 20 μl containing DEET, ethanol, water, and/or permethrin were dosed according to the treatments ($n = 5$ replicates) listed in Table 1. Perfusate samples were collected using the same times already given for the IPPSFs, and were assayed for DEET as described later.

Chemical Analysis

A modified solid-phase extraction method (Qiu & Jun, 1996) was used to extract DEET from the sample media. Solid-phase extraction discs (Ansys SPEC PLUS 3 ml C18 15 mg, Lake Forest, CA) were preconditioned with 500 μl acetonitrile followed by 500 μl water. The discs were not allowed to dry out during the preconditioning or sample loading steps. The 1 ml samples were loaded onto the discs and drained slowly (1 to 2 drops/s) using low pressure or gravity. This was followed by a solvent (10:90 acetonitrile: pH 4.5 ammonium acetate buffer, 0.03 *M*) wash and strong vacuum (15 in Hg) to dry out the discs before the final elution step. Samples were then eluted with 1 ml elution solvent (40:60 acetonitrile: pH 4.5 ammonium acetate buffer 0.03 *M*) and placed directly into HPLC vials for analysis. The Waters HPLC system (Waters, Inc., Milford, MA) was equipped with a 996 PDA detector, 717 plus autosampler, 600 controller, temperature control module, and model 60 F solvent pumping system. All data were collected on a Gateway E3110 computer utilizing Waters Millennium 32 version 3.05.01 software. A Waters Symmetry Shield RP18 (3.5 μm, 4.6 × 150 mm) column was used for the separations. The mobile phase consisted of 36% acetonitrile and 64% pH 4.5 ammonium acetate buffer, 0.03 *M*. The column temperature was 35 °C, detector wavelength 220 nm, flow rate 1.0 ml/min, and injection volume 10 μl. Recoveries and blanks were run with every batch of samples. The standard curves, prepared by spiking the same volumes of elution solvent with the same amounts of DEET used for spiking the media, revealed a minimum R^2 of .999. The average recoveries for sample media spiked with 0.5 to 5.0 ppm DEET ranged from 93 to 117%, with coefficient of variations generally averaging 2–13%. The limit of detection of the assay was 0.02 μg/ml and the limit of quantitation was 0.08 μg/ml.

Calculation and Statistics

DEET absorption (μg) into perfusate was quantitated by determining the area under the curve (AUC), peak flux, and total mass of DEET absorbed. Total mass absorbed is the summation of the DEET in the perfusate samples and final venous effluent. All parameters are expressed as mean ± standard error. Statistical significance ($p \leq .05$) between treatments was determined by analysis of variance (ANOVA) with multiple-comparison tests performed using the LSD method (SAS 8.1 for Windows, SAS Institute, Inc., Cary, NC).

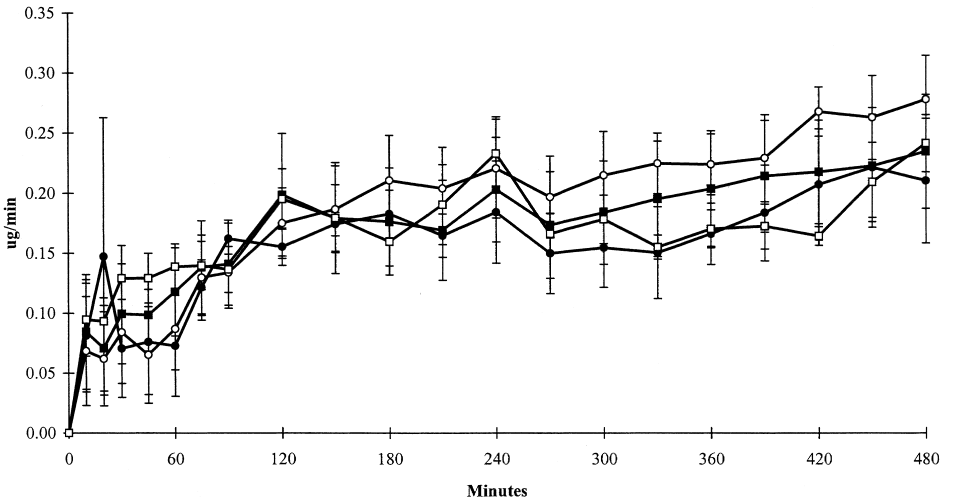
The in vitro diffusion cell data were further analyzed to determine diffusion parameters. Extrapolated steady state flux, J_{ss} (μg/cm^2/h), was determined at pseudosteady state from the slope of the cumulative mass per unit area versus time (h) curve. Steady state was determined as that time interval in the DEET concentration versus time plots when there is no significant change in DEET concentration with time. Permeability, P(cm/h), was determined from the ratio of individual fluxes to the concentration (μg/cm^3) of applied dose. Diffusivity, D (cm^2/h), was determined by obtaining the lag time before steady-state flux is reached. This lag time (τ) was obtained by extrapolating the steady state portion of the curve back to the time or x axis. Lag time is related to diffusivity (D) and membrane thickness (L) by the following equation: $D = L^2/6\tau$, where $L = 250\,\mu$m for silastic and $500\,\mu$m for porcine skin. The partition coefficient between the skin and dosing vehicle (K_{sv}) was then determined as $K_{sv} = P \times (L/D)$.

RESULTS

The results of these exposures are presented in graphical and tabulated forms as Figures 1 through 7. The effects are best assessed by examining the tabulated data where the statistical comparisons are presented. The graphics in turn illustrate the effect that additives have on the shape of the DEET flux profiles in both model systems. Statistical comparisons are not shown on the flux profiles, but rather in the tabulated parameters. In examining these data, one must take into consideration that the vehicle in which DEET is dosed, ethanol and ethanol/water (aqueous ethanol), also affects disposition, making comparisons of additive effects within vehicles necessary.

Isolated Perfused Porcine Skin Flaps

The percutaneous absorption parameters of DEET are tabulated and depicted in Figures 1 through 5. Note that some of the specific treatment combinations are repeated in these tabulations to facilitate making statistical comparisons between treatments. DEET absorption was not significantly different (this cross-figure comparison is not shown in the tabulations) between ethanol (Figure 1) and ethanol/water (Figure 2) solutions (absorption 69 vs. 67 μg; AUC 77 vs. 83 μg-min/ml; Peak Flux 0.22 vs. 0.27 μg/min). Permethrin added to aqueous ethanol solutions (Figure 2) had no marked effect on DEET absorption.

