JPP Journal of Pharmacy and Pharmacology

JPP 2004, 56: 621–628

Received November 19, 2003
Accepted February 16, 2004
DOI 10.1211/0022357023402
ISSN 0022-3573

In-vitro permeation of the insect repellent N,N-diethyl-m-toluamide (DEET) and the sunscreen oxybenzone

Xiaochen Gu, Sreeneeranj Kasichayanula, Daryl J. Fediuk and Frank J. Burczynski

Abstract

The permeation behaviours of the insect repellent N,N-diethyl-m-toluamide (DEET) and the sunscreen oxybenzone were assessed in a series of in-vitro diffusion studies, using piglet skin and poly (dimethylsiloxane) (PDMS) membrane. The transmembrane permeability of DEET and oxybenzone across piglet skin and PDMS membrane was dependent on dissolving vehicles and test concentrations. An enhanced permeation increase across piglet skin was found for DEET and oxybenzone when both compounds were present in the same medium (DEET: 289% in propylene glycol, 243% in ethanol and 112% in poly(ethylene glycol) (PEG-400); oxybenzone: 139% in PEG-400, 120% in propylene glycol and 112% in ethanol). Permeation enhancement was also observed in PDMS membrane (DEET: 207% in ethanol, 124% in PEG-400 and 107% in propylene glycol; oxybenzone: 254% in PEG-400, 154% in ethanol and 105% in propylene glycol). PDMS membrane was found to be a suitable candidate for in-vitro diffusion evaluations. This study shows that the permeations of the insect repellent DEET and the sunscreen oxybenzone were synergistically enhanced when they were applied simultaneously.

Introduction

Increased public awareness of protection against the West Nile virus and skin cancer has led to a dramatic rise in the application of topical insect repellent and sunscreen preparations. Concurrent use of insect repellents and sunscreens has gradually become a widely accepted practice in many countries. As they are intended to be topical use only preparations, active repellent and sun-blocking ingredients such as DEET and oxybenzone should remain on the surface of the skin with minimal systemic absorption. This is of particular importance for insect repellents because there are a large number of consumers who have concerns about the safety and toxicity of DEET in humans. Furthermore, insect repellents and sunscreens are mainly used at the discretion of individuals without fixed doses and application sequences. Potential chemical and physical interactions among active compounds from concurrent applications could not only alter the percutaneous characteristics of these components across various skin layers, but also compromise the protection efficacy of both products.

The transdermal absorption and systemic adverse effects of DEET have been studied and documented to a certain extent with variable conclusions (Staub et al 2002). It has been reported that systemic absorption of DEET following topical application ranged between 8 and 59% (Robbins & Cherniak 1986). Qiu et al (1997) reported a dermal absorption of DEET with a 2.5-h half-life and 18% systemic bioavailability in beagle dogs. The pharmacokinetic data for DEET in humans is relatively limited. The extent of transdermal absorption from topical application was reported to be 11–51% (Smith et al 1963; Feldmann & Maibach 1970; Spencer et al 1979). Systemic absorption in humans is responsible for the known adverse effects of DEET, including cutaneous or allergic reactions, hypotension, headaches, disorientation and encephalopathy (Miller 1982; Baynes et al 1997; Fradin 1998; Chaney et al 2002). Because there is no effective human vaccine currently available against the West Nile virus and most other repellents are

Faculty of Pharmacy, University of Manitoba, 50 Sifton Road, Winnipeg, Manitoba, Canada R3T 2N2

Xiaochen Gu, Sreeneeranj Kasichayanula, Daryl J. Fediuk, Frank J. Burczynski

Correspondence: Xiaochen Gu, Faculty of Pharmacy, University of Manitoba, 50 Sifton Road, Winnipeg, Manitoba, Canada R3T 2N2.
E-mail: xgu@cc.umanitoba.ca

Acknowledgments and funding: This study was supported through an operating grant from the Manitoba Institute of Child Health (MICH) and an infrastructure grant from the Canada Foundation for Innovation (CFI). Sreeneeranj Kasichayanula is the recipient of a graduate fellowship from the University of Manitoba and Daryl J. Fediuk is the recipient of a summer studentship from Merck Research Foundation.

either short-lasting or ineffective, Health Canada started the recommendation in summer 2003 that DEET could be used in infants as young as 6 months old (Koren et al 2003).

Commercial sunscreen preparations intended for summer outdoor activities contain at least five sun-blocking components as the active ingredients. The long-term toxicology of these sunscreen compounds has been poorly defined and quantified because of study data limitations. It is now known that active sunscreen ingredients permeate across the skin after topical application. Using the excised hairless mouse skin, Brand et al (2002) found that some commercial sunscreen preparations enhanced the dermal penetration of the herbicide 2,4-dichlorophenoxyacetic acid by as much as 81%. Concurrent application of two sunscreen compounds, oxybenzone and octyl methoxycinnamate, was shown to produce synergistic absorption of each across the stratum corneum in excised pig skin (Gupta et al 1999). Sunscreen chemicals have also been detected in the urine and breast milk of humans 48 h after topical application, indicating systemic absorption and distribution of these compounds in vivo (Hayden et al 1997). While dermatologists stress the importance of frequent and generous application of sunscreens for effective skin protection, the consequences of the percutaneous permeation and systemic absorption of active sunscreen ingredients should be addressed, especially when they are applied simultaneously with other topical preparations.

