IN VITRO PERCUTANEOUS PERMEATION OF REPELLENT PICARIDIN AND SUNSCREEN OXYBENZONE

BY

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STATEMENT OF ORIGINALITY

The work presented in this thesis is, to the best of my knowledge and belief, original, except as acknowledged in the text, and the materials have not been submitted, either in whole or in part of a degree at this or any other university.

Ting Chen

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LIST OF ABBREVIATIONS

Brij®98: *Polyoxyethylene 20-oleyl ether*

CDC: *United States Center for Disease Control*

DEET: *N,N-diethyl-m-toluamide*

DMP: *Dimethyl phthalate*

FDA: *The United States Food and Drug Administration*

HDPE: *High density polyethylene*

HPLC: *High performance liquid chromatography*

IR3535: *Ethyl butylacetylaminopropionate*

KBR3023: *Picaridin (Bayrepel™, Icaridin)*

MED: *Minimal erythema dose*

OTC: *Over-the-Counter*

OBZ: *Oxybenzone*

PABA: *P-aminobenzoic acid*

PBS: *Phosphate buffered solution*

PCR: *Picaridin*

PDMS: *Polydimethylsiloxane*

SC: *Stratum corneum*

SPF: *Sun Protection Factor*

SSF: *Steady-state flux*

US-EPA: *The United States Environmental Protection Agency*

UV: *Ultraviolet*

UVA: *Ultraviolet radiation-Type A*

UVB: *Ultraviolet radiation-Type B*

UVR: *Ultraviolet radiation*

WNV: *West Nile virus*

LIST OF SYMBOLS

A: *Diffusion area (cm^2)*

C: *Drug concentration (g/ml)*

D: *Diffusion coefficient (cm^2/h)*

J: *Flux (g/cm^2h)*

K: *Permeability coefficient (cm/h)*

P: *Transmembrane penetration percentage (%)*

Q: *Drug amount (g)*

t: *Diffusion time (h)*

V: *Diffusion volume (cm^3)*

x: *Membrane thickness (cm)*

ABSTRACT

Increased public awareness of protection against the West Nile virus and skin cancer has led to extensive application of topical insect repellent and sunscreen preparations. Concurrent application of the insect repellent DEET or picaridin with various sunscreen components has become prevalent. Previous studies have described percutaneous absorption of DEET and oxybenzone both individually and concurrently after topical skin application. However, no study has been carried out to assess the percutaneous characterization of the newer alternative repellent picaridin and its concurrent application with the sunscreen oxybenzone.

In this thesis, a series of *in vitro* diffusion studies were performed to evaluate the transmembrane permeation of picaridin and oxybenzone across human epidermis and poly(dimethylsiloxane) (PDMS) membrane. Transdermal permeation of picaridin and oxybenzone from four commercially available repellent and sunscreen products was also investigated by using different application concentrations and sequences. The results obtained were then compared to those of the repellent DEET and the sunscreen oxybenzone under identical experimental conditions.

Permeation of picaridin and oxybenzone across human epidermis was suppressed when both compounds were used concurrently. Increasing concentration of the test compounds further reduced the permeation percentage of picaridin and oxybenzone. While permeation characteristics were correlative between human epidermis and artificial PDMS membrane, permeability of PDMS membrane was significantly larger than that of human epidermis. This finding was different from concurrent use of DEET and oxybenzone in which a synergistic permeation enhancement was observed between the two substances.

Transdermal permeation of picaridin across human epidermis from various commercially available spray preparations was significantly lower than that of DEET from similar spray products, both alone and in combination with sunscreen oxybenzone. Concurrent application of the commercial products resulted in either no change or suppression of permeation of picaridin and oxybenzone. This finding was also different from concurrent application of DEET and oxybenzone using commercial preparations. In addition, permeation of picaridin and oxybenzone across human epidermis was dependent on application concentration, use sequence, and preparation type.

It was concluded from this thesis that picaridin would be a better candidate for concurrent application with sunscreen preparations in terms of percutaneous permeation. Further studies are needed to understand permeation mechanisms and interactions between picaridin and oxybenzone, and to develop a potential composite picaridin/sunscreen preparation that produces dual protection efficacy but possesses minimal transdermal permeation.

Chapter 1

Introduction

1.1. Skin Structure

The skin is the largest and outmost organ of the human body. It comprises approximately one tenth of the total body weight of an average adult, and covers a surface area of two square meters [Schaefer and Redelmeier, 1996]. Human skin is recognized as a complex organ with unique metabolic, immunologic, and sensory capabilities. One of the primary functions of the skin is to create a flexible and protective barrier between the interior organs and the exterior environment. This barrier further extends its functionality by preventing the human body from excessive water loss, bacteria entry, physical and chemical assault, and ultraviolet (UV) radiation [Berti and Lipsky, 1995]. In addition, the skin regulates body temperature, produces the senses of touch and sensation, and synthesizes vitamin D with the help of sunlight. Overall the skin is a vital and integrated human organ that makes our body a complete functioning object.

Anatomically, human skin is essentially composed of four functional layers from outside inward (*Figure 1.1*): stratum corneum (non-viable epidermis), epidermis, dermis, and hypodermis (subcutis). Each layer possesses its own unique physiological and biological characteristics, and their integrity is essential to the overall health of the skin and the human body.

Structurally, the thickness of complete epidermis measures approximately 0.1 mm on average, which may range from around 0.05 mm for the eyelids to up to 1.5 mm for the soles of feet. Epidermis is further categorized into four to five sublayers depending on the region of the human body, i.e., stratum corneum, stratum lucidum, stratum granlosum, stratum spinosum, and stratum basale. Stratum corneum is the top epidermis layer that is also non-viable in nature. Stratum corneum is only 10-20 μm in thickness, composed of

several layers of dead skin cells that are embedded in a lipid matrix. Even though stratum corneum is thin and non-viable, it is the primary limiting barrier against skin permeation by chemical substances and bacteria. A compromise to the integrity of stratum corneum will result in enhanced skin permeation and transdermal absorption, which is sometimes beneficial to effective drug application using the skin. The dermis is typically 3-5 mm in thickness, and composed of two layers, papillary dermis and reticular dermis. Subcutis, also known as hypodermis, is a layer of connective tissue and elastin that attaches the skin to the underlying bones and muscles. Subcutis also supplies the skin with blood vessels and nerves for other physiological functions.

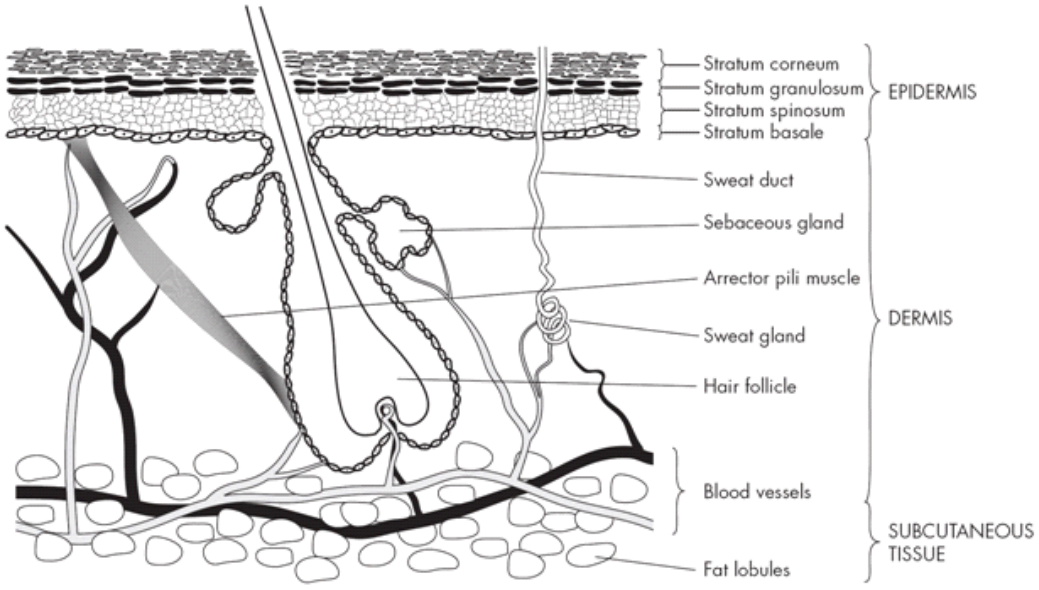

Figure 1.1. A diagrammatical cross section of the human skin [Williams, 2003]

1.1.1. Stratum Corneum (Non-Viable Epidermis)

The barrier property of the human skin is primarily accredited to stratum corneum, a top dead layer of the epidermis that is also referred to as "non-viable epidermis". Stratum corneum is often treated as a separate skin layer, due mainly to its protective characters against all external invasions. The barrier property of the stratum corneum was first demonstrated by Blank in 1953 using a series of tape stripping experiments; the diffusion of water across the skin increased significantly after the removal of the stratum corneum [Blank, 1953]. A better understanding of stratum corneum and its roles in transdermal drug delivery has been subsequently acquired, and numerous chemical and physical approaches have been devised for effective systemic drug delivery and absorption across the skin [Wildnauer et al., 1975; Elias, 1981 & 1983; Wertz and Downing, 1989; Marjukka et al., 1999; Wertz, 2000].