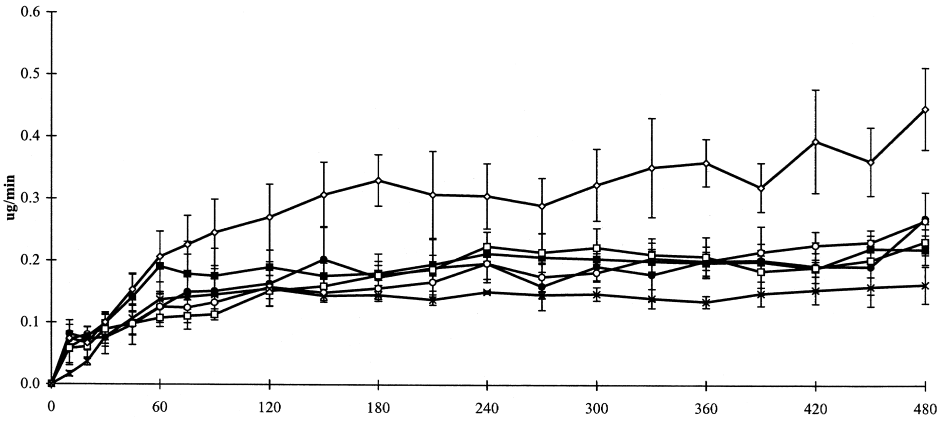


	75%DEET 25%EtOH	75%DEET 25%EtOH Permethrin	Pyridostigmine 75%DEET 25%EtOH	Pyridostigmine 75%DEET 25%EtOH Permethrin
Absorption (ug)	69 (17) [a]	81 (14) [a]	75 (20) [a]	81 (10) [a]
AUC (ug-min/ml)	77 (13) [a]	92 (13) [a]	85 (19) [a]	82 (9) [a]
Peak flux (ug/min)	0.22 (0.12) [a]	0.27 (0.05) [a]	0.23 (0.05) [a]	0.24 (0.03) [a]

FIGURE 1. Mean (SEM) DEET IPPSF perfusate absorption flux (μg/min) profiles and absorption parameters for nonaqueous mixtures. Tabulated means with the same letters (a, b, or c) are not significantly different across treatments.

As seen in Figures 1 and 2; there were no statistically significant differences in DEET absorption parameters when pyridostigmine or when permethrin and pyridostigmine were present in 75% DEET/25% ethanol or 75% DEET/15% ethanol vehicles. However, coinfusion of DFP with the complete chemical exposure scenario (Figure 2) resulted in statistically significant, almost twofold, increased DEET absorption compared to all other treatment conditions. Finally, application of a low concentration (7.5%) of DEET resulted in similar levels of DEET absorption.

The final comparisons involve the effect of sulfur mustard (HD) coexposure. In these sets of experiments, aqueous vehicles could not be employed due to the hydrolysis of HD when water is present. Figure 3 compares the effect of HD in permethrin–ethanol–DEET vehicles, with and without simultaneous pyridostigmine infusions. Figure 4 adds DFP infusions to this matrix. As can be seen in Figure 3, HD significantly increased DEET absorption when pyridostigmine was not present. Similarly, in Figure 4, DFP blocked this HD effect. However, unlike all other DEET perfusate flux profiles previously compared, the full mixture profile (Figure 4 with DFP+pyridostigmine) appeared different with



	75%DEET 15%EtOH 10%Water	75%DEET 15%EtOH 10%Water Permethrin	Pyridostigmine 75%DEET 15%EtOH 10%Water	Pyridostigmine 75%DEET 15%EtOH 10%Water Permethrin	Pyridostigmine+DFP 75%DEET 15%EtOH 10%Water Permethrin	7.5%DEET 55.5%EtOH 37%Water Permethrin
Absorption (ug)	67 (10) [b]	66 (8) [b]	82 (11) [b]	81 (6) [b]	148 (22) [a]	61 (2) [b]
AUC (ug-min/ml)	83 (16) [b]	83 (7) [b]	89 (7) [b]	83 (7) [b]	141 (21) [a]	66 (4) [b]
Peak flux (ug/min)	0.27 (0.06) [b]	0.27 (0.03) [b]	0.22 (0.06) [b]	0.23 (0.03) [b]	0.45 (0.08) [a]	0.16 (0.03) [b]

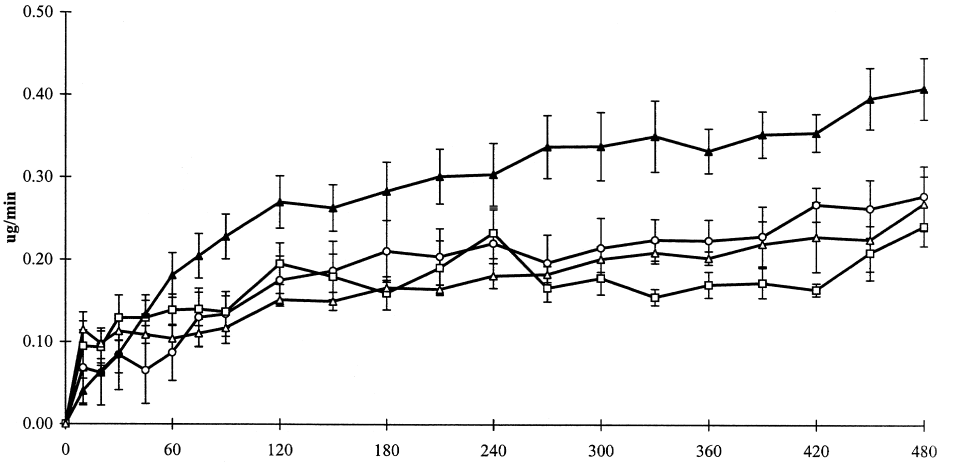
FIGURE 2. Mean (SEM) DEET IPPSF perfusate absorption flux (μg/min) profiles and absorption parameters for aqueous mixtures. Tabulated means with the same letters (a, b, or c) are not significantly different across treatments.

a peak level around 180 min, albeit with a relatively high error bar. A similar but delayed peak was seen at 420 min in the DFP profile, again with a high degree of variation, but it is noteworthy that a kinetic plateau was less apparent overall. In summary, HD alone clearly enhances DEET absorption. The presence of DFP creates a flux pattern different from that seen for all other treatment combinations in Figures 1 through 3.