Studies have indicated that concurrent use of DEET and sunscreen reduced the protection efficacy (SPF) of the sunscreen by 33%, but not that of the repellent (Montemarano et al 1997; Murphy et al 2000). However, there are no data available regarding the rate and extent of percutaneous absorption from the concurrent use of insect repellents and sunscreens. The objective of this study was to evaluate the transdermal profiles of the active components from concurrent application of insect repellent and sunscreen preparations. Using both excised piglet skin and artificial poly (dimethylsiloxane) (PDMS) membrane, we measured the in-vitro transmembrane permeation characteristics of the insect repellent DEET and the sunscreen oxybenzone from a series of diffusion studies. The influence of vehicle, temperature and concentration of active ingredients on their permeation profiles was also assessed.

Materials and Methods

Chemicals and other materials

DEET and oxybenzone were obtained from Fluka Chemika GmbH (Buchs, Switzerland) and Riedel-de Haër GmbH (Seelze, Germany), respectively. Acetonitrile (HPLC grade), ethanol (HPLC grade), poly(ethylene glycol) (PEG-400), potassium phosphate monobasic, propylene glycol and sodium hydroxide were obtained from Fisher Chemicals (Fair Lawn, NJ). Glacial acetic acid was obtained from Mallinckrodt Chemicals (Paris, KY). Polyoxyethylene 20-oleyl ether (Brij 98) was obtained from Sigma Chemical Co (St Louis, MO). All chemicals, unless otherwise mentioned, were analytical grade. Deionized water was obtained from a Milli-Q pure water system (Millipore, Nepean, ON, Canada). PDMS membrane (0.005″) was purchased from Advanced Biotechnologies Inc (Silverdale, WA).

Skin preparation

Full-thickness skin of 3-week-old piglets was obtained from the University of Manitoba Glenlea Research Station. The animal use protocol was approved by the University of Manitoba Fort Garry Campus Protocol Management and Review Committee, and conducted according to current guidelines published by the Canadian Council on Animal Care. The piglet skin was kept at $-20\,^{\circ}C$ after collection. Before each study, the skin was thawed, rinsed with water and dried by blotting the surface with paper towels. The hair was shaved with a razor to provide a smooth skin surface, and the fat and underlayer skin tissues were removed with a scalpel. Skin was dermatomed to a thickness of $250\,\mu m$ using an electric dermatome (Padgette Instruments, Kansas City, MO). The integrity of the skin was carefully examined and only undamaged skin sections with even thickness were selected for the diffusion studies.

Diffusion study with piglet skin

The dissolving vehicles tested in the diffusion study included ethanol (50%), PEG-400 and propylene glycol. These compounds were selected because of their common use in the formulation of commercial insect repellents and sunscreens. DEET and oxybenzone were dissolved in the vehicles at $5\,mg\,mL^{-1}$, either individually or as a combination. Three replicates were conducted for each experiment.

Skin samples were presoaked in deionized water before being mounted onto static Franz-style diffusion cells (PermeGear Inc, Bethlehem, PA). The cell consisted of a 2-mL donor compartment and a 3.5-mL receptor compartment, with a $1.1\text{-}cm^2$ surface available for diffusion. Test solution (1 mL) was placed in the donor cell, while 3.5 mL of receptor medium was placed in the receptor cell. Receptor fluid was composed of pH 7.4 phosphate buffer containing 4% Brij 98. Use of a surfactant in the receptor medium was to facilitate the solubility of the highly lipophilic DEET and oxybenzone, thus maintaining 'sink conditions' in the diffusion experiments. The receptor fluid enhanced the solubility of the test components without affecting membrane or transmembrane diffusion characteristics (Lazar et al 1996; Gupta et al 1999). The receptor fluid was stirred continuously at 650 rpm and $45\,^{\circ}C$ using a Variomag magnetic stirrer (Daytona Beach, FL) to maintain homogeneity. Receptor fluid ($100\,\mu L$) was collected hourly for 6 h, followed by replenishment of the same volume of fresh buffer medium after each sampling interval. Samples were directly injected to the HPLC for concentration analysis.

Diffusion study with PDMS membrane

In-vitro diffusion studies were also conducted using PDMS membrane. The purposes of the studies were to compare the permeation characteristics of the compounds between

biological and artificial membranes, and to obtain detailed information on the transmembrane profiles of DEET and oxybenzone. The experimental conditions were identical to those described above. The concentrations tested were 1, 2.5, 5 and 10 mg mL^{-1} for DEET and oxybenzone, either individually or in combination. The experimental temperatures were 25, 37 and 45 °C, respectively. Each experiment was conducted in five replicates.

