Concurrent application of insect repellent picaridin or DEET with sunscreens has become prevalent due to concerns on West Nile virus and skin cancer. The objectives of this study were to characterize the percutaneous permeation of picaridin and sunscreen oxybenzone from commercially available preparations and to compare the differences in permeability between picaridin and DEET in association with oxybenzone. In vitro diffusion studies were carried out to measure transdermal permeation of picaridin and oxybenzone from four different products, using various application concentrations and sequences. Results were then compared to those of repellent DEET and sunscreen oxybenzone under identical conditions. Transdermal permeation of picaridin across human epidermis was significantly lower than that of DEET, both alone and in combination with oxybenzone. Concurrent use resulted in either no changes or suppression of transdermal permeation of picaridin and oxybenzone. This finding was different from concurrent use of DEET and oxybenzone in which a synergistic permeation enhancement was observed. In addition, permeation of picaridin, DEET and oxybenzone across human epidermis was dependent on application concentration, use sequence, and preparation type. It was concluded from this comparative study that picaridin would be a better candidate for concurrent use with sunscreen preparations in terms of minimizing percutaneous permeation of the chemicals.

1. Introduction
Insect repellents and sunscreens are the most practical, cost-effective and well-accepted defence against vector-borne diseases and skin cancer, and have been extensively used as over-the-counter specialty products by the general public for decades (Dadlani and Orlow 2008; Fradin 1998; Katz et al. 2008; Keeney et al. 2009). Concurrent use of repellents and sunscreens has become prevalent among special work forces whose job responsibilities require extended daily outdoor exposure and for those seeking healthy and active lifestyles. Designed as topical preparations, active repellent and sunscreen ingredients should remain on the skin surface for optimal protection efficacy. Systemic absorption of the active ingredients is considered neither productive nor desirable (Abdel-Rahman et al. 2004; Hexsel et al. 2008; Koren et al. 2003; Robbins and Cherniak 1986). Previous studies have found a systemic absorption of repellent DEET (N,N-diethyl-m-toluamide, OFF®) and sunscreen oxybenzone from topical skin applications (Hayden et al. 1997; Qiu et al. 1997). We have previously reported a synergistic percutaneous enhancement between DEET and oxybenzone from a series of studies (Gu et al. 2005; Kasichayanula et al. 2007; Wang and Gu 2007).

DEET has been the dominant insect repellent on the market for more than five decades. Since 2000, several newer insect repellents have been registered and approved for civil use in Europe and the US. These include synthetic picaridin (2-(2-hydroxyethyl)-1-piperidinecarboxylic acid 1-methylpropyl ester, Bayrepel®, Icaridin®) and IR3535® (ethyl butylacetylaminopropionate), and natural lemon eucalyptus oil (active ingredient p-menthane 3,8-diol) and Citronella oil (Carroll et al. 2008; Katz et al. 2008; Naucke et al. 2007; Zhu et al. 2008). Recently we reported the percutaneous characteristics of picaridin and oxybenzone in 50% ethanolic solution across human skin and artificial PDMS membrane (Gu and Chen 2009). In this study, we characterized the transdermal permeation of picaridin and oxybenzone from several commercially available products and compared the results to those of DEET and oxybenzone under identical experimental conditions. We also investigated the influences of application concentrations, treatment sequences, and formulation types on the permeability of picaridin and oxybenzone from commercial products. The primary objective of the study was to determine whether or not picaridin would be a practical candidate for developing composite repellent/sunscreen preparations.

2. Investigations, results and discussion
2.1. Permeation of picaridin
We tested three commercially available picaridin-based repellent sprays that contained picaridin at 5%, 7%, and 20%,
Figure 1 shows the accumulated permeation percentage of picaridin across human epidermis from the three repellent sprays after 6 h. Adding sunscreen lotion to Product A (5% picaridin) and Product B (7% picaridin) did not significantly alter permeation characteristics of the repellent. However, product C (20% picaridin) did produce significant suppression of picaridin permeation across epidermis. The permeation suppression ranged from 201–214% for single use and 163–191% for concurrent use. In comparison to a previous study using ethanolic solution (Gu and Chen 2009) picaridin from commercial preparations produced a similarly low permeability across human epidermis. We also tested product B (7% picaridin) by mixing it with sunscreen preparation at different proportions and application sequences. Fig. 2 shows the accumulated permeation percentage of picaridin across human epidermis from these experiments.
significant in comparison to single use. Placing picaridin spray on top of sunscreen without premixing also significantly reduced the permeation by 82%. This was mainly attributed to a physical sunscreen barrier between the repellent spray and the skin membrane. On the other hand, mixing picaridin and sunscreen at 2:1 (w:w) did not alter its transdermal permeation. When picaridin spray was placed beneath sunscreen lotion without premixing, transdermal permeation of picaridin was increased by 32%. This might have resulted from the occlusive effect of having a sunscreen layer on top of the repellent spray. Diffusion across the epidermis would be an easier path for the picaridin molecules under this condition.