The effects of occlusion on DEET absorption parameters and flux profiles is presented in Figure 5. As expected, complete occlusion with cellophane significantly increased all parameters of DEET absorption. Fabric occlusion, as would be seen with wearing a uniform, resulted in an intermediate state of enhanced absorption that was not statistically different from the nonoccluded treatment. The flux profile clearly shows this intermediate absorption rate.

In Vitro Diffusion Cell Studies

Figure 6 depicts the porcine skin flow-through diffusion-cell data. The primary effect observed in porcine skin diffusion cell studies was a significant increase in DEET absorption, perfusate AUC, and peak flux when DEET was dosed in ethanol vehicles containing water. Figure 6 depicts an almost twofold increase in DEET AUC when dosed in an aqueous ethanol vehicle.

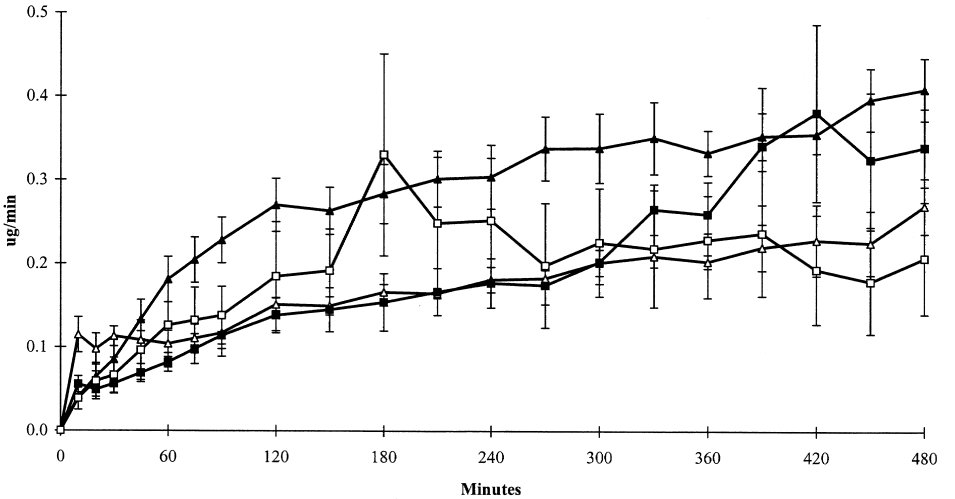


		Pyridostigmine	HD	HD Pyridostigmine
	75%DEET 25%EtOH Permethrin	75%DEET 25%EtOH Permethrin	75%DEET 25%EtOH Permethrin	75%DEET 25%EtOH Permethrin
Absorption (ug)	81 (14) [b]	81 (10) [b]	145 (16) [a]	77 (6) [b]
AUC (ug-min/ml)	92 (13) [b]	82 (9) [b]	138 (13) [a]	84 (5) [b]
Peak flux (ug/min)	0.27 (0.05) [b]	0.24 (0.03) [b]	0.41 (0.04) [a]	0.27 (0.04) [b]

FIGURE 3. Mean (SEM) DEET IPPSF perfusate absorption flux (μg/min) profiles and absorption parameters for sulfur mustard (HD) versus no HD mixtures. Tabulated means with the same letters (a, b, or c) are not significantly different across treatments.

Figure 7 depicts DEET absorption parameters from silastic membrane diffusion cell experiments. DEET absorption through silastic was orders of magnitude greater than through skin. Consistent with the porcine skin, DEET absorption was significantly increased in aqueous ethanol vehicles as assessed by absorption, perfusate AUC, and peak flux parameters. In contrast, the effect of codosed permethrin was variable, showing an increase in ethanol and a decrease in the aqueous vehicle. This is similar to the apparent blunting by permethrin of the DEET aqueous flux profile in porcine skin diffusion cells seen from 45 to 150 min (Figure 6). This blunting in ethanol vehicle was not seen in the aqueous IPPSF data in Figure 2. In contrast, the increase in DEET flux with permethrin in ethanol was not seen in porcine skin diffusion cells.

Diffusion parameters of DEET in both porcine skin and silastic diffusion cells are tabulated in Table 2. The noteworthy observations in this analysis are that permeability in skin changed slightly under different solvent conditions, which was a function of altered diffusivity and stratum corneum to vehicle partition coefficients. Second, as observed with the raw flux data, permeability

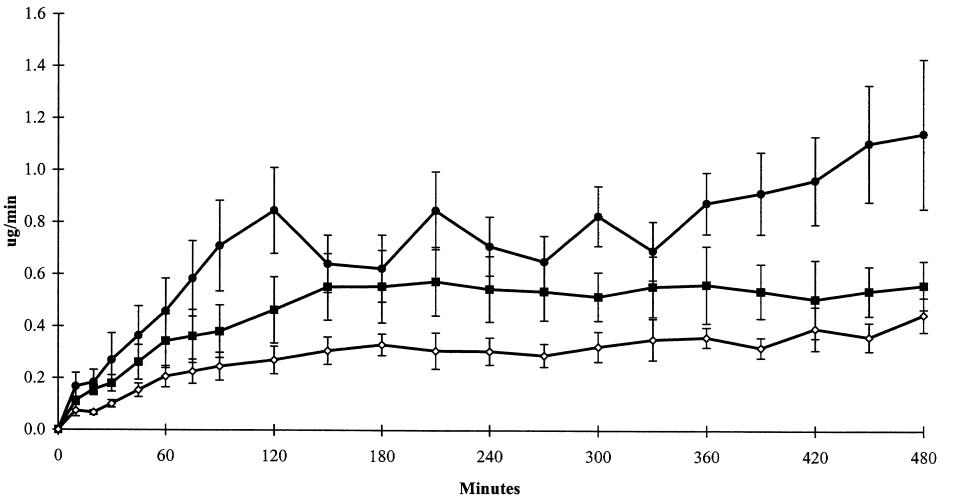


	HD 75%DEET 25%EtOH Permethrin	HD Pyridostigmine 75%DEET 25%EtOH Permethrin	HD DFP 75%DEET 25%EtOH Permethrin	HD Pyridostigmine + DFP 75%DEET 25%EtOH Permethrin
Absorption (ug)	145 (16) [a]	77 (6) [b]	87 (9) [ab]	87 (39) [ab]
AUC (ug-min/ml)	138 (13) [a]	84 (5) [b]	95 (8) [ab]	94 (27) [ab]
Peak flux (ug/min)	0.41 (0.04) [a]	0.27 (0.04) [a]	0.38 (0.11) [a]	0.33 (0.12) [a]

FIGURE 4. Mean (SEM) DEET IPPSF perfusate absorption flux (μg/min) profiles and absorption parameters for nonaqueous sulfur mustard (HD) mixtures. Tabulated means with the same letters (a, b, or c) are not significantly different across treatments.