Solubility of DEET and oxybenzone

Separate tests were carried out to determine the solubility of both compounds in three dissolving vehicles as well as in the receptor fluid. In the single component tests, an excessive amount of DEET or oxybenzone was added to 10 mL of solvent to maintain two phases (liquid phase for DEET and solid phase for oxybenzone). The mixture was kept at 37 °C under constant agitation for 48 h. In the combined component tests, only one compound was added to 10 mL of solvent in an excessive amount to achieve a two-phase stage while the amount of the other compound was fixed at 10 mg mL^{-1} (i.e. the maximal test concentration in the diffusion studies). Samples were centrifuged for 10 min after 48 h, and the supernatant was further diluted for analysis. Four replicates were conducted for each study, and the concentrations of DEET and oxybenzone in each solvent were determined by HPLC.

HPLC assay

The concentrations of DEET and oxybenzone in all test samples were analysed simultaneously using an HPLC assay developed in our laboratory. The HPLC system consisted of Waters components (Waters Corporation, Milford, MA), including a 600 controller, a 616 pump, a 717 autosampler, a 996 photodiode array detector, and a Nova-Pak C_{18} column (3.9 × 150 mm, 4 μm). The system was operated by Millennium 32 software. The mobile phase was composed of acetonitrile and water (pH 2.83, adjusted with glacial acetic acid) at a ratio of 80:20. At a flow rate of 1 mL min^{-1}, DEET and oxybenzone were eluted at 1.7 and 2.4 min, respectively. Both compounds were detected at 254 nm with a detection limit of approximately 20 ng for each. The calibration linearity ranged between 50 and 2000 ng for both components. Validation and calibration were performed before and during analysis. The concentrations of DEET and oxybenzone in the samples were calculated based on average calibration curves ($n = 6$).

Data analysis

The permeation amount and percentage of the two test compounds were calculated based on their concentrations at each sampling interval. A steady-state diffusion flux was obtained from the linear portion of the diffusion curve (Martin 1993). Statistical analysis was performed using two-way ANOVA and Tukey's test (PC-SAS 8.02, SAS Institute Inc., Cary, NC). The following statistical analyses of the data were conducted: (a) overall permeation percentage and steady-state flux of DEET and oxybenzone across piglet skin among three test vehicles; (b) overall permeation percentage of DEET and oxybenzone across PDMS membrane among four test concentrations at 45 and 37 °C; (c) overall permeation percentage of DEET and oxybenzone across PDMS membrane between 45 and 37 °C; (d) solubility of DEET and oxybenzone among four test solvents. All differences were considered statistically significant at $P \leq 0.05$.

Results

Permeation across piglet skin

Three vehicles were studied in the diffusion study for their dissolving capacity and percutaneous characteristics. PEG-400 and propylene glycol are commonly used in emulsion-based lotions as auxiliary agents. While most commercial sunscreen products are now labelled as 'alcohol-free', ethanol and/or alcohol substitutes are still used in some insect repellent formulations either as solubilizing agents or for facilitating the quick drying of the application through instant evaporation. Figure 1 shows the overall permeation percentage of DEET and oxybenzone after 6 h in test vehicles across piglet skin. Table 1 shows the steady-state diffusion flux of DEET and oxybenzone in piglet skin.

The synergistic permeation of both DEET and oxybenzone was observed when the compounds were present in combination. The average increment in permeability of DEET was 289% in propylene glycol, 243% in ethanol and 112% in PEG-400. The average increment in permeability of oxybenzone was 139% in PEG-400, 120% in propylene glycol and 112% in ethanol. Compared to individual components, there was a significant increase in the permeation of DEET in ethanol and propylene glycol when oxybenzone was simultaneously present. Permeation of both DEET and oxybenzone in ethanol and propylene glycol was also significantly higher than in PEG-400. The impact of DEET and oxybenzone in combination was more significant on DEET than on oxybenzone. PEG-400 appeared to be an inert vehicle that produced minimal percutaneous permeation of DEET and oxybenzone, whether the compounds were tested individually or in combination.

Permeation across PDMS membrane

The permeation profiles of DEET and oxybenzone across PDMS membrane were similar to those obtained from piglet skin. Permeation of the compounds was dependent on the dissolving vehicles and test concentrations. Tables 2 and 3 show the overall permeation percentage of DEET and oxybenzone after 6 h in test vehicles at 45 and 37 °C, respectively. Diffusion results at 25 °C showed similar patterns for both DEET and oxybenzone but lower overall permeation percentage (data not shown).