In general, stratum corneum comprises 15-20 layers of dead cells with an average thickness of 10-20 μm. In comparison to the viable epidermis, stratum corneum is thicker on the palms and the soles, but is thinner on the lips [Williams, 2003]. The stratum corneum is made up of an intact layer of large, flattened, envelope-shaped cells filled with keratin, which are migrating dead cells from stratum granulosum, and shed at a rate of approximately once every 2 weeks. Architecturally, stratum corneum resembles a wall-like structure, which can be graphically described using a "Brick and Mortar" Model (*Figure 1.2*). The horny cells of hydrated keratin comprise the bricks while the lipids fill the space between the dead cells like mortar [Elias, 1983]. The lipid mortar plays a more important role in the barrier function of stratum corneum. Water permeability across stratum corneum is approximately 1000 times lower than that to most other lipid

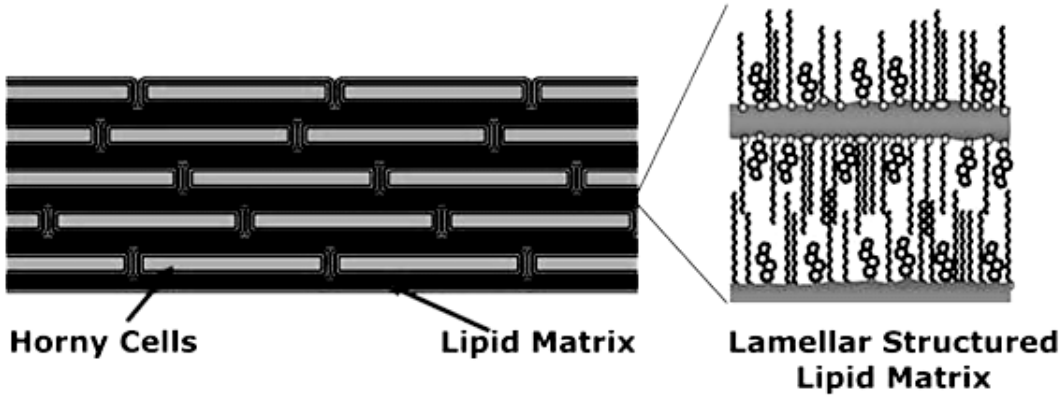

Figure 1.2. Schematic structure of stratum corneum according to "Brick and Mortar" Model [Daniels, 2004, with permission from www.scf-online.com, Cognis GmbH]

membranes in the body. This barrier function and insolubility of other substances in stratum corneum greatly contribute to the uniqueness of the stratum corneum [Grubauer et al., 1989; Potts and Francoeur, 1991].

In terms of composition, stratum corneum contains 75-80% of proteins on a dry weight basis. They primarily include fibrous α-keratin (approximately 70%) and amorphous β-keratin (approximately 10%) [Marjukka et al., 1999]. These protein components collaborate to make the corneocytes dense and nearly impermeable to a solute. In addition, there are approximately 10-15% of protein fractions that are water soluble. The remaining constituents of the stratum corneum are lipids (5-15%) and unidentified materials (5-10%) [Wilkes et al., 1973]. The viable epidermis (50-100 μm in thickness) that lies under the stratum corneum is responsible for the generation and replenishment of the stratum corneum.

The lipid components present in the stratum corneum also play an important role in skin barrier properties. Unlike other biological membranes, stratum corneum is virtually absent of phospholipids. The essential content of lipids of the stratum corneum comprises ceramides (41%), cholesterol (27%), cholesteryl ester (10%), fatty acids (9%), and cholesterol sulfate (2%) [Wertz and Downing, 1989]. While the lipid composition in stratum corneum may vary depending on body sites and individuals, a group of seven ceramides with unique structure of long, straight, saturated aliphatic chains forms the foundation of the highly ordered, impermeable stratum corneum that is resistant to temperature variation, UV exposure, and skin oxidation [Lampe et al., 1983; Schurer and Elias, 1991]. Removal of the lipids with solvent extraction may lead to increased transepidermal water loss and enhanced skin permeability [Matoltsy et al., 1968; Sweeney and Downing, 1970; Scheuplein and Blank, 1971; Squier et al., 1991].

1.1.2. Viable Epidermis

The epidermis is a complex and multilayer membrane. The primary cell type within the epidermis is the keratinocyte. Viable epidermis refers to the layers underlying stratum corneum which, from the inside to the outside, include stratum basale, stratum spinosum, and stratum granlosum. These layers of viable epidermis can be differentiated according to the morphological characteristics of maturating keratinocytes (*Figure 1.3*).

Keratinocytes of the viable epidermis possess two special physiological functions. The first function is to act as stem cells to generate new skin cells, and the second function is to act as an adhesive to band the epidermis to the basement membrane [Eckert, 1989]. The development of stratum corneum from keratinocytes requires several stages of

cellular differentiation, in which the keratinic cells advance through a series of transformations that feature stratum spinosum, stratum granulosum, and stratum lucidum. The normal turnover time for the skin keratinocytes is around 30 days.

In addition to keratinocytes, the viable epidermis also consists of melanocytes, Langerhans cells, and Merkel cells. These components are responsible for the production of pigmentation (melanin), the immune defense system, and the sensory perception in the skin, respectively [Williams, 2003].

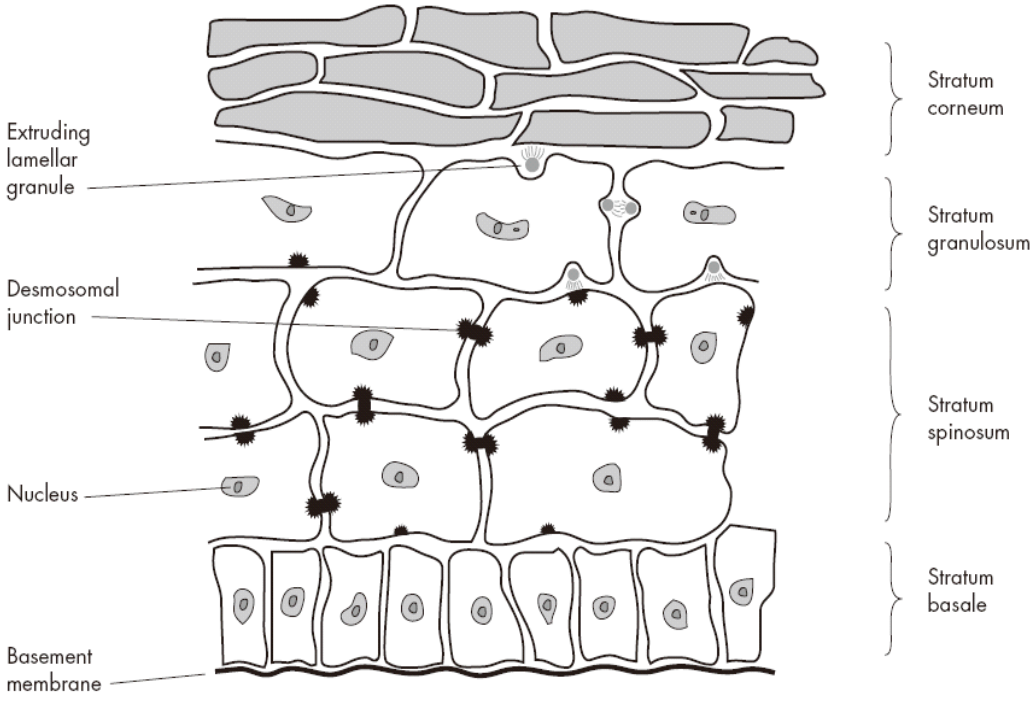

Figure 1.3. A representation of human epidermal cell differentiation [Williams, 2003]

1.1.3. Dermis and Hypodermis

Dermis is the layer beneath the epidermis that measures about 1-5 mm in thickness. The primary components of the dermis are connective tissues such as collagen, elastic fiber, and extrafibrillar matrix. This complex network of connective tissues protects the body from external stresses and strains. Structurally, dermis can be categorized into two distinctive layers, i.e., the upper papillary layer and the lower reticular layer. The former contains a thin complex of collagen fibers, whereas the latter is a thicker layer made of collagen fibers that are assembled parallel to the surface of the skin [Wilkes et al., 1973].

There are numerous organelles embedded within the dermis, which include blood and lymphatic vessels, nerve endings, pilosebaceous units (e.g., hair follicles and sebaceous glands), and sweat glands (*Figure 1.1*). Body temperature is regulated by the entire vasculature system in the dermis, which also delivers oxygen and other nutrients to the skin and removes toxins and waste metabolites from the skin. In addition, the blood vessels are responsible for repairing skin wound and damage, by providing vascular exchanges to the papillary layer, the subcutaneous region, and the skin appendages [Williams, 2003].

Under the dermis is the hypodermis, also known as subcutaneous layer. It connects the overlying dermis to the underlying body constituents. The hypodermis is composed primarily of adipose tissue, and it also houses larger blood vessels and nerves. This layer of tissue provides essential insulation and protection of the body against external temperature change and physical shock.