TABLE 2. Flux, Permeability, and Diffusivity of DEET Following Topical Doses of 75% DEET in Porcine and Silastic Flow-Through Diffusion Cells

Pig skin	Ethanol ($n = 5$)	Permethrin:ethanol ($n = 5$)	Permethrin:ethanol/water ($n = 5$)	Ethanol/water ($n = 5$)
Flux (μg/cm^2/h)	10.5 (3.7)	10.4 (2.8)	11.2 (7.6)	18.5 (10.4)
Permeability (cm/h $\times 10^{-5}$)	1.4 (0.5)	1.4 (0.4)	1.5 (1.0)	2.5 (1.4)
Diffusivity (cm^2/h $\times 10^{-5}$)	24.2 (1.5)	25.4 (2.2)	155 (70)	114 (50)
$K_{sv} \times 10^{-3}$	2.90 (0.95)	2.73 (0.72)	0.49 (0.19)	1.26 (0.81)
Silastic	**Ethanol ($n = 5$)**	**Permethrin:ethanol ($n = 5$)**	**Permethrin:ethanol/water ($n = 4$)**	**Ethanol/water ($n = 5$)**
Flux (μg/cm^2/h)	1336 (483)	1836 (291)	988 (35)	1757 (60)
Permeability (cm/h $\times 10^{-5}$)	179 (65)	246 (39)	132 (5)	235 (8)
Diffusivity (cm^2/h $\times 10^{-5}$)	23.2 (9.5)	53.4 (22.0)	10.8 (0.7)	13.9 (3.9)
$K_{sv} \times 10^{-3}$	197 (35)	130 (53)	308 (25)	450 (122)



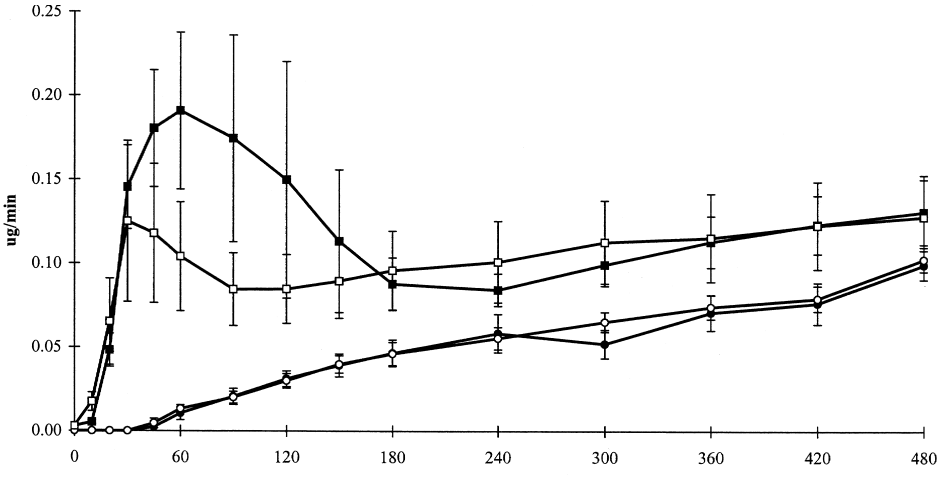
	Occluded Pyridostigmine+DFP 75%DEET 15%EtOH:10%Water Permethrin	Fabric Occluded Pyridostigmine+DFP 75%DEET 15%EtOH:10%Water Permethrin	Nonoccluded Pyridostigmine+DFP 75%DEET 15%EtOH:10%Water Permethrin
Absorption (ug)	343 (30) [a]	256 (70) [ab]	148 (22) [b]
AUC (ug-min/ml)	352 (26) [a]	230 (51) [b]	141 (21) [b]
Peak flux (ug/min)	1.14 (0.29) [a]	0.57 (0.15) [b]	0.45 (0.08) [b]

FIGURE 5. Mean (SEM) DEET IPPSF perfusate absorption flux (μg/min) profiles and absorption parameters for aqueous mixtures with complete cellophane tape or fabric occlusion versus control. Tabulated means with the same letters (a, b, or c) are not significantly different across treatments.

and flux through silastic were two orders of magnitude greater than through porcine skin.

Integration of Results

There are significant differences between model systems, relative to absolute absorption as well as relative to the effects of concomitant chemical administration. When one compares total DEET absorption over 8 h normalized to surface area, between the IPPSF and the porcine skin diffusion cell model, using a comparable aqueous ethanol vehicle control group, absorption in the porcine skin diffusion cells was approximately 90 $\mu g/cm^2$, compared to 13 $\mu g/cm^2$ in the IPPSF. Across all treatment combinations in the IPPSF, DEET absorption into the perfusate was enhanced by coinfusion of pyridostigmine bromide and the organophosphate nerve agent simulant DFP, in the presence of HD, and by dosing under occlusive conditions. Maximum extents of enhanced absorption (treatment/control) under nonoccluded conditions ranged from approximately 1.8- to 2.2-fold. When dose sites were occluded, absorption increased another twofold. The "worst-case" maximal enhancement scenario