The increment in permeability of DEET was 207 ± 18% (mean ± s.e.m., range 152–308%) in ethanol, 124 ± 22% (0–250%) in PEG-400 and 107 ± 2% (0–118%) in propylene glycol. The increment in permeability of oxybenzone

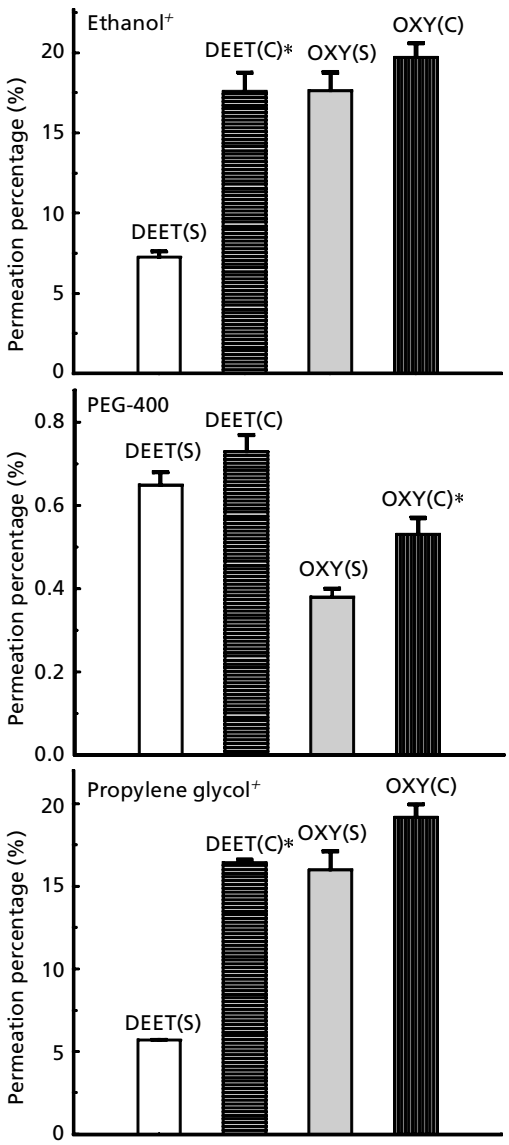

Figure 1 Overall permeation percentages of DEET and oxybenzone (OXY) across piglet skin after 6 h at 5 mg mL^{-1} and 45 °C (S, single; C, combined; *significant difference from single component; $^{+}$ significant difference from PEG-400).

was 254 ± 62% (124–555%) in PEG-400, 154 ± 7% (123–179%) in ethanol and 105 ± 2% (0–120%) in propylene glycol. The permeation amount of DEET and oxybenzone increased with the increase in test concentration. Figure 2 shows the relationship between steady-state flux and test concentration of DEET and oxybenzone at 45 °C.

The transmembrane permeation of both DEET and oxybenzone in combination increased significantly in ethanol compared to their individual single values at 45 and 37 °C. There were no changes in permeation of DEET and oxybenzone in propylene glycol. Permeation characteristics of DEET and oxybenzone in ethanol were significantly different from those in PEG-400 and propylene glycol. Between PEG-400 and propylene glycol the permeation profiles of oxybenzone were significantly different, but the permeation profiles of DEET were very similar.

Comparing identical experimental concentration and temperature (5 mg mL^{-1} and 45 °C), the permeability results from PDMS membrane in ethanol and PEG-400 were higher than those obtained from piglet skin, while the permeability results from PDMS membrane in propylene glycol were lower than those obtained from piglet skin. The values were 2.2 ± 0.4 (mean ± s.e.m., range 1.4–3.3) in ethanol, 5.6 ± 0.9 (4.0–7.8) in PEG-400 and 0.6 ± 0.1 (0.3–0.8) in propylene glycol. As an in-vitro membrane model commonly used for transdermal evaluation, PDMS membrane was found to be useful for in-vitro evaluations from which preliminary information on transmembrane profiles was obtained. The difference in permeation results between biological and artificial membranes may be attributed to variations in the thickness of the piglet skin and the PDMS membrane, and the variable permeability of the test vehicles across the study membranes.

Solubility of DEET and oxybenzone

Table 4 shows the solubility of DEET and oxybenzone in the three dissolving media and the receptor fluid. Compared to the single compound in the same vehicle, there was no significant change in solubility of DEET and oxybenzone in PEG-400 and in the receptor fluid when the two compounds were combined. This suggests that there was no molecular interaction between DEET and oxybenzone in these two media. The solubility of DEET in propylene glycol was significantly reduced when oxybenzone was

Table 1 Steady-state flux (μg cm^{-2} h^{-1}) of DEET and oxybenzone in piglet skin.

Solvent	DEET		Oxybenzone	
	Single	Combined	Single	Combined
Ethanol**	69.68 ± 7.37	142.41 ± 5.82*	194.90 ± 29.53	220.62 ± 22.82
Propylene glycol**	15.94 ± 1.36	34.75 ± 1.23*	32.32 ± 0.29	112.73 ± 1.88*
PEG-400**	1.63 ± 0.26	2.82 ± 0.09*	0.93 ± 0.25	1.82 ± 0.63

n = 3; mean ± s.e.m. *Significant difference from single-component test ($P \leq 0.05$). **Significant difference among vehicles ($P \leq 0.05$).