2.2. Permeation of oxybenzone

We tested one commercial sunscreen lotion that contained 4% oxybenzone as one of the active ingredients. Fig. 3 shows the accumulated permeation of oxybenzone across human epidermis after 6 h. No significant change in oxybenzone permeation occurred when the sunscreen was mixed with picaridin spray of product A (5% picaridin) and product B (7% picaridin). However, permeation of oxybenzone was significantly suppressed by 34% when picaridin concentration was increased to 20% (product C). Significant suppression of oxybenzone permeation was also observed when repellent and sunscreen were mixed at ratios of 1:2 and 2:1 (w:w), and when repellent and sunscreen preparations were placed on either the top or the bottom of each other without mixing. Transdermal suppression from these four application conditions ranged 17-74% in comparison to single sunscreen use.

Commercial sunscreen products are composed of multiple active and auxiliary compounds to produce effective and stable preparations. It was apparent that the presence of other excipients and auxiliary compounds to produce effective and stable preparations. It was apparent that the presence of other excipients modified diffusion and permeation of oxybenzone within the skin membrane. Compared to a single ethanolic solution of oxybenzone investigated in a previous study (Gu and Chen 2009), the overall permeation of oxybenzone investigated from sunscreen lotion was much lower. This might have been attributed to the lotion medium and associated viscosity from which oxybenzone had to diffuse through before reaching the skin surface. Partition of oxybenzone between lotion vesicles and skin membrane might have also suppressed the diffusivity of the molecules, subsequently leading to low permeation of oxybenzone across human epidermis.

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2.3. Comparison of picaridin and DEET

We were particularly interested in comparing transdermal permeation between picaridin and DEET from commercially available preparations, because we had observed a synergistic enhancement between DEET and oxybenzone from previous studies (Gu et al. 2005; Wang and Gu 2007), and the general public would buy and apply these products. Therefore, we compared the results obtained from this study to those of DEET and oxybenzone under identical experimental conditions. Figure 4 shows the comparative results of picaridin and DEET in association with oxybenzone from 5 different study groups. Permeation of DEET was significantly larger than that of picaridin in all study groups (3–66 times). The synergistic enhancement between DEET and oxybenzone and the occlusive effect of sunscreen lotion on DEET permeation were both evident from the data, as mixing DEET and sunscreen at 1:2 (w:w) and placing the sunscreen on top of the DEET spray led to an over 60-fold increase in DEET permeation. DEET also demonstrated a significantly greater diffusivity than picaridin. Its permeation across both sunscreen layer and epidermis was 46 times higher than picaridin under the same conditions. Mixing DEET and oxybenzone at 2:1 (w:w) also produced a 36-fold permeation increase than mixing picaridin and oxybenzone.

Figure 5 shows the comparative results of oxybenzone permeation in association with either picaridin or DEET spray. Permeation of oxybenzone with DEET was significantly larger (6–30 times) than that of oxybenzone with picaridin. The enhancement effect of DEET on oxybenzone permeation was clearly evident when DEET and sunscreen were mixed at ratios of 2:1 or 1:2 (w:w, 18 and 30-fold, respectively), and when DEET spray was placed under sunscreen lotion (a 14-fold increment). On the other hand, mixing picaridin spray with sunscreen lotion generally showed no change or even suppression of oxybenzone permeation from these study groups. Comparison of DEET and picaridin demonstrated different characteristics of skin permeation between the two repellent compounds. Their permeation properties also affected sunscreen ingredients when both repellent and sunscreen preparations were applied concurrently. DEET and oxybenzone permeated synergistically across the skin when mixed together, while picaridin and oxybenzone were either unaffected or showed a suppressive skin permeation when used concurrently. Reports have
also indicated that mixing DEET-based repellents and sunscreen products reduced Sun Protection Factor (SPF) of the sunscreens but not the repellent efficacy of DEET (Montemarano et al. 1997; Murphy et al. 2000). It is unknown whether or not mixing picaridin and a sunscreen preparation would alter protection efficacy.

Concurrent use of insect repellents and sunscreens during the summer months has become both necessary and beneficial in protecting individuals from vector-borne diseases and sunburns. Based on comparative data obtained from our studies, and from perspective of avoiding unnecessary systemic exposure to the chemicals, picaridin would be a more appropriate choice than DEET for concurrent application with sunscreen products.