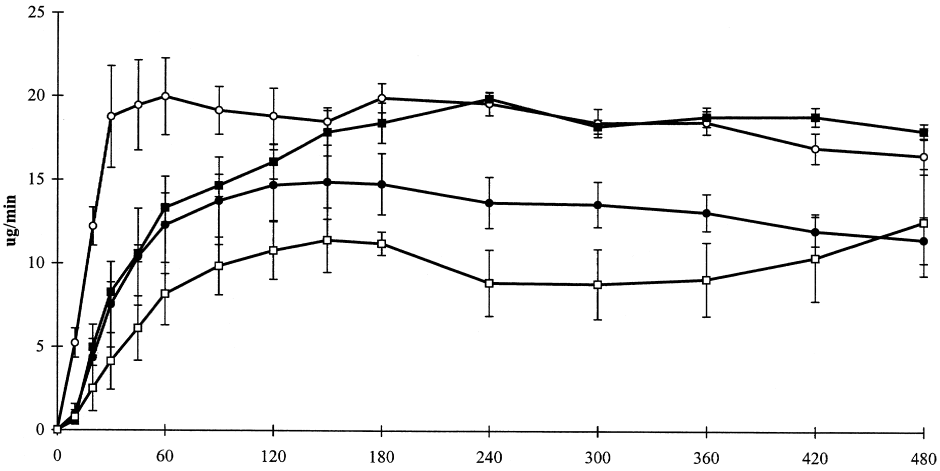
	75%DEET 25%EtOH	75%DEET 25%EtOH Permethrin	75%DEET 15%EtOH:10%Water	75%DEET 15%EtOH:10%Water Permethrin
Absorption (ug)	28 (3) [bc]	28 (2) [c]	57 (10) [a]	51 (11) [ab]
AUC (ug-min/ml)	343 (46) [b]	339 (33) [b]	793 (149) [a]	700 (173) [a]
Peak flux (ug/min)	0.10 (0.01) [b]	0.10 (0.01) [b]	0.19 (0.07) [a]	0.13 (0.05) [ab]

FIGURE 6. Mean (SEM) DEET porcine skin flow-through diffusion-cell perfusate absorption flux (μg/min) profiles and absorption parameters in aqueous and nonaqueous mixtures. Tabulated means with the same letters (a, b, or c) are not significantly different across treatments.

seen in this study was a fivefold increase when DEET absorption in the occluded/pyridostigmine/DFP/permethrin/15% ethanol/10% water group (approximately 340 μg/5 cm^2; Figure 5) was compared to its vehicle control (approximately 67 μg/5 cm^2; Figure 2). This would be equivalent, in dosed surface area normalized units, to 68 versus 13 μg/cm^2, a fivefold increase.

DISCUSSION

The focus of these studies was to assess the effects of coexposure factors on the percutaneous absorption of topically applied DEET. These studies clearly indicate that DEET is well absorbed when topically applied to porcine skin. Depending on the exposure conditions in the IPPSF, 13 to 68 μg/cm^2 was absorbed into perfusate over an 8-h period. This is the amount of DEET that would be considered bioavailable to cause a systemic effect. Selim et al. (1995) reported on DEET absorption in vivo in humans applying neat DEET, or 15% DEET in ethanol, to a 24-cm^2 dosing site. Absorption was determined by quantitative collection of urine and feces over various time periods for up to 128 h. Using the Selim et al. (1995) urine data through 8 h to take into account



	75%DEET 25%EtOH	75%DEET 25%EtOH Permethrin	75%DEET 15%EtOH:10%Water	75%DEET 15%EtOH:10%Water Permethrin
Absorption (ug)	6,116 (663) [b]	8,749 (248) [a]	8,208 (157) [a]	4,588 (488) [c]
AUC (ug-min/ml)	83,741 (9,518) [b]	121,864 (3,730) [a]	114,737 (2,012) [a]	65,209 (3,888) [c]
Peak flux (ug/min)	15 (3) [b]	20 (3) [a]	20 (1) [a]	13 (3) [b]

FIGURE 7. Mean (SEM) DEET silastic flow-through diffusion-cell perfusate absorption flux (μg/min) profiles and absorption parameters in aqueous and nonaqueous mixtures. Tabulated means with the same letters (a, b, or c) are not significantly different across treatments.

the IPPSF study duration, and normalizing to unit surface area to correct for the IPPSF 5-cm^2 dosing site compared to the 24-cm^2 human site, DEET absorption in humans ranged from 14 to 20 $\mu g/cm^2$. These values are essentially identical to the approximately 13–16 $\mu g/cm^2$ absorption, from 7.5% and 75% DEET vehicle controls, seen in the IPPSF experiment. This concurrence supports previous work with other drugs and chemicals (Riviere & Monteiro-Riviere, 1991; Riviere et al., 1992; Wester et al., 1998) that the IPPSF data reported here should be predictive of DEET absorption in vivo in humans.

These studies suggest that for the exposure conditions examined, DEET absorptive flux is relatively constant at a rate of approximately 2 $\mu g/cm^2/h$, even when 7.5% and 75% concentrations are compared. This is also supported by a similar rate seen for DEET absorption in humans when neat or 15% DEET was applied (Selim et al., 1995). These data suggest that transdermal DEET flux is operationally saturated through in vivo skin. Viewed in a pharmacokinetic context, DEET transdermal flux across human skin and in the IPPSF can be viewed as a zero-order kinetic process with a k_0 of 2 $\mu/cm^2/h$. Some workers have suggested the formation of a DEET depot in skin (Moody et al., 1995), which could affect the interpretation of these data. Factors that could further

increase this absorption would have to do so by altering the inherent permeability of the skin barrier and increasing k_0. For example, increased permeability of DEET though the skin of children could modulate these conclusions concerning saturation. Unfortunately, such studies have not been conducted. In the present experiments, flux was increased significantly when low levels of topical sulfur mustard were present, when systemic exposure to both pyridostigmine bromide and DFP occurred, or when occlusive dosing conditions were present.

The enhanced DEET flux seen with coexposure to low-level sulfur mustard is not surprising, as this highly reactive vesicant would be expected to alter epidermal barrier function based on it mechanism of action in skin (Monteiro-Riviere & Inman, 1995, 1997). Low-level sulfur mustard in the IPPSF has previously been shown to alter enzyme activity, detected using histochemistry techniques, in all layers of the epidermis, supporting the nonspecific biochemical toxicologic effects of this compound (Monteiro-Riviere & Inman, 2000). Sulfur mustard exposure can also result in release of inflammatory mediators from skin. This was detected in IPPSFs after relatively low-level sulfur mustard exposure, which could modulate vascular permeability and enhance percutaneous absorption (Zhang et al., 1995). In a toxicokinetic study of sulfur mustard percutaneous absorption in IPPSFs, absorbed sulfur mustard induced changes in vascular permeability inferred by time-varying mustard fluxes and directly assessed by altered inulin (extracellular space marker) distribution (Riviere et al., 1995). Therefore, changes either in epidermal permeability due to mustard-induced structural or biochemical damage to keratinocytes, or of increased vascular uptake due to increases in capillary permeability (direct toxicity or secondary to cytokine release), would be consistent with the increased DEET absorption seen in this study.