Table 2 Permeation percentage of DEET and oxybenzone across PDMS membrane after 6 h at 45 °C.

Solvent	DEET		Oxybenzone	
	Single	Combined	Single	Combined
Ethanol** ($mg\,mL^{-1}$)				
1	16.9 ± 1.2	26.2 ± 0.9*	55.6 ± 1.5	76.6 ± 2.8*
2.5	11.9 ± 0.6	29.6 ± 0.8*	47.0 ± 1.4	73.1 ± 1.4*
5	13.3 ± 1.3	24.4 ± 0.6*	36.6 ± 2.6	65.5 ± 1.1*
10	10.7 ± 0.3	20.2 ± 0.5*	28.8 ± 0.8	38.3 ± 1.1*
PEG-400 ($mg\,mL^{-1}$)				
1	3.9 ± 0.2	5.4 ± 0.2	1.7 ± 0.1	8.7 ± 0.8*
2.5	3.5 ± 0.1	5.4 ± 0.2	1.7 ± 0.1	3.8 ± 0.1
5	4.1 ± 0.1	5.7 ± 0.2	1.7 ± 0.1	2.1 ± 0.1
10	5.2 ± 0.3	5.5 ± 0.2	1.0 ± 0.0	1.6 ± 0.1
Propylene glycol*** ($mg\,mL^{-1}$)				
1	4.8 ± 0.1	5.4 ± 0.4	9.5 ± 1.0	9.8 ± 1.2
2.5	4.7 ± 0.2	4.7 ± 0.2	8.9 ± 1.0	8.9 ± 0.5
5	4.7 ± 0.2	4.9 ± 0.2	10.6 ± 0.5	10.9 ± 1.0
10	4.6 ± 0.3	4.6 ± 0.1	9.3 ± 0.5	9.2 ± 0.4

n = 5, mean ± s.e.m. *Significant difference from single-component test ($P \leq 0.05$). **Significant difference from PEG-400 and propylene glycol ($P \leq 0.05$). ***Significant difference from PEG-400 (oxybenzone only, $P \leq 0.05$).

Table 3 Permeation percentage of DEET and oxybenzone across PDMS membrane after 6 h at 37 °C.

Solvent	DEET		Oxybenzone	
	Single	Combined	Single	Combined
Ethanol** ($mg\,mL^{-1}$)				
1	16.5 ± 0.7	25.0 ± 2.6*	49.7 ± 2.5	61.2 ± 2.2*
2.5	9.8 ± 0.4	30.2 ± 1.1*	39.3 ± 0.6	62.3 ± 6.2*
5	11.3 ± 0.8	23.1 ± 1.5*	31.7 ± 0.8	52.1 ± 0.8*
10	9.6 ± 0.6	20.9 ± 0.6*	22.4 ± 0.6	40.0 ± 1.3*
PEG-400 ($mg\,mL^{-1}$)				
1	5.0 ± 0.2	12.5 ± 1.9*	1.1 ± 0.1	6.1 ± 0.1*
2.5	8.4 ± 0.2	6.3 ± 1.6	1.5 ± 0.1	2.8 ± 0.1
5	7.8 ± 0.5	4.4 ± 0.7	1.4 ± 0.0	1.9 ± 0.1
10	5.7 ± 0.4	4.0 ± 0.1	0.9 ± 0.0	1.2 ± 0.0
Propylene glycol*** ($mg\,mL^{-1}$)				
1	4.0 ± 0.1	4.7 ± 0.3	7.4 ± 0.7	8.5 ± 0.8
2.5	3.9 ± 0.2	4.2 ± 0.0	8.4 ± 0.2	8.4 ± 0.1
5	3.6 ± 0.1	3.9 ± 0.1	7.4 ± 0.3	8.9 ± 0.7
10	4.5 ± 0.1	4.5 ± 0.2	9.4 ± 0.8	9.4 ± 0.6

n = 5, mean ± s.e.m. *Significant difference from single-component test ($P \leq 0.05$). **Significant difference from PEG-400 and propylene glycol ($P \leq 0.05$). ***Significant difference from PEG-400 (oxybenzone only, $P \leq 0.05$).

added, but DEET did not affect the solubility of oxybenzone. The solubility of both DEET and oxybenzone in ethanol significantly increased when the compounds were present in combination, which may be attributed to the polarity of the solvent. The solubility profiles of DEET and oxybenzone were distinctly different among the four test solvents. 'Sink condition' was maintained in all diffusion studies based on the solubility data.