1.1.4. Skin Appendages

Human skin also comprises various appendages including hair follicles and associated sebaceous glands, sweat glands, and nails. The origin of skin appendages is located in the dermis. Hair follicles are extensively spread all over the skin surface except for the lips, the palms, and the soles. They are directly associated with sebaceous glands that secrete sebum (fatty acids and triglycerides) to lubricate skin surface and maintain a healthy skin pH of approximately 5.0. Sweat glands are normally referred to as eccrine glands and apocrine glands. Eccrine sweat glands discharge sweat, a dilute salt solution of pH 5.0. Heat and emotional stress can often trigger the secretion of sweat from eccrine glands. Apocrine sweat glands are coiled tubular glands that are only distributed in certain specific locations of the skin such as axillae, nipples, and ano-genital regions. The exact function of apocrine glands is not yet clear, but it has been suggested that they are scent-releasing glands that produce a noticeable odorous secretion [Katz and Poulsen, 1971]. Nails are composed of keratin, one type of tough protein produced by living skin cells in the areas of fingers and toes. In general, skin appendages are regarded as an insignificant route for drug delivery even though they are capable of providing a quick passage to the general circulation bypassing the barrier of the stratum corneum.

1.2. Skin Transport

1.2.1. Routes of Skin Penetration

As discussed above, the skin is a multilayered organ with its primary function of protecting the human body from the surrounding environment. The barrier property of the

skin relies essentially on the stratum corneum, the uppermost layer of the dead skin cells. Drug diffusion across the skin is generally believed to be a passive process, during which the diffusion flow rate is proportional to the concentration gradient of the drug [Scheuplein, 1965; Scheuplein and Blank, 1971; Potts et al., 1992; Walker and Smith, 1996]. There are two diffusional routes existing in skin penetration, i.e., the transepidermal route and the transappendageal route. The former pathway involves diffusion through the continuous stratum corneum while the latter route comprises transport via hair follicles and sweat glands. Because skin appendages cover approximately 0.1% of the entire skin surface, transappendageal route is generally regarded marginally important in drug delivery through the skin [Williams et al., 1992; Marjukka et al., 1999; Daniels, 2004]. This route might be beneficial in delivering unique preparations such as liposomes, microspheres, and small crystals due to high permeability [Flynn, 1990; Hueber et al., 1994]. Once passing through stratum corneum or skin appendages, a substance can further diffuse across the dermis and reach the general blood circulation (*Figure 1.4*) [Squier et al., 1991; Marjukka et al, 1999; Daniels, 2004].

Transepidermal route is further categorized into two diffusional pathways depending on how a drug molecule moves across the stratum corneum. Intercellular diffusion utilizes the lipid domains between the corneocytes; transcellular diffusion allows direct passage across both the corneocytes and the lipid matrix (*Figure 1.5*) [Marjukka et al., 1999; Barry, 2002; Daniels, 2004]. It has been widely accepted that intercellular diffusion is the principal pathway taken by a majority of penetrants across stratum corneum, while transcellular diffusion is only important to certain compounds

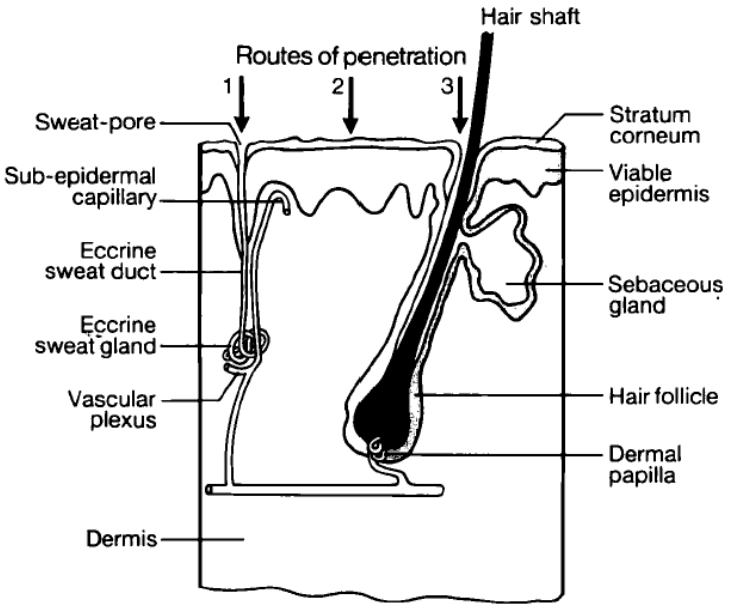

Figure 1.4. Possible routes of penetration through the skin (1: sweat gland, 2: transepidermal, 3: hair follicle) [Wiechers, 1989, with permission from Springer]

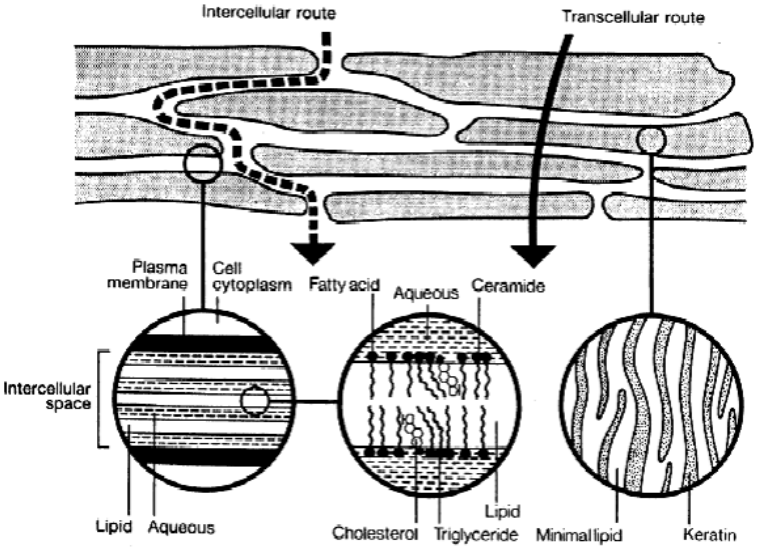

Figure 1.5. Drug permeation across stratum corneum [Marjukka et al., 1999, with permission from Elsevier]

[Perkins and Heard, 1999]. Skin penetration is strongly dependent on the lipophilicity and molecular size of a substance [Marjukka et al., 1999]. Lipophilic compounds possess a greater tendency for transdermal permeation than hydrophilic compounds, due primarily to intercellular diffusion [Potts et al., 1992; Fartasch et al., 1993; Morimoto et al., 1994; Daniels, 2004]. In addition, drug solubility in a dosage form, skin hydration, application site, and skin integrity may also influence the rate and extent of drug diffusion across the skin [Riviere and Heit, 1997].

1.2.2. Factors Affecting Skin Transport

Percutaneous diffusion and penetration is influenced by a variety of factors. Among them partition coefficient, diffusivity and molecular size of the penetrants, concentration gradient of drug compounds within the skin, the site of skin application, skin temperature, and skin hydration are all important parameters in drug delivery across the skin.

The partition coefficient is a physicochemical property that is directly associated with specific drug molecules. A partition coefficient is defined as the ratio of drug solubility in an organic phase (commonly octanol) to that in an aqueous phase (commonly water). Being lipophilic in nature, the skin shows a tendency to associate with compounds that have high partition coefficients. Therefore, lipophilic substances generally demonstrate higher transdermal permeation potentials than hydrophilic counterparts owing to their favorable solubility in the skin and the underlying tissues [Surber et al., 1990]. Molecular size also plays an important role in transdermal penetration. Large molecules tend to permeate less than small molecules due to size

restriction. In addition, transport from the skin to blood circulation may also be influenced by partition coefficient. Lipophilic compounds with high partition coefficients are preferentially distributed to lipophilic compartments such as lipid bilayers of cells, while hydrophilic compounds with low partition coefficients may be readily transported to hydrophilic compartments such as blood serum [Thomas and Finnin, 2004]. A balance of partition coefficient and suitable molecular size is therefore needed in order to achieve effective drug permeation and systemic absorption through the skin.

The establishment of an adequate concentration gradient within the skin will facilitate passive drug diffusion along this concentration path according to the Fick's Laws of Diffusion [Wiechers, 1989]. This results from the random molecular movement from a zone of high concentration to a zone of low concentration to reach an equilibrium. To achieve effective transdermal permeation, special preparations are formulated to create a desirable concentration gradient for optimal drug diffusion. The diffusivity of a compound is also influenced by preparation type and concentration gradient within both the dosage form and the skin. Diffusivity of a lipophilic compound from a high concentration gradient is generally larger than that of a hydrophilic compound from a low concentration gradient. Diffusivity is also a parameter that can be manipulated by different formulation approaches.

The site of skin application also influences transdermal drug delivery across the skin. While the skin possesses the advantage of large surface for drug application, regions that have thickened stratum corneum can significantly limit percutaneous drug penetration. These regions include the fingertips, the palms of hands, and the soles of feet. On the other hand, areas of the chest, the back of ears, inside of the arms and thighs have

soft and smooth skin surface, which are commonly selected for skin applications to maximize percutaneous drug penetration. Application convenience and visibility may also play an important role in selecting application site; skin surface where it is difficult for drug administration or the application is easily visible might not be a desirable site for applying transdermal dosage forms.