In contrast to this enhancing effect of sulfur mustard exposure on DEET, [^{14}C]permethrin absorption was slightly decreased when coexposed to this toxicant (Riviere et al., 2002). In the case of [^{14}C]permethrin, it is probably a metabolite of permethrin that is actually being absorbed across the skin (Baynes et al., 2002). Permethrin absorption is thus limited by both a diffusion barrier and a metabolic barrier. The parent compound does not readily penetrate skin. Consistent with the effects of sulfur mustard on enzyme histochemistry discussed earlier, damage to epidermal enzymes would be expected to result in a decreased transdermal flux of [^{14}C]permethrin if a metabolite were the primary absorbed moiety. In the case of DEET, HD-induced enhanced permeability through a diffusion barrier would increase transdermal flux.

The enhanced DEET absorption seen with coinfusion of pyridostigmine bromide and DFP is in this case similar to that seen with [^{14}C]permethrin absorption (Baynes et al., 2002). [^{14}C]Permethrin absorption increased 5.6-fold above control in a scenario where both compounds were coinfused. Unlike DEET, infusion of DFP or pyridostigmine alone only slightly increased [^{14}C]permethrin absorption compared to controls. The mechanism behind this effect is not clear. Pyridostigmine and DFP are both drugs targeted to the cholinergic

nervous system, making modulation of the skin's microcirculation a potential mechanism. A second potential target of interaction would be on enzyme metabolism. The significant finding of these studies is that coinfusion of these two compounds also enhances DEET absorption, as was seen for [^{14}C]permethrin. The interactions of pyridostigmine bromide and DFP on the sulfur mustard-induced DEET effect, seen in Figure 4, would also point to a more complex pharmacologic mechanism whose explanation at this time would be speculative.

The final significant effect seen with DEET was the increased absorption observed after complete occlusion. This may have been secondary to a combined effect of occlusion that increases skin permeability due to hydration, as well as some retention of evaporated DEET. This effect was not as pronounced when fabric occlusion was employed. Occlusion-induced hydration of the epidermal permeability barrier may also be similar to the hydration seen in conditions of high humidity, perspiration, or water exposure.

The exposure variables that proved significant in these studies may shed light on the vehicle effects seen in the in vitro diffusion cells that were not present in the IPPSFs. In vitro absorption studies were conducted to probe mechanisms of interactions that might shed light on the absorption changes seen in the IPPSF experiments. Absorption and permeability through silastic membranes were orders of magnitude above those seen in the skin systems, suggesting that this membrane is not an appropriate model for studying DEET in skin. In contrast, percutaneous absorption in the in vitro porcine skin diffusion cells is in the low end of the range seen for DEET absorption reported using in vitro human skin diffusion cells (Ross & Shah, 2000; Stinecipher & Shah, 1997; Moody et al., 1995). These lower fluxes are consistent with differences in techniques, where the human data was often conducted using fully hydrated static Franz cells with prolonged experimental durations. However, all in vitro results were much higher than those seen in the IPPSF and, by extension, also in humans.

In the in vitro studies, changes in vehicle composition generally altered permeability by changing K_{sv} and diffusivity. This change in K_{sv}, secondary to formulation, resulting in a change in permeability, is the same effect reported in other in vitro formulation studies (Ross & Shah, 2000). Aqueous vehicles significantly increase DEET absorption in both porcine skin and silastic diffusion cell experiments. The permeability of DEET in silastic membranes is very large and probably precludes these model membranes from being used to study factors that modulate DEET absorption in vitro.

These vehicle effects did not alter DEET absorption in the IPPSF. It is possible that changes in vehicle to membrane partitioning were decreasing DEET diffusion in the in vitro experiments. These effects are not detectable with the lower absorption fluxes seen in the more biologically complex IPPSF model. That is, in the IPPSF and most probably in humans based on the similar fluxes seen with in vivo studies with DEET vehicles, the rate-limiting step for absorption is the intact skin. Permeability through this intact barrier modulates overall

absorptive flux. Factors that further increase DEET availability on the surface of skin may not translate into increased transdermal flux, much as increasing the applied concentration from 7.5% to 75% does not result in increased flux.

From the perspective of pharmacokinetics, DEET diffusion in the in vitro models is usually governed by Fick's law of diffusion, which specifies a first-order kinetic process (Riviere, 1999). Factors that alter partitioning may modulate permeability, described by a first-order rate constant, which when multiplied by available dose yields the observed transdermal flux ($\mu g/cm^2/h$). In contrast, it appears that in the IPPSF and in vivo, transdermal flux is now fixed ($k_0 = 2\,\mu g/cm^2/h$) and insensitive to vehicle modulation. However, conditions where k_0 are increased, such as with sulfur mustard or occlusion, result in an increase in transdermal flux to levels approaching $10\,\mu g/cm^2/h$.

This phenomenon is often encountered in formulation of dermatologic drugs where the inherent skin permeability is too low for such manipulations to actually increase transdermal flux. Instead, topical activity may increase in the absence of an increase in systemic absorption. The only effects that resulted in enhanced absorption in the IPPSF were those that changed permeability, effectively increasing absorption above the plateau seen with all other DEET exposure conditions. In the case of in vitro studies, this "base flux" is large enough to allow first-order kinetics to be operative, and thus altered transdermal flux due to changing formulations.

What are the practical applications of these findings? First one must assume that the "base level" of DEET flux seen under normal application conditions in the IPPSF and in vivo human studies, of $14–20\,\mu g/cm^2/8\,h$, or approximately $1–3\,\mu g/cm^2/h$, does not result in significant systemic toxicity. Adverse reactions to DEET are apparently not common, considering the extremely large numbers of people in the general population who routinely apply this as an insect repellent in the summer. Signs of acute toxicity, when it does occur, include tremor, restlessness, slurred speech, seizures, impaired cognitive functions, and coma (Young & Evans, 1998). Cases of toxicosis are related to extensive and repeated topical applications, or ingestion with suicidal intent. The focus of the present study was not to relate DEET absorption to acute toxicosis, but rather to assess factors that might modulate percutaneous absorption, and thus affect DEET's role in the Gulf War Illness. Are there exposure scenarios that would result in higher levels of DEET systemic exposure than anticipated under normal conditions?