Discussion

The permeability of DEET and oxybenzone across piglet skin was clearly dependent on the dissolving vehicle, which differentiated from the steady-state flux values (Table 1). This may be associated with the ability of a solvent to solubilize the solutes as well as to interact with the skin membrane. A higher flux from a given vehicle probably reflects an increased permeability of the solute across the barrier due to vehicle–membrane interaction (Jiang et al 1998). Our results showing permeability in the ranking order of ethanol > propylene glycol > PEG-400 are identical to those reported in previous studies in which the effect of vehicles on permeation of either DEET or sunscreen components was evaluated (Jiang et al 1998; Ross & Shah 2000). The increase in permeability when DEET and oxybenzone were concurrently present clearly suggests the potential for both compounds to act as permeation enhancers, which would result in transdermal absorption synergy, an undesirable property in real-life application situations. Ethanol and other alcohol substitutes are known as percutaneous penetration enhancers, so they are not appropriate vehicles for any insect repellent and sunscreen formulations. Vehicles such as PEG-400 and propylene glycol may be suitable for topical repellent and sunscreen preparations because both DEET and oxybenzone showed relatively low overall permeation percentage in these two vehicles. The solubilizing capacity of PEG-400 may also result in a strong affinity to lipophilic compounds such as DEET and oxybenzone, rendering them incapable of penetrating across the membrane to a significant extent. Furthermore, the high vehicle viscosity of PEG-400 and propylene glycol can reduce the permeation process of DEET (Ross & Shah 2000). Studies have indicated greater penetration of sunscreen chemicals from emulsion-based formulations than from petrolatum-based preparations (Treffel & Gabard 1996). This information will benefit formulators in designing new insect repellent and/or sunscreen preparations in which further improvements can be made by selecting appropriate excipients and/or formulations to increase local retention and minimize the percutaneous systemic absorption of the various active ingredients.

The permeability of DEET and oxybenzone across the PDMS membrane was also dependent on the dissolving vehicles and application concentrations. Twist and Zatz (1990) reported that solvents such as water, PEG-400 and propylene glycol behave as ideal vehicles across PDMS

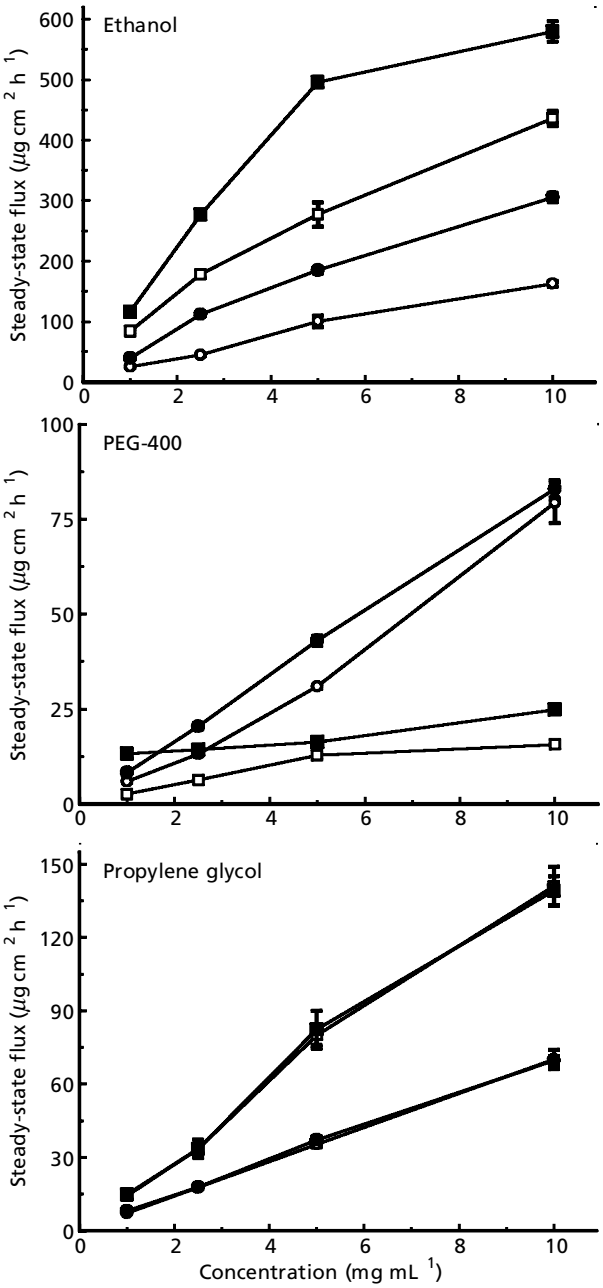

Figure 2 Steady-state flux of DEET and oxybenzone (OXY) across PDMS membrane at 45°C (○, single DEET; □, single OXY; ●, DEET combined with OXY; ■, OXY combined with DEET).

membrane, and that the permeation and diffusion flux of a solute from these vehicles are functions of permeant activity independent of the solvent. The ranking order of permeation from PDMS membrane was identical to what was determined in the piglet skin, which indicated the suitability of PDMS membrane as an in-vitro model for transmembrane characterization. Commercially available repellent and sunscreen products are commonly composed of multiple active and non-medicinal ingredients to facilitate protection efficacy and improve formulation stability, elegancy and application convenience. By using artificial membranes for initial and individual evaluation of the transmembrane properties of certain active compounds and excipients, it is possible to reduce the experimental cost associated with using a large quantity of animal or human skin, and minimize the data variations frequently encountered with biological membranes.