Percutaneous absorption of chemical compounds across the skin may also be affected by surface temperature and relative humidity of the skin. Even though the concept that increasing skin temperature and skin hydration would generally facilitate transdermal permeation has been widely accepted by researchers, there is little available experimental data confirming or refuting these notions. Increasing skin surface temperature might improve blood circulation within the skin. However, a chemical still needs to overcome the barrier function of the stratum corneum. The normal water content of the stratum corneum is 30-50% of the dry stratum corneum weight *in vivo*, and this may vary depending on different skin conditions. For example, an occlusion of the skin using a water-impermeable membrane would prevent water loss on the skin surface and enhance drug permeation. In addition, the dried and flattened corneocytes of the stratum corneum may result in disrupted lipid bilayers once absorbing moisture. This will subsequently alter the barrier function of the stratum corneum and often increase the percutaneous absorption [Caspers et al., 2001; Hikima and Maibach, 2006].

1.2.3. Penetration Principles (Fick's Laws of Diffusion)

Even though human skin is a complex structure that is composed of various layers including the stratum corneum, epidermis, dermis, and subcutaneous tissues, it is

regarded as a simple and homogeneous membrane configuration in terms of mass diffusion. The mathematical description of a passive diffusion process was initially formalized by Adolf E. Fick in 1855. It was postulated that the flux of a mass diffusion across a given membrane is proportional to the concentration gradient across the membrane and the distance of diffusion [Flynn et al., 1974; Crank, 1975]. Thus, Fick's First Law of Diffusion is expressed in the following equation,

$$J = -D\frac{dC}{dx}$$

Where *J:* diffusion flux

D: diffusion coefficient (diffusivity, length^2/time)

dC: concentration gradient

x: thickness of the membrane

The negative sign in above equation indicates that the diffusant moves in the direction of decreasing concentration. Because the concentration (*C*) across a membrane varies, the diffusion flux is a variable that corresponds to the diffusion time and location. Fick's Second Law of Diffusion was further derived to describe this changing situation, which predicts how diffusion causes the concentration gradient to change with time,

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2}$$

A steady-state diffusion process can be reached when an indefinite diffusion time is allowed based on both Fick's Laws of Diffusion. The steady-state flux (J_s) and concentration gradient (C_s) across the membrane can then be viewed as constant values when this equilibrium of diffusion has been reached,

$$J = DK\frac{Cs}{x}$$

Where *D:* diffusion coefficient

K: partition coefficient of permeant

C_s*:* saturated concentration in donor cell

x: thickness of the membrane

In most *in vitro* diffusion experiments, the measurement of permeability coefficient (K_p) is correlative to the permeability coefficient of a permeant from the formulation vehicle to the membrane, the diffusion coefficient of the compound in the membrane, and the thickness of the membrane. The steady-state flux J_s is therefore simplified to the following equation,

$$J_s = K_p C_s$$

Where K_p*:* apparent permeability coefficient of permeant

C_s: saturated concentration of permeant in donor cell under "sink condition"

J_s of a substance can usually be determined by measuring the cumulative amount of the permeant permeating through the membrane, the diffusional surface area, and the diffusion time. The relationship is described in the following equation,

$$Q_t = J_s A t$$

Where Q_t: accumulative permeation amount

A: surface area of diffusion

t: diffusion time

subsequently,

$$J_s = \frac{Q_t}{At}$$

In this thesis, the cumulative permeation amount (Q_t) was measured using an HPLC assay, the diffusion surface area in a diffusion cell (A) was a predetermined value, and the diffusion time (t) for all diffusion studies was 6 hours. In addition, the percentage of the transmembrane permeation of a compound was calculated by the following equation,

$$P = \frac{Q_t}{C_s V}$$

Where P: percentage of transmembrane permeation

C_s: saturated concentration of permeant in donor cell

V: volume of formulation applied

1.3. In Vitro Methods for Studying Skin Transport

In vitro diffusion studies are a simple and cost-effective approach for investigating and predicting percutaneous diffusion and permeation of a chemical substance in the skin. The primary aim of an *in vitro* diffusion experiment is to characterize permeation of a compound in terms of transmembrane rate and extent. The experimental conditions of an *in vitro* diffusion study can be precisely controlled and reproduced, so that it is possible to standardize assessment criteria and simulate or correlate to drug diffusion properties from skin preparations. Designing and performing an *in vitro* diffusion study requires careful consideration of various factors in order to obtain correlative data and to achieve specific objectives. These factors may include diffusion cell design, test membrane, receptor medium, diffusion and sampling duration, permeant concentration and vehicle.

1.3.1. Diffusion Cell Design

In vitro diffusion cells are composed of two main components, i.e., the donor compartment and the receptor compartment. The test membrane is to be placed between the two compartments with the appropriate surface facing the donor cell, allowing for proper drug diffusion and permeation. There are various designs of diffusion cells ranging from simple "static diffusion cell (e.g., Franz-style cell) to highly complex "flow-through diffusion cell" (*Figure 1.6*). It is very important to select the right diffusion cell for a study so that specific research objectives can be achieved.

Static diffusion cells usually include two types, i.e., vertical cell and horizontal cell. Upright Franz-style diffusion cell is perhaps the most widely used diffusion cell for

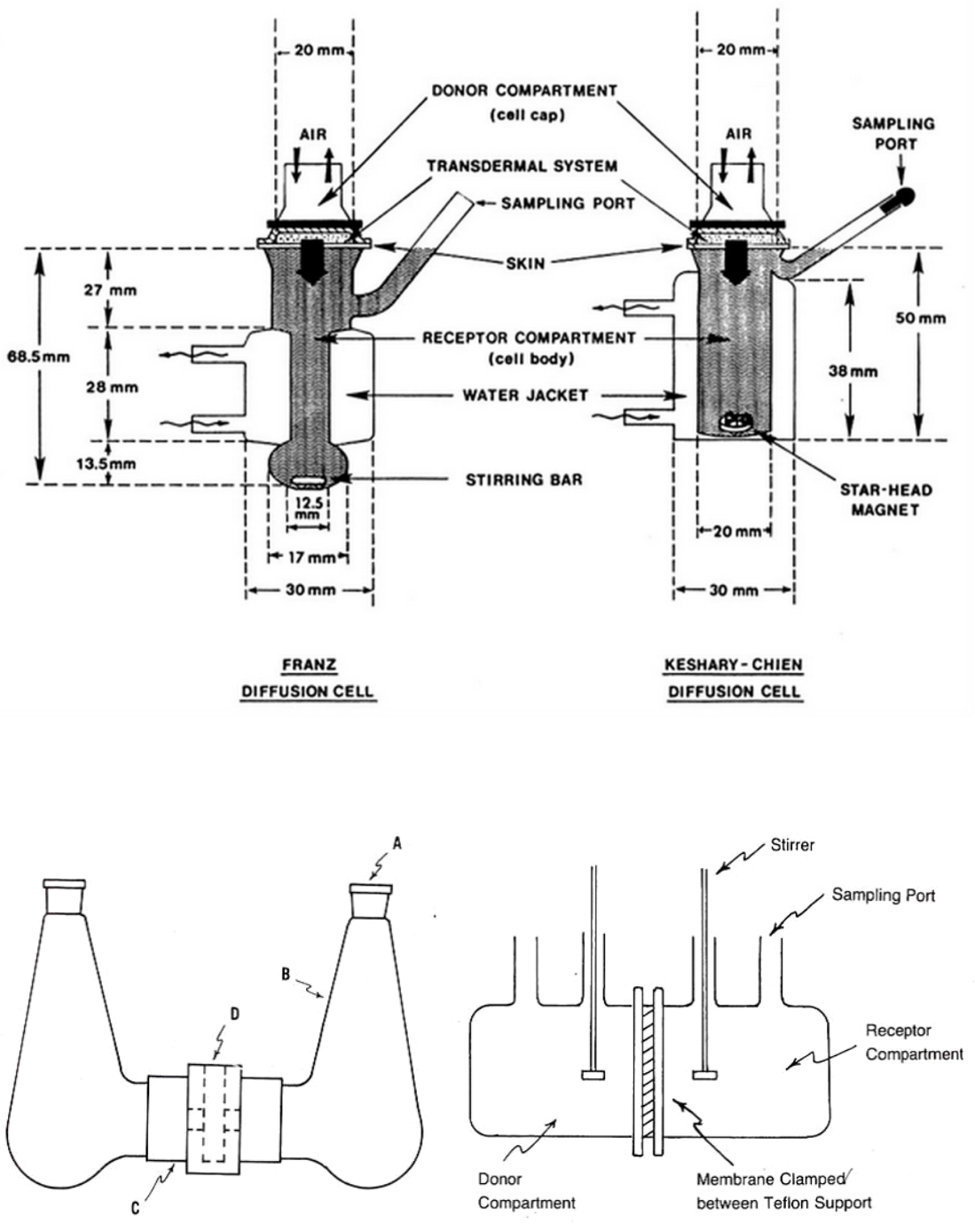

Figure 1.6. Diffusion cells of different designs
(top: vertical, bottom: horizontal) [Martin, 1993]

a variety of diffusion studies. This type of diffusion cells is normally made of glass material, with a diffusional area of approximately 0.2-2 cm^2 and a receptor volume of 2-10 ml. The receptor medium is continuously agitated with a magnetic stirring bar to ensure its uniformity. Accurate samples are collected easily through a side arm of the receptor chamber. The Franz-style diffusion cell has been extensively utilized for evaluating drug uptake by membranes, finite dose permeation, and steady-state flux of a drug either alone or in a formulation. Its relatively large and open donor compartment allows simple application of semi-solid formulations to the test membrane and facilitates transmembrane permeation. Franz-style diffusion cells were utilized for all diffusion experiments in this thesis.