Our data suggests that coexposure to low-level mustard, systemic pyridostigmine, or the organophosphate DFP, and occlusion may increase base-level DEET absorption up to fivefold over normal levels. Systemic exposure is a function of applied surface area, as well as integrity of the skin barrier, and not primarily the concentration of DEET applied. In contrast, efficacy against mosquitoes is related to applied surface concentration, as evaporative loss is directly related to surface concentration. Taking our worst-case scenario, these modulating factors would be equivalent, in terms of systemic DEET exposure, to increasing the applied surface area by a factor of five. It must be stressed

that all of the conditions selected for study in the present report were not those that were actually encountered in the Gulf War, except for pyridostigmine bromide, permethrin, and fabric (uniform) occlusion. Instead, they included examples of toxic chemicals that might be encountered in a military environment.

The important finding is that vehicle and concentration seemed to have minimal effects on absorption, while exposure to systemic drugs or a topical toxicant such as subvesicating doses of sulfur mustard did increase absorption, thereby accentuating any role that DEET might play in a syndrome such as the Gulf War Illness. The most obvious finding, and one that is easily addressable in an occupational environment, was that covering DEET dose areas with an occlusive dressing, or even fabric, enhances absorption. Care should be taken to avoid this scenario after DEET has been applied.

The relevance of these findings to Gulf War Illness is not known except for confirming that significant amounts of DEET may be absorbed across the skin. From a scientific perspective, coadministration of a cutaneous toxicant may modulate DEET absorption. Significantly, systemic exposure to certain chemicals, such as pyridostigmine and DFP, can modulate the percutaneous absorption of an unrelated chemical. This scenario is not usually assumed to be relevant to dermal risk assessment, and further supports the contention that risk assessment of chemical mixtures cannot be based solely on individual chemical studies.

REFERENCES

Abou-Donia, M. B., Wilmarth, K. R., Jensen, K. F., Oehme, F. W., and Kurt, T. L. 1996. Neurotoxicity resulting from coexposure to pyridostigmine bromide, DEET, and permethrin. *J. Toxicol. Environ. Health* 48:35–56.

Abou-Donia, M. B., Goldstein, L. B., Dechovskaia, A., Bullman, S., Jones, K. H., Herrick, E. A., Abdel-Rahman, A. A., and Khan, W. A. 2001. Effects of daily dermal application of DEET and permethrin, alone or in combination, on sensorimotor performance, blood–brain barrier, and blood–testis barrier in rats. *J. Toxicol. Environ. Health A* 62:523–541.

Abu-Qare, A. W., and Abou-Donia, M. B. 2001a. Combined exposure to DEET (*N,N*-diethyl-*m*-toluamide) and permethrin induced release of rat brain mitochondrial cytochrome *c*. *J. Toxicol. Environ. Health A* 63:243–252, 2001.

Abu-Qare, A. W., and Abou-Donia, M. B. 2001b. DEET (*N,N*-diethyl-*m*-toluamide) alone or in combination with permethrin increased urinary excretion of 6β-hydroxycortisol in rats, a marker of hepatic CYP3A induction. *J. Toxicol. Environ. Health A* 64:373–384.

Baynes, R. E., Halling, K. B., and Riviere, J. E. 1997. The influence of diethyl-*m*-toluamide (DEET) on percutaneous absorption of permethrin and carbaryl. *Toxicol. Appl. Pharmacol*. 144:332–339.

Baynes, R. E., Monteiro-Riviere, N. A., and Riviere J. E. 2002. Pyridostigmine bromide modulates the dermal disposition of ^{14}C-permethrin. *Toxicol. Appl. Pharmacol*.181:164–173.

Bowman, K. F., Monteiro-Riviere, N. A., and Riviere, J. E. 1991. Development of surgical techniques for preparation of *in vitro* isolated perfused porcine skin flaps for percutaneous absorption studies. *Am. J. Vet. Res*. 52:75–82.

Bronaugh, R. L., and Stewart, R. F. 1985. Methods for *in vitro* percutaneous absorption studies IV: The flow-through diffusion cell. *J. Pharm. Sci*. 74:64–67.

Bronaugh, R. L., Hod, H. L., Kraeling, M. E., Yourick, J. J. 1999. Determination of percutaneous absorption by in vitro techniques. In *Percutaneous absorption. Drugs–cosmetics–mechanisms–methodology*, eds. R. L. Bronaugh and H. I. Maibach, 3rd ed. pp. 229–233. New York: Marcel Dekker.

Carver, M. P., Williams, P. L., and Riviere, J. E. 1989. The isolated perfused porcine skin flap. III. Percutaneous absorption pharmacokinetics of organophosphates, steroids, benzoic acid and caffeine. *Toxicol. Appl. Pharmacol*. 97:324–337.

Fulco, C. E., Liverman, C. T., and Sox, H. C. 2000. *Gulf War and health*, Vol. I, *Depleted uranium, sarin, pyridostigmine bromide, vaccines*. Washington, DC: National Academy Press.

Hoy, J. B., Cornell, J. A., Karlix, J. L., Tebbett, I. R., and van Haaren, F. 2000. Repeated coadministrations of pyridostigmine bromide, DEET, and permethrin alter locomotor behavior of rats. *Vet. Human Toxicol*. 42:72–76.

Jagannathan, R., Husain, K., and Somani, S. M. 2000. Interaction of pyridostigmine and physical stress on antioxidant defense system in skeletal muscle of mice. *J. Appl. Toxicol*. 21:341–348.

Marino, M. T., Schuster, B. G., Brueckner, R. P., Lin, E., Kaminskis, A., and Lasseter, K. 1998. Population pharmacokinetics and pharmacodynamics of pyridostigmine bromide for prophylaxis against nerve agents in humans. *J. Clin. Pharmacol*. 38:227–235.