The steady-state diffusion flux of DEET and oxybenzone across PDMS membrane increased with the increasing test concentration used (Figure 2). Compared to single component, the flux of DEET and oxybenzone in combined samples was higher in ethanol and PEG-400. Propylene glycol did not change the diffusion flux of DEET and oxybenzone. No definitive correlation was observed between overall permeation percentage and test concentration. The general tendency was that the permeation percentage decreased when concentration increased. High concentration results in the presence of more solute molecules in the vehicle, which consequently leads to a reduction in the thermodynamic activity of the solute as well as availability of the solute from the vehicle to the diffusional process. The recommendation of using sunscreen liberally in ample amounts has practical merits. A large amount of sunscreen spread over the surface of the skin not only effectively diffuses or absorbs UV radiation, but also saturates the stratum corneum, preventing the active components from further diffusing and penetrating into deeper skin layers. If possible, it is always appropriate to apply insect repellent sparsely or wear protective clothing. This is of clinical importance to children and the elderly, as skin conditions as well as metabolic functions in these population groups are unique because of age factors. When repellent and sunscreen are applied concurrently, sunscreen should be applied first to block the surface of the skin before repellent is used on top of the sunscreen. This application practice normally results in maximal protection effects for both products.

The effect of temperature on the transmembrane permeation of DEET and oxybenzone is perhaps more complex in nature than that of preparation vehicles and application concentrations. High temperatures theoretically promote the diffusion process by increasing molecular movement, resulting in enhanced transmembrane permeability. Numerous studies have achieved improved percutaneous penetration of various compounds when they were applied at elevated temperatures (Klemsdal et al 1992; Clarys et al 1998). In our PDMS study, 45°C conditions produced slightly higher permeation of the compounds than 37°C, and the temperature appeared to influence oxybenzone more than DEET, especially when using ethanol and PEG-400 as the vehicle. However, statistical analysis of the data between the two temperatures did not reveal statistically significant differences in the overall permeation percentage of DEET and oxybenzone. Clarys et al (2001) have recently concluded from an in-vivo human study that temperature does not significantly alter the adsorption of sunscreen into the stratum corneum. The study was conducted at 31 and 40°C, and only the penetration patterns of sunscreen into the stratum corneum were measured through skin stripping. The authors did find a loss of sunscreen from stratum corneum over time, but did not provide an explana-

Table 4 Solubility ($g\,mL^{-1}$) of DEET and oxybenzone in various dissolving media.

Solvent	DEET		Oxybenzone	
	Single	Combined	Single	Combined
Ethanol**	1.63 ± 0.01	2.22 ± 0.10*	0.36 ± 0.05	0.65 ± 0.04*
PEG-400**	3.86 ± 0.17	3.65 ± 0.07	1.58 ± 0.11	1.34 ± 0.06
Propylene glycol**	0.57 ± 0.03	0.25 ± 0.01*	0.46 ± 0.04	0.55 ± 0.02
Receptor fluid**	0.07 ± 0.01	0.01 ± 0.00	0.002 ± 0.000	0.002 ± 0.000

n = 4, mean ± s.e.m. *Significant difference from single-component test ($P \leq 0.05$). **Significant difference among media ($P \leq 0.05$).

tion. Insect repellents and sunscreens are usually applied to exposed skin at elevated environmental temperatures. Most users also take part in physical activities such as running, swimming and jogging after application. There are some other physiological factors that could contribute to the overall transdermal absorption of these preparations in association with elevated environmental and/or body temperatures, such as increased blood flow within the skin layers, sweating and skin hydration, the loss of applied doses through evaporation or washing, UV intensity and exposure time. All these factors have to be considered as a whole in order to realistically assess the skin penetration and absorption of insect repellents and sunscreens. While in-vitro diffusion studies can provide useful information to a certain degree, in-vivo studies in either animals or humans would be more appropriate for such transdermal absorption assessment.

Conclusions

The diffusion results from this preliminary study indicate an increased permeation of the insect repellent DEET and the sunscreen oxybenzone when used concurrently. This synergistic permeation is dependent on the dissolving vehicles and application concentrations. Ethanol was not considered a satisfactory vehicle for insect repellents or sunscreens because it enhances the permeation of DEET and oxybenzone across the membranes. The permeability of the insect repellent DEET was also affected more significantly than that of the sunscreen oxybenzone. PDMS membrane was found to be a suitable artificial membrane for preliminary evaluation of the transmembrane characterization of DEET and oxybenzone. Although the study was conducted under in-vitro conditions, percutaneous patterns of this kind would be considered undesirable in-vivo. As a result, concurrent application of DEET-based insect repellents and sunscreens could potentiate systemic transdermal absorption in consumers when both products are applied simultaneously. Since both insect repellents and sunscreens are now extensively used by the general public, the percutaneous characteristics of concurrent application of both preparations warrant further systematic evaluations, both in-vitro and in-vivo.