Horizontal side-by-side diffusion cells enable continuous agitation of both donor and receptor compartments during an experiment. This is of a great use for evaluating diffusion and permeation of preparations in which solid permeant exists. The mechanical movement in a donor cell prevents the sedimentation and accumulation of solid material on the surface of the test membrane, and allows for realistic estimation of molecular diffusion of a solution. A flow-through diffusion cell usually consists of a donor compartment and a receptor compartment within which the receptor fluid is continuously replaced with fresh solution. In contrast to a static diffusion cell, the flow-through diffusion system attempts to mimic *in vivo* application conditions where drug permeated across the stratum corneum is carried away by the blood flow. Both side-by-side diffusion cells and flow-through diffusion cells are useful in assessing drug diffusion and absorption from specific preparations [Clowes et al., 1994]. All *in vitro* results are then

compared or correlated to *in vivo* data in order to establish a reasonable relationship between drug delivery from a preparation and drug absorption across the skin.

1.3.2. Membrane Models

The most suitable and realistic membrane model for *in vitro* assessment of percutaneous penetration is unquestionably the human skin. Specimens of human skin can be obtained from various sources, including surgical procedures, skin banks, or commercial sources [Friend, 1992]. No matter where the skin specimens are collected, there are certain variations and limitations that the investigators have little control over the samples prior to preparing the skin for a diffusion experiment. For instance, the skin surface is normally cleaned using disinfectants prior to a surgical procedure as an attempt to minimize potential infection of the wound. Alcohol, a primary ingredient in a majority of disinfectants, is capable of extracting skin lipids from the stratum corneum, subsequently altering the barrier property of the membrane. This pretreatment of the skin is largely unknown to the investigators. The selection of skin site and the history of skin samples are also beyond the control of research scientists in many cases [Bronaugh et al., 1986]. To make the matter worse, it is often difficult to acquire sufficient amount of human skin specimens from living subjects due to considerable ethical and legal restrictions. As a result, alternative membrane models such as biological membranes from different animals and artificial membranes are also frequently utilized for *in vitro* diffusion studies.

A wide variety of animals have been recommended as suitable alternatives to humans in obtaining biological membrane models for *in vitro* transdermal delivery

studies. These include primate, porcine, mouse, rat, guinea pig, and snake. Among these biological models, porcine skin is the most common substitute to human skin because of its physiological similarities and ready availability. Histologically, the thickness of porcine stratum corneum is approximately 21-26 μm [Jacobi et al., 2007], which resembles that of human stratum corneum (10-20 μm). Porcine skin has also demonstrated similar permeability properties as human skin, even though its lipid content differs from that of human tissues. One of the most obvious advantages of using porcine skin is its easy availability and low cost; porcine skin specimens can be obtained from slaughter houses or farmer's markets. For example, porcine ear skin is often chosen for permeation studies, and has demonstrated comparable results to those obtained using human skin [Jacobi et al., 2007]. Using biological animal skin specimens also offers the flexibility of collecting skin membranes from specific body site. As a result, skin variation is minimized and diffusion reproducibility is improved. Overall, animal skin models are capable of providing predictive and satisfactory correlation to the human skin in terms of percutaneous characterization.

Artificial membranes have also been extensively utilized as substitute models for *in vitro* skin permeation investigation. The most commonly encountered synthetic membranes include polydimethylsiloxane (silastic or PDMS), cellulose acetate, silicone, and high density polyethylene (HDPE). Structurally, artificial membranes are much more simplistic than biological membranes. Hence they offer advantages in accessibility, variability, and reproducibility. Transmembrane diffusion and permeation across a synthetic membrane can also be characterized by using simple mathematical equations. On the other hand, the simplicity of artificial membranes is disadvantageous in

correlating *in vitro* diffusion results to *in vivo* absorption characteristics because human skin is a multilayered, heterogeneous membrane with viability and metabolism. Human skin also possesses hair follicles and other shunt routes in which the percutaneous permeation can take place. It has been proven that certain types of synthetic membranes are able to mirror penetration of drugs through biological skin [Houk and Guy, 1988; Mulder, 1996]. However, use of artificial membranes in diffusion experiment is primarily restricted for studying formulation variables and validating diffusion reliability and reproducibility. Artificial membranes should not be utilized for evaluating mechanisms of molecular penetration across human skin.

1.3.3. Receptor Medium

Human skin is a complex tissue that plays an important role in transdermal absorption and transportation. Dermis contains various cutaneous microvasculature and lymph vessels, which are capable of removing chemicals that have permeated the stratum corneum and viable epidermis, thus maintaining a sink condition for the skin permeation. As a result, it is important to ensure that the concentration of a permeant in the receptor compartment does not exceed 10% of its solubility in the receptor medium. Otherwise, diffusion flux through the skin may be significantly altered [Skelly et al., 1987]. Ideally a receptor medium selected for an *in vitro* diffusion experiment should grant an accurate simulation of the conditions pertaining to *in vivo* application. Aqueous receptor solution and phosphate buffered solution (PBS, pH 7.4) are the two most commonly used receptor mediums that are capable of mimicking realistic *in vivo* physiological conditions in humans. Aqueous receptor solution is often used for hydrophilic and moderately

lipophilic permeants (up to a log $P_{octanol/water}$ of around 2.0), while phosphate buffered solution is generally good for ionized substances [Brain et al., 2002]. When a permeant is much more lipophilic with low aqueous solubility (less than 10 μg/ml), it becomes necessary to select a more appropriate receptor medium for the diffusion experiment. Adding solubilizing agents such as surfactants or bovine serum albumin to the receptor medium is one of the most effective approaches, as such measures can effectively increase the solubility of a permeant in the receptor medium [Cross et al., 2003]. Nevertheless, the use of solubilizing agents should be carefully chosen so as not to influence the integrity of the test membrane, or damage the barrier nature of the membrane, especially for biological membranes, for the entire duration of a diffusion experiment.

1.4. Conclusion

Human skin is a complex and flexible organ that guards against the entry of many foreign substances into the human body. The primary barrier of the human skin relies on the outmost stratum corneum, a highly-packed, dry, multi-layering of dead skin cells. Once diffusing across the stratum corneum, a chemical compound is capable of permeating the epidermis and reaching the blood circulation. Diffusion and permeation are utilized to design and evaluate drug absorption across the skin membrane from topical or transdermal drug delivery systems. It is possible to manipulate various formulation parameters in order to achieve desirable and optimal preparation characteristics for skin applications.

Franz-style diffusion cells are commonly used for *in vitro* evaluation of drug permeation across the skin. A properly-designed and executed diffusion setting allows pharmaceutical scientists to characterize mechanisms of drug transport across the skin and to simulate drug penetration from a formulation for quality control purposes. In this thesis, an established *in vitro* diffusion study was used to evaluate the transdermal permeation of the insect repellent picaridin and the sunscreen oxybenzone from a series of experiments, as both products are likely to be applied simultaneously, the transdermal properties of the two chemicals alone and in combination were also characterized.

Chapter 2

Repellents and Sunscreens

2.1. Insect Repellents

Historically, humans have invented and utilized various approaches to ward off biting arthropods for centuries. Some of the natural methods range from smoke, plant extracts, tars and muds, and many of these methods are still being applied in certain parts of the world today. As our society and technologies advance, synthetic chemical compounds as active insect repellent ingredients have become the preferred method of prevention. In particular, the military has significantly invested its resources in the research and development of chemical repellents, in order to prevent the troops from insect-borne diseases [Debboun et al., 2005]. Some of the noteworthy examples include citronella oil (discovered in 1901), dimethyl phthalate (DMP, patented in 1929), Indalone® (patented in 1937), and 2-ethyl-1,3-hexane diol (Rutgers 612, 1939) [Peterson and Coats, 2001]. However, the most significant breakthrough in the history of synthetic repellents belongs to N,N-diethyl-*m*-toluamide (DEET), which was synthesized by the U.S. Army in 1953 [Peterson and Coats, 2001]. This compound has become a dominant insect repellent ingredient for both military and civil applications for the past six decades. *Figure 2.1* lists some of the structures of primary insect repellents.