Repellent and sunscreen products are generally applied under the discretion of individual users, with no medically recommended dose and application approach. It became evident from this study that application sequence is particularly important to repellent and sunscreen products, as inadvertent misuse may not only compromise protection efficacy but also increase systemic absorption of the active ingredients. Sunscreens should always be applied prior to repellents to reduce transdermal penetration of the active repellent ingredient. This is also an appropriate and practical approach because sunscreens are designed for skin protection while repellents are intended for averting insect bites. Applying repellent underneath sunscreens would diminish the repellence efficacy and promote transdermal absorption, which has been proven for both picaridin and DEET. It would be beneficial to illustrate correct application methods on repellent and sunscreen product labels so that consumers can follow these directions safely and effectively.

In conclusion, percutaneous permeation characteristics of the newer repellent picaridin in association with sunscreen oxybenzone were different from those of DEET and oxybenzone in vitro. Picaridin possessed smaller permeability across human epidermis than DEET, and its permeation was not overly affected when sunscreen preparation was simultaneously present. Permeability of oxybenzone was generally reduced when mixed with commercially available picaridin spray products. It was therefore concluded that picaridin would be a better candidate for concurrent use with sunscreen preparations in terms of minimizing percutaneous permeation. This might also provide a justification for developing combined repellent/sunscreen products that would be beneficial to specialty outdoor workforces and recreational individuals alike. More formulation exploration and product testing are ongoing to produce a quality picaridin/sunscreen preparation with satisfactory stability, protection efficacy, and acceptable cost.

3. Experimental

3.1. Materials

The following materials were used for the experiments: picaridin standard (Lanxess Corporation, Pittsburgh, Pennsylvania, USA), oxybenzone standard (Rest-de-Hans GmbH, Seefze, Germany), glacial acetic acid (Millipore Specialty Chemical Company, Paris, Kentucky, USA), methanol, phosphoric acid, potassium phosphate monobasic, sodium hydroxide (Fisher Scientific, Fair Lawn, New Jersey, USA), and polyoxyethylene 20-stearyl ether (Brij® 98, Sigma-Aldrich Co., St. Louis, Missouri, USA). Three picaridin-based repellent sprays and one sunscreen lotion were purchased and tested: OFF® Skintastic Clean Feel Spray (Product A, 5% picaridin, S.C. Johnson and Son Ltd., Racine, Wisconsin, USA), Cutter Advanced Repellent Spray (Product B, 7% picaridin, Spectrum, Division of United Industries Corporation, St. Louis, Missouri, USA), Repel Sportsman Formula Spray (Product C, 20% picaridin, WPC Brands, Inc., Bridgeton, Missouri, USA), and Coppertone® Oil-Free Sunblock Lotion (Product D, 4% oxybenzone, SPF 50, Schering-Plough HealthCare Products, Point-Claire, Quebec, Canada).

3.2. Diffusion studies

In vitro diffusion experiments were performed in an automated transdermal system (Jenway Instruments Corporation, Somerset, New Jersey, USA) using vertical Franz-style diffusion cells (0.84 cm² diffusion surface and 7.0 ml receptor volume). Diffusion studies were carried out at 37 ± 0.05°C and 300 rpm. 1 ml of sample was tested for the experiment. Full human skin specimens (400 μm thickness) were used as the membrane model (Gu and Chen 2009; Wang and Gu 2007). The skin collection protocol was approved by Research Ethics Boards at both the University of Manitoba and the St. Boniface General Hospital of Winnipeg.

3.3. HPLC assay

Picaridin and oxybenzone were simultaneously analyzed using an HPLC assay. The chromatographic conditions were: column, µBondapak® C18, mobile phase, methanol/water (pH 3.0 (65:35, v:v); flow rate, 1.0 ml/min; column temperature, 37°C for picaridin and 55°C for oxybenzone, detection wavelength, 210 nm for picaridin and 287 nm for oxybenzone. Diffusion samples were directly injected for analysis without further pretreatment.

3.4. Data analysis

Statistical analyses were performed using one-way ANOVA (PC-SAS® 8.02, SAS Institute Inc., Cary, North Carolina, USA) to compare: overall permeation percentages of picaridin and oxybenzone among various study groups; overall permeation percentages of picaridin, DEET and oxybenzone under similar experimental conditions. Differences were considered statistically significant at p < 0.05.

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References


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Fig. 5: Comparative permeation percentages of oxybenzone in association with picaridin or DEET across human epidermis after 6 h (from corresponding picaridin study groups; ±SEM, n = 4; R: repellent, S: sunscreen, B: bottom, T: top. DEET/oxybenzone data from Wang and Gu 2007)