References

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

Brand, R. M., Spalding, M., Mueller, C. (2002) Sunscreen can increase dermal penetration of 2,4-dichlorophenoxyacetic acid. *J. Toxicol.* **40**: 827–832

Chaney, L. A., Rockhold, R. W., Hume, A. S. (2002) Cardiorespiratory effects following acute exposure to pyridostigmine bromide and/or N,N-diethyl-m-toluamide (DEET) in rats. *Int. J. Toxicol.* **21**: 287–300

Clarys, P., Alewaeters, K., Jadoul, A., Manadas, R. O., Preat, V. (1998) In-vitro percutaneous penetration through hairless rat skin: influence of temperature, vehicle and penetration enhancers. *Eur. J. Pharm. Biopharm.* **46**: 279–283

Clarys, P., Gabard, B., Lambrecht, R., Barel, A., Bieli, E., Ludi, S. (2001) There is no influence of a temperature rise on in-vivo adsorption of UV filters into the stratum corneum. *J. Dermatol. Sci.* **27**: 77–81

Feldmann, R. J., Maibach, H. I. (1970) Absorption of some organic compounds through the skin in man. *J. Invest. Dermatol.* **54**: 39–404

Fradin, M. S. (1998) Mosquitoes and mosquito repellents: a clinician's guide. *Ann. Intern Med.* **128**: 931–940

Gupta, V. K., Zatz, J. L., Rerek, M. (1999) Percutaneous absorption of sunscreen through micro-yucatan pig skin in-vitro. *Pharm. Res.* **16**: 1602–1607

Hayden, C. J., Roberts, M. S., Benson, H. A. E. (1997) Systemic absorption of sunscreen after topical application. *Lancet* **350**: 863–864

Jiang, R., Benson, H. A. E., Cross, S. E., Roberts, M. S. (1998) In-vitro human epidermal and polyethylene membrane penetration and retention of the sunscreen oxybenzone from a range of solvents. *Pharm. Res.* **15**: 1863–1868

Klemsdal, T. O., Gjesdal, K., Bredesen, J. E. (1992) Heating and cooling of the nitroglycerine patch application area modify the plasma level of nitroglycerine. *Eur. J. Clin. Pharmacol.* **43**: 625–628

Koren, G., Matsui, D., Bailey, B. (2003) DEET-based insect repellents: safety implications for children and pregnant and lactating women. *Can. Med. Assoc. J.* **169**: 209–212

Lazar, G. M., Baillet, A., Fructus, A. E., Arnaud-Battandier, J., Ferrier, D., Marty, J. P. (1996) Evaluation of in-vitro percutaneous absorption of UV filters used in sunscreen formulations. *Drug Cosmet. Ind.* **158**: 50–62

Martin, A. (1993) *Physical pharmacy*. 4th edn, Lea & Febiger, Philadelphia, p 325

Miller, J. D. (1982) Anaphylaxis associated with insect repellent. *N. Engl. J. Med.* **307**: 1341–1342

Montemarano, A. D., Gupta, R. K., Burge, J. R., Klein, K. (1997) Insect repellents and the efficacy of sunscreen. *Lancet* **349**: 1670–1671

Murphy, M. E., Montemarano, A. D., Debboun, M., Gupta, R. (2000) The effect of sunscreen on the efficacy of insect repellent: a clinical trial. *J. Am. Acad. Dermatol.* **43**: 219–222

Qiu, H., Jun, H. W., Tao, J. (1997) Pharmacokinetics of insect repellent N,N-diethyl-m-toluamide in beagle dogs following intravenous and topical routes of administration, *J. Pharm. Sci.* **86**: 514–516

Robbins, P. J., Cherniak, M. G. (1986) Review of the biodistribution and toxicity of the insect repellent N,N-diethyl-m-toluamide (DEET). *J. Toxicol. Environ. Health* **18**: 503–525

Ross, J. S., 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**: 211–221

Smith, C. N., Gilbert, I. H., Gouck, H. K., Bouman, M. C., Acree Jr, F., Schmidt, C. H. (1963) Factors affecting the protection period of mosquito repellents. *USDA Tech. Bull.* No. 1285

Spencer, T. S., Hill, J. A., Feldmann, R. J., Maibach, H. I. (1979) Evaporation of diethyltoluamide from human skin in-vivo and in-vitro. *J. Invest. Dermatol.* **72**: 317–319

Staub, D., Debrunner, M., Amsler, L., Steffen, R. (2002) Effectiveness of a repellent containing DEET and EBAAP for preventing tick bites. *Wilderness Environ. Med.* **13**: 12–20

Treffel, P., Gabard, B. (1996) Skin penetration and sun protection factor of ultra-violet filters from two vehicles. *Pharm. Res.* **13**: 770–774

Twist, J. N., Zatz, J. L. (1990) A model for alcohol-enhanced permeation through poly(dimethylsiloxane) membranes. *J. Pharm. Sci.* **79**: 28–31