Proper protection again biting insects is both critical and effective in minimizing vector-borne diseases such as malaria, yellow fever, Lyme disease, and West Nile fever. While some of the diseases were previously restricted to certain parts of the world, they have gradually spread around the globe recently due mainly to extensive travel by both humans and migrating animals. It is not uncommon to see previously exotic diseases reported in all continents, and subsequently prompting health warnings for the general public to prevent potential epidemic conditions.

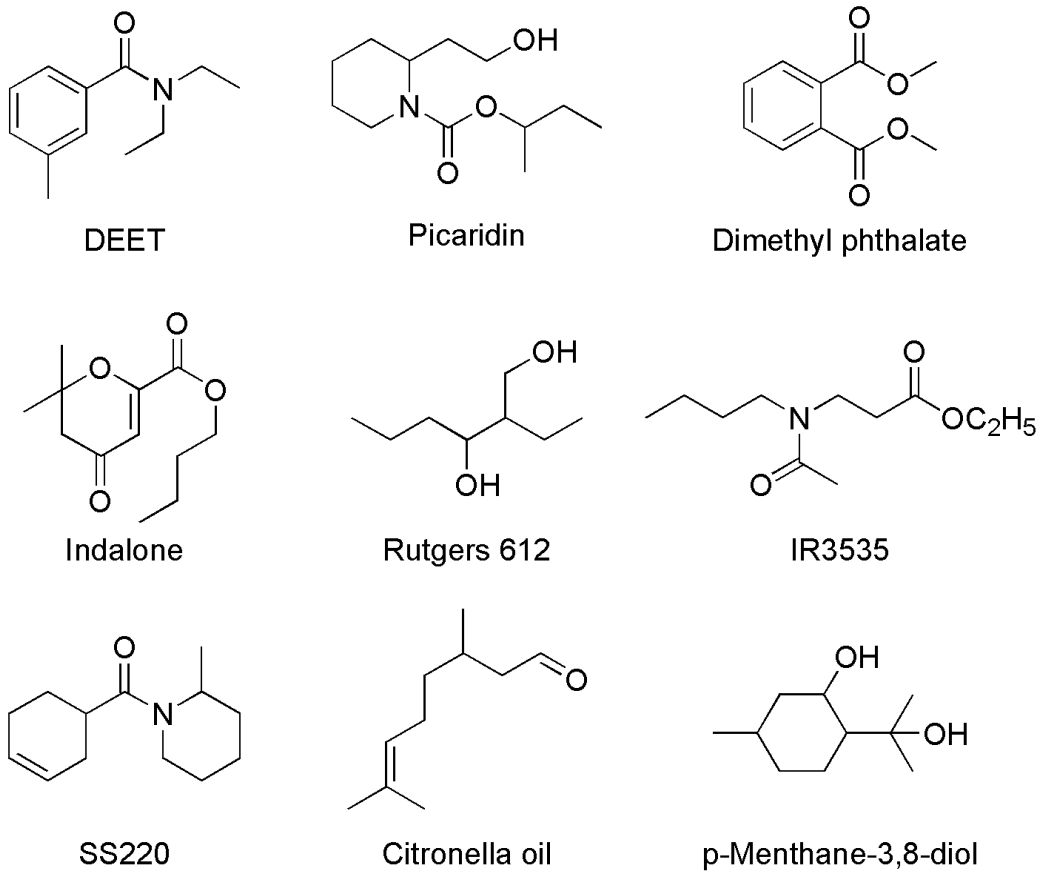

Figure 2.1. Chemical structures of primary insect repellents

The West Nile virus (WNV) is a typical example of vector-borne disease that was transported by migrating birds and mosquitoes across the world. First identified and documented in 1937 from a human patient of febrile illness in the West Nile district of Uganda, the virus was primarily found in parts of Africa, the Middle East, and the Mediterranean region. The human infection of WNV is transmitted through the bites of infected mosquitoes that have previously bitten birds carrying the virus. The symptoms of WNV can vary greatly among humans, ranging from fever and flu-like symptoms to neuroinvasive symptoms of meningitis or encephalitis. Not everyone who contracts the

virus will have a severe reaction, but elderly patients are generally more vulnerable to the virus than other population groups [Hayes, 1989].

The dissemination of WNV in North America first occurred during the summer of 1999, when 62 infected patients were hospitalized in the State of New York, and 7 people subsequently died of the infection [Garmendia et al., 2001]. Since then the virus has spread rapidly across the continent by migrating birds and mosquitoes [Gubler, 2007]. According to data collected by the Center for Disease Control and Prevention (CDC), 9862 cases and 264 deaths were reported in the 2003 outbreak in the United States. But the number of cases of human infection has been gradually declining thereafter. In 2008 there were 1356 total cases and 44 deaths recorded in the United States. In Canada, 14 deaths and 1494 confirmed and probable human WNV cases were reported in 2003. The infection has increased by more than 50% in 2007. In Manitoba, WNV was first detected in the birds (crows), horses and mosquitoes in July 2002. Human cases of WNV were initially detected in Manitoba in the summer of 2003. In 2007, most human infection cases were recorded in the provinces of Manitoba and Saskatchewan.

Prevention against insect biting is perhaps best achieved by taking an integrated and practical approach, which may range from staying away from the infested habitats, utilizing physical barriers such as protective clothing or bed nets, to applying personal insect repellents [Curtis, 1992]. Application of insect repellents is the only practical and effective way of protecting against arthropod bites for military personnel and specialized field workers. The use of personal repellents for recreational purposes is variable depending on consumer preference and regional infestation levels. According to a survey conducted by the CDC in the fall of 2004, approximately 40% of individuals in the

United States reported using mosquito repellents regularly. The CDC has advocated for regular application of personal insect repellents to prevent WNV fever and Lyme disease.

2.1.1. Picaridin

Picaridin (see *Figure 2.1*) is a newer, synthetic insect repellent that possesses similar efficacy to the repellent DEET. Its full chemical name is 1-piperidine carboxylic acid, 2(2-hydroxyethyl)-, 1-methylpropylester. This compound was first developed by the German chemical company Bayer Inc. in the 1980s. Some other names are also used for picaridin, including Bayrepel^{TM}, KBR3023, and Icaridin. Picaridin has been commercially available in Europe and Australia since its debut. It was initially approved and registered for use by the United States Environmental Protection Agency (US-EPA) in 2001. Commercial picaridin preparations became available in the U.S. and Canadian market starting in 2006 [Frances, 2006].

Compared to the repellent DEET, picaridin possesses many desirable characteristics of an ideal insect repellent, as it is almost odorless, does not cause dermal and eye irritation (EPA Grade IV) or skin sensitization, and does not feel sticky or greasy upon topical application. In addition, picaridin does not damage plastics or fabrics, which is a major drawback of DEET products. *Table 2.1* lists major physical and chemical properties of picaridin. Its low toxicity, comparable efficacy and desirable applicability have all contributed to an increased acceptance by the consumers in selecting alternative insect repellent products; the CDC has also endorsed picaridin products for general public application.

Table 2.1. Physical and chemical properties of picaridin*

Parameter	Value / Property
Molecular Weight	229.3 g/mole
Physical	Liquid, clear
Solidifying Point	< -170 °C
Initial Boiling Point	296 °C at 1013 hPA
Color/Form	Colorless
Odor	Nearly odorless
Water Solubility (25 °C)	Insoluble
Partition Coefficient (log K_{ow}, 20 °C)	2.11
Soil Sorption Coefficient (K_{oc})	Estimated at 389
Vapor Pressure (25 °C)	5.9×10^{-2} pa
EPA Toxicity Classification	Grade III
Viscosity (30 °C)	30.7 sec. flow time

* from WHO Specifications and Evaluations for Public Health Pesticides - Icaridin

Picaridin has been proven to be effective against a wide variety of mosquitoes, ticks, flies and other biting insects in both laboratory and field tests. *Table 2.2* refers to some common known biting insects that are susceptible to picaridin. A series of field studies have demonstrated that picaridin is effective against mosquitoes in tropical regions such as Malaysia and Florida [Yap et al., 1998 & 2000; Barnard et al., 2002]. Numerous studies have compared the efficacy of repellents containing picaridin, DEET and other active ingredients against different insects; one study conducted in Australia indicated that a formulation containing 19.2% picaridin was as good as or better than DEET against *Verrallina lineate* (Taylor) in a rainforest habitat [Frances et al., 2002].