McCain, W. C., Lee, R., Johnson, M. S., Whaley, J. E., Ferguson, J. W., Beall P., and Leach, G. 1997. Acute oral toxicity study of pyridostigmine bromide, permethrin, and DEET in the laboratory rat. *J. Toxicol. Environ. Health* 50:113–124.

Monteiro-Riviere, N. A. 1990. Specialized technique: Isolated perfused porcine skin flap. In *Methods for skin absorption*, eds. B. W. Kemppainen and W. G. Reifenrath, pp. 175–189. Boca Raton, FL: CRC Press.

Monteiro-Riviere, N. A. 1991. Comparative anatomy, physiology, and biochemistry of mammalian skin. In *Dermal and ocular toxicology: Fundamentals and methods*, ed. D. W. Hobson, pp. 3–71. Boca Raton, FL: CRC Press.

Monteiro-Riviere, N. A., and Inman, A. O. 1995. Indirect immunohistochemistry and immunoelectron-microscopy distribution of eight epidermal-dermal junction epitopes in the pig and in isolated perfused skin treated with bis(2-chloroethyl) sulfide. *Toxicol. Pathol*. 23:313–325.

Monteiro-Riviere, N. A., and Inman, A. O. 1997. Ultrastructural characterization of sulfur-mustard-induced vesication in isolated perfused skin. *Microsc. Res. Technique* 37:229–241.

Monteiro-Riviere, N. A., and Inman, A. O. 2000. Characterization of sulfur mustard-induced toxicity by enzyme histochemistry in porcine skin. *Toxicol. Methods* 10:127–142.

Moody, R. P., Nadeau, B., and Chu, I. 1995. In vitro dermal absorption of *N,N*-diethyl-*m*-toluamide (DEET) in rat, guinea pig, and human skin. *In Vitro Toxicol*. 8:263–275.

National Research Council. 1994. *Health effects of permethrin-impregnated Army battle-dress uniforms*. Washington, DC: National Academy Press.

Osimitz, T. G., and Grothaus, R. H. 1995. The present safety assessment of DEET. *J. Am. Mosquito Control Assoc*. 11:274–278.

Qiu, H., and Jun, H. W. 1996. Solid-phase extraction and liquid chromatographic quantitation of insect repellent *N,N*-diethyl-toluamide in plasma. *J. Pharm. Biomed. Anal*. 15:241–250.

Qiu, H., Jun, H. W., and McCall, J. W. 1998. Pharmacokinetics, formulation, and safety of insect repellent *N,N*-diethyl-3-methylbenzamide (DEET): A review. *J. Am. Mosquito Control Assoc*. 14:12–27.

Reifenrath, W. G., and Robinson, P. B. 1982. *In vitro* skin evaporation and penetration characteristics of mosquito repellents. *J. Pharm. Sci*. 71:1014–1018.

Riviere, J. E. 1999. *Comparative pharmacokinetics: Principles, techniques and applications*. Ames: Iowa State University Press.

Riviere, J. E., and Monteiro-Riviere, N. A. 1991. The isolated perfused porcine skin flap as an in vitro model for percutaneous absorption and cutaneous toxicology. *Crit. Rev. Toxicol*. 21:329–344.

Riviere, J. E., Bowman, K. F., Monteiro-Riviere, N. A. Dix, L. P., and Carver, M. P. 1986. The isolated perfused porcine skin flap (IPPSF). I. A novel *in vitro* model for percutaneous absorption and cutaneous toxicology studies. *Fundam. Appl. Toxicol*. 7:444–453.

Riviere, J. E., Williams, P. L., Hillman, R., and Mishky, L. 1992. Quantitative prediction of transdermal iontophoretic delivery of arbutamine in humans using the in vitro isolated perfused porcine skin flap (IPPSF). *J. Pharm. Sci*. 81:504–507.

Riviere, J. E., Brooks, J. D., Williams, P. L., and Monteiro-Riviere, N. A. 1995. Toxicokinetics of topical sulfur mustard penetration, disposition, and vascular toxicity in isolated perfused porcine skin. *Toxicol. Appl. Pharmacol*. 135:25–34.

Riviere, J. E., Monteiro-Riviere, N. A., and Baynes, R. E. 2002. Gulf War related exposure factors influencing topical absorption of ^{14}C-permethrin. *Toxicol. Lett.*, 135:61–71.

Ross, J. S., and Shah, J. C. 2000. Reduction in skin permeation of *N,N*-diethyl-*m*-toluamide (DEET) by altering the skin/vehicle partition coefficient. *J. Control. Release* 67:211–212.

Selim, S., Hartnagel, R. E., Osmitz, T. G., Gabriel, K. L., and Schoenig, G. P. 1995. Absorption, metabolism, and excretion of *N,N*-diethyl-*m*-toluamide following dermal application to human volunteers. *Fundam. Appl. Toxicol*. 25:95–100.

Stinecipher, J., and Shah, J. 1997. Percutaneous permeation of N,N-diethyl-m-toluamide (DEET) from commercial mosquito repellents and the effect of solvent. *J. Toxicol. Environ. Health* 52:119–135.

Wessely, S. 2001. Ten years on: What do we know about the Gulf War Syndrome? *Clin. Med. JRCPL* 1:28–37.

Wester, R. C., and Maibach, H. I. (1993). Animal models for percutaneous absorption. In *Health risk assessment: Dermal and inhalation exposure and absorption of toxicants*, ed. R. G. M. Wang, J. B. Knowls, and H. I. Maibach, pp. 89–103. Boca Raton, FL: CRC Press.

Wester, R. C., Melendres, J., Sedik, L., Maibach, H. I., and Riviere, J. E. 1998. Percutaneous absorption of salicylic acid, theophylline, 2,4-dimethylamine, diethyl hexylphthalic acid, and *p*-aminobenzoic acid in the isolated perfused porcine skin flap compared to man. *Toxicol. Appl. Pharmacol*. 151:159–165.

Young, D. G., and Evans, S. 1998. Safety and efficacy of DEET and permethrin in the prevention of arthropod attack. *Military Med*. 163:324–330.

Zhang, Z., Riviere, J. E., and Monteiro-Riviere, N. A. 1995. Topical sulfur mustard induces changes in prostaglandins and interleukin-1α in isolated perfused porcine skin. *In Vitro Toxicol*. 8:149–158.