Table 2.2. Insects that are susceptible to picaridin*

Name	Common Name
Aedes *albopictus*	Asian Tiger Mosquito
Aedes *aegypti*	Yellow Fever Mosquito
Aedes *taeniorhynchus*	Black Salt Marsh Mosquito
Anopheles *stephensi*	3 species of mosquito found throughout Asia and considered vectors for Malaria
Anopheles *sinensis*	
Anopheles *dirus*	
Culex *pipiens fatigans*	House Mosquito
Ixodes *ricinus*	Common tick (aka sheep, pasture or castor-bean tick)
Ixodes *scapularis*	Deer Tick (carrier of Lyme disease)
Rhipicephalus *sanguineus*	Brown Dog Tick
Biting midges	Biting gnats
Culex *quinquefasciatus* *Tabanidae*	Horsefly
Musca *domestica*	Housefly
Simulium *venustum*	Black Fly

* from http://www.picaridin.info

As with all other insect repellents, the repellent mechanism of picaridin is still unknown. As a matter of fact, there has been an ongoing debate on whether or not common mechanisms do exist among different insect repellents towards various species of arthropods. It has been generally hypothesized that most active insect repellent compounds work by interfering with insects' homing system located on the antennae, which is made of a great number of chemical receptors. Mosquitoes are able to track the emission of carbon dioxide and lactic acid to their hosts. An insect repellent would

interrupt such tracking action by forming a physical vapor shield between the skin surface and the insect. The efficacy of a repellent is related to the boiling point of the compound, and would diminish over the time due to ambient evaporation of the substance [Fradin, 1998]. Compounds with a low boiling point may vaporize too rapidly, leading to quick dissipation of the preparation. On the contrary, those with high boiling point may not vaporize sufficiently to create a desired repellent environment. A boiling point between 230-260 °C is the most desirable range for an effective repellent [Garson and Winnike, 1968]. In addition, the repellent efficacy of a preparation may also be significantly reduced from abrasion, sweating, washing with water, warm temperatures, and high winds [Khan et al., 1973; Maibach et al., 1974].

Insect repellents are commonly formulated in the form of pump spray, aerosol, lotion, and impregnated wipe. A liquid formulation is able to facilitate application and evaporation of the active ingredient, leading to improved application acceptance. Formulations of picaridin commercially available in the market include spray, aerosol, and impregnated wipe. The concentrations of picaridin used in these repellent products range between 5% and 20%, providing a wide variety of product choices for different applications and protections.

2.2. Sunscreens

The sun emits sunlight into the universe, and brings the essential energy for all living things on the earth. In accordance to their wavelength, sunlight can be categorized into three different spectra, i.e., infrared radiation (780-5000 nm), visible light (400-780

nm), and ultraviolet (UV) rays (100-400 nm). While visible light and infrared radiation from the sun are not considered harmful to humans, the UV radiation emitted by the sun is capable of causing detrimental effects on living cells. In particular, a prolonged exposure to UV radiation may induce acute and chronic damages of the human skin. Most common acute skin damage from sunlight includes skin inflammation, sunburn, pigmentation, and hyperplasia. Chronic skin damage from extensive sun exposures ranges from untimely photoaging to skin cancer.

Skin cancer is a malignant growth on the skin surface, and it generally develops in the outermost layer of the skin, the epidermis. Skin cancer can be detected in the early stages of development, so that it is rarely a fatal disease when treated timely and appropriately. It takes many years for skin cancer to develop and progress, because chronic skin damage by UV exposure at young ages is normally revealed as one grows older, commonly in people over 50 years old. The three types of skin cancer, from the least to the most dangerous, are basal cell carcinoma, squamous cell carcinoma, and melanoma.

Basal cell carcinoma and squamous cell carcinoma are collectively known as "non-melanoma skin cancer". Basal cell carcinoma is the most common form of skin cancer, and squamous cell carcinoma occurs roughly one-quarter as often as basal cell carcinoma. Non-melanoma skin cancer accounts for more than 90% of all skin cancers detected annually in North America. Pathologically, basal cell carcinoma grows slowly and almost never spreads to other tissues. Squamous cell carcinoma, on the other hand, is capable of spreading to other parts of the body. In general, the signs of non-melanoma skin cancer appear excessively on the body areas where exposure to sunlight is possible

and extensive, most likely the head, face, neck, arms, and hands. Both basal and squamous carcinoma can be cured with proper medical treatment [Šitum et al., 2008].

Melanoma is the most serious type of skin cancer, and it also causes the most deaths among skin cancer patients. Cancerous cells of melanoma originate from melanocytes, which are located at the bottom layer of the epidermis and between the basal cells. Melanocytes produce the pigment melanin that gives the skin its color. Although less common than basal carcinoma and squamous carcinoma, melanoma is capable of spreading quickly to other regions of the body through lymph system or blood stream if left untreated. When discovered and treated in the early stages, melanoma is still curable in most cases [Dennis et al., 2003; Saladi and Persaud, 2005].

There are various risk factors or precursors that are frequently associated with skin aging and damage. They can subsequently lead to an increased potential for developing skin cancer in humans. Excessive exposure to UV radiation is one of the primary causes of skin cancer. Other risk factors include climate, history of sunburns, skin moles, precancerous skin lesions, family history, skin aging, and exposure to other environmental hazards. People with fair skin are generally at a higher risk of developing skin cancer than people of color, because the former has less melanin in their skin system than the latter, a compound known to provide protection against UV radiation. In addition, the rate of developing skin cancer is elevated in persons with increased UV exposure through tanning; a suntan is actually a response of skin injury to excessive UV radiation. The risk of developing skin cancer from sun-tanning has been considered great enough to prompt the United States Food and Drug Administration (FDA) in considering regulations to ban children and adolescents under 18 years old from using tanning beds.

One of the most practical and cost-effective approaches to reduce and prevent skin damage by sunlight is to apply sunscreens to the skin surface, which is capable of blocking or absorbing harmful UV radiation. The application of active sunscreen substances began in the early 20th century, with the first reported sunscreen preparation containing benzyl salicylate and benzyl cinnamate. In the 1940s, p-aminobenzoic acid (PABA) was patented and incorporated into sunscreen formulations as an effective sun-blocking agent. Since its debut, formulations and derivates of PABA had been introduced to the sunscreen market for many decades [Moloney et al., 2002]. Sunscreen applications have also experienced a progressive expansion for the past 30 years, accompanied by an increasing public awareness of sun safety as well as a better understanding of the relationship between skin cancer and prolonged sunlight exposure. Currently, FDA has approved 17 active sunscreen ingredients for use in over-the-counter sunscreen products (*Table 2.3*). A variety of UV filters are commonly incorporated into a broader range of consumer care products such as facial cosmetics, hair sprays and dyes, perfumes, shampoos, and shaving creams.

Active sunscreen ingredients are broadly classified as either chemical absorbers or physical blockers according to their mode of UV protection. Chemical sunscreens absorb certain wavelengths of the UV spectrum, while physical sunscreens reflect most of UV radiation back to the space. A majority of commercially available sunscreen products are capable of providing broad-spectrum protection by combining UVA (320-400 nm) and UVB (280-320 nm) filters together with physical blockers. Therefore, an effective sunscreen preparation frequently comprises numerous sun-blocking agents, and requires careful formulation processing to achieve efficacy, stability, and applicability.

Table 2.3 FDA-approved active ingredients for sunscreens*

Ingredient	Concentration*	UV Absorbance
Aminobenzoic acid (PABA)	up to 15%	UVB
Avobenzone	up to 3%	UVA
Cinoxate	up to 3%	UVB
Dioxybenzone	up to 3%	UVA/UVB
Ecamsule* (terephthalylidence dicamphor sulfonic acid)	up to 10%	UVA
Homosalate	up to 15%	UVB
Menthyl anthranilate	up to 5%	UVA
Octocrylene	up to 10%	UVB
Octyl methoxycinnamate	up to 7.5%	UVB
Octyl salicylate	up to 5%	UVB
Oxybenzone	up to 6%	UVA/UVB
Padimate O	up to 8%	UVB
Phenylbenzimidazole sulfonic acid	up to 4%	UVB
Sulisobenzone	up to 10%	UVA/UVB
Trolamine salicylate	up to 12%	UVB
Titanium dioxide	up to 25%	Physical
Zinc oxide	up to 25%	Physical

* from http://www.healthhype.com/supposedly-safe-sunscreen-ingredients.html
Use concentration in grams of total mass of a sunscreen product
Ecamsule was approved by the FDA on July 24, 2006. It is commercially available as Anthelios SX in the US, and Mexoryl SX in EU and AU.

2.2.1. Mechanisms of Sunscreens

Active sunscreen agents are classified as either chemical or physical sunscreens depending on their interactions with the UV radiation. A chemical sunscreen works primarily by absorbing UV energy, whereas a physical sunscreen works by scattering or reflecting UV energy. Chemical sunscreens are generally aromatic molecules conjugated with carbonyl groups. This structural configuration allows the molecules to absorb high-energy UV radiation and transfer it as lower-energy rays, thereby preventing the damaging radiation from reaching deep into the skin. Furthermore, most of chemical sunscreens (with the notable exception of avobenzone) do not undergo significant chemical change (photodegradation) upon exposure to UV radiation, allowing them to retain the UV-absorbing potency for an extended period of time. Physical sunscreens, on the other hand, are normally recognized as the opaque components in sunscreen preparations. They are able to create a physical barrier between the skin and the external environment thus reflecting or scattering UV radiation from the skin surface. The efficacy of physical sunscreens in a sunscreen product is directly related to the diameter and size of their particles and the thickness of the sunscreen film upon topical application [Pustišek et al., 2005]. The newer micro-sized forms of physical sunscreens may also function partly by absorbing UV radiation in addition to physical reflection and scattering. Titanium dioxide (TiO_2) and zinc oxide (ZnO) are the two most commonly used physical sunscreens, which protect against UV radiation ranging 250-340 nm. A combination of chemical and physical sunscreen ingredients is utilized to formulate commercially available sunscreen products in order to achieve broad-spectrum UV protection.

The protection efficacy of a sunscreen preparation is measured by its ability to reduce sunburn in terms of the Sun Protection Factor (SPF). SPF is defined as the ratio of the UV energy dose required to produce a minimal erythema dose (MED) on sunscreen-protected skin to that required to produce the same MED on unprotected skin in the same individual [Moloney et al., 2002]. The standard measurement of SPF is obtained by applying a sunscreen preparation at a dose of 2 mg/cm^2. However, several studies have also indicated that people do apply variable amounts of sunscreen to their skin surface (0.5-1.0 mg/cm^2), which could lead to actual SPF lower than the laboratory-measured standards [Stenberg and Larkö, 1985]. A sunscreen product with a higher SPF rating allows individuals to be exposed to sunlight for longer duration without suffering sunburn than a sunscreen product with a lower SPF rating. Of course, sun exposure is also dependent upon the length of time one spends under the sun, exposure time of the day, geographic location, and other weather conditions. Dermatologists generally recommend using a broad-spectrum sunscreen product with SPF 15 or higher for minimal skin protection; products of SPF 30-50 are needed for extended outdoor activities or for summer sports and recreations.

The protection efficacy and cosmetic acceptability of a sunscreen product are also influenced by the vehicle in which active sunscreen ingredients are incorporated. A properly selected and prepared vehicle determines not only how sunscreen substances remain effective upon skin application but also how long the protection is able to sustain under unique use conditions such as sweating and swimming. Commonly-used sunscreen preparations include emulsions, gels, sticks and aerosols, and each formulation requires special processing to achieve optimal protection efficacy. Some water-resistant sunscreen

products are capable of maintaining sufficient sun protection for 40 minutes in the water, whereas a waterproof sunscreen preparation extends the same protection for 80 minutes after the application. It is common for a sunscreen formulation to be composed of multiple additives and excipients in order to obtain these skin-retention characteristics.

2.2.2. Oxybenzone

Oxybenzone is one of the most commonly used active sun-blocking agents in commercially available sunscreen products. Oxybenzone is also known as benzophenone-3 or 2-hydroxy-4-methoxybenzophenone, and its chemical structure is shown in *Figure 2.2*. *Table 2.4* lists primary physical and chemical properties of oxybenzone.

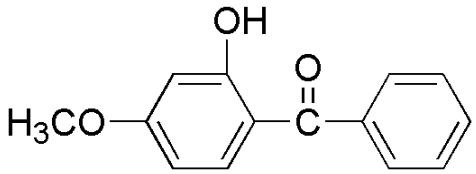

Figure 2.2. Chemical structure of oxybenzone

Oxybenzone is a derivative of benzophenone; benzophenone has numerous variants designated from benzophenone-1 to benzophenone-12. All benzophenone variants are utilized as active UV absorbers. Oxybenzone can well absorb UV radiation in the range between UVB (290-320 nm) and UVA2 (320-340 nm, λ_{max} 326 nm). It is recognized as a broad-spectrum sun-blocking compound. It is estimated that 20-30% of commercial sunscreen products contain oxybenzone at various use concentrations. In

Table 2.4. Physical and chemical properties of oxybenzone*

Parameter	Value / Property
Molecular Weight	228.8 g/mole
Purity	98% minimum oxybenzone
Specific Gravity (25 ºC)	1.32
Color/Form	White to yellow powder
Odor	Faint
Water Solubility (25 ºC)	Insoluble
Vapor Pressure (20 ºC)	Not determined
Toxicity	LD_{50}: 7400 mg/kg (oral, rat)
Boiling Point	150-160 ºC (1 mmHg)
Stability	Stable under normal conditions

* from http://www.spectrumchemical.com

addition, oxybenzone is also frequently incorporated in other types of conventional cosmetic preparations (0.05-0.5%) to act as a photoprotection agent.

Oxybenzone shows low acute toxicity in animal models [Cosmetic Ingredient Review, 1983]. However, little is known about the chronic toxicity and disposition of oxybenzone in humans from topical application of sunscreen preparations. It has been demonstrated that oxybenzone is one of the common sunscreen agents to induce photoallergic contact dermatitis, due partially to its extensive presence as a primary active ingredient in the commercial sunscreen products [Knobler et al., 1989]. One case report also described an incident in which a 22-year-old female developed anaphylaxis after applying an oxybenzone-containing sunscreen preparation; the allergic reaction to oxybenzone was later confirmed through skin testing [Lenique et al., 1992; Collin and

Ferguson, 1994]. Systemic absorption of oxybenzone from topical skin application of sunscreens in humans has been proven from several studies [Hayden et al., 1997; Gonzalez et al., 2002 & 2006; Janjua et al., 2004 & 2008]. Moreover, oxybenzone is capable of enhancing transdermal permeation of other chemicals as a percutaneous absorption enhancer [Hayden et al., 1997]. These characteristics are neither productive nor desirable for oxybenzone, because sunscreen products are generally designed as topical preparations and their protection efficacy is intended only for the skin surface.

2.3. Percutaneous Absorption of Insect Repellents and Sunscreens

Investigation on the percutaneous characterization of active insect repellents and sunscreens from topical skin applications was very limited in the past, because a majority of transdermal research has focused on developing formulation strategies to achieve effective systemic absorption of medications across the skin using novel transdermal drug delivery systems. Furthermore, percutaneous characterization of cosmetic and other consumer care skin products has always been lacking and under-appreciated, even though the general public utilizes these over-the-counter products extensively on a daily basis. As a result, little information has been available regarding transdermal permeation and systemic absorption of the active repellent and sunscreen compounds from topical skin applications.

Increased public awareness of the necessity and benefit from using repellent and sunscreen products to prevent vector-borne diseases and skin cancer has led to expansive application of these products over the past two decades. In particular, concurrent

application of both repellent and sunscreen preparations has become a common summer practice in Canada and the US since 2000, when the West Nile virus first landed in North America continent. Subsequently, studies were initiated to investigate the percutaneous characteristics of the active repellent and sunscreen compounds from topical skin applications.

Designed as "Topical Use Only" preparations, repellent and sunscreen preparations should ideally remain on the surface of skin or in the upper layers of the stratum corneum with minimal percutaneous penetration and systemic absorption of the active ingredients. The protection efficacy of both products is designated for the external environment, i.e., insect bites and UV radiation. Therefore, systemic distribution of any active ingredients is neither productive nor necessary. As a matter of fact, safety consideration would require minimal systemic exposure of the chemicals, because repellents and sunscreens are commonly applied at the discrepancy of individual users without medically recommended doses and frequencies. Adverse effects might result from inappropriate application of the repellents and sunscreens in susceptible subjects.

Transdermal permeation of several insect repellent and sunscreen compounds from topical skin application had been studied and reported separately [Hayden et al., 1997; Qiu et al., 1997; Jiang et al., 1998; Gonzalez et al., 2002; Abdel-Rahman et al., 2004A & 2004B; Janjua et al., 2004]. Numerous studies have focused on the insect repellent DEET (N,N-diethyl-*m*-toluamide), an effective and long-lasting repellent that has been in commercial use for over 50 years, and the sunscreen oxybenzone, one of the primary UVA/UVB sun-blocking agents present in sunscreen preparations. Both substances are capable of permeating systemically from topical skin applications in

humans, and their percutaneous permeation is dependent on various factors including use concentration, preparation type, and application duration. In particular, adverse neurological effects have been detected from concurrent application of DEET and several pesticides that were commonly used in military personnel during the first Gulf War, which was also speculated as one possible contributing cause to the Gulf War Syndrome reported in the veterans [Brown and Hebert, 1997]. Oxybenzone was also found to be capable of permeating systemically from topical skin application in humans and of enhancing permeation of several other fertilizers or pesticides *in vitro* [Hayden et al., 1997]. In addition, studies have confirmed that mixing DEET-based repellents and sunscreen preparations reduced the sun protection efficacy of the sunscreens but not that of the DEET, which could be of concern to individuals who are unaware of this efficacy compromise [Montemarano et al., 1997; Murphy et al., 2000].

In our laboratory, we have reported a synergistic percutaneous permeation between DEET and oxybenzone when the two ingredients were applied simultaneously, both *in vitro* and *in vivo* [Gu et al., 2004 & 2005; Wang and Gu, 2007]. Furthermore, it was also observed that the transdermal permeation of DEET was affected more than that of oxybenzone from a concurrent application, and that the permeation was dependent upon preparation type, use concentration, and application sequence. These percutaneous characteristics are considered neither desirable nor productive for both topical preparations.

As a newly-introduced alternative repellent, picaridin became commercially available in the North American market starting summer of 2006. Its percutaneous properties from topical skin applications, individually or in combination with other

sunscreen substances, have been largely unknown. Therefore, we carried out a series of *in vitro* studies in this thesis to characterize the transdermal profiles of picaridin and oxybenzone from concurrent application of the two substances. We also compared the transdermal permeation of picaridin to that of DEET in association with the sunscreen oxybenzone from commercially available preparations under identical experimental conditions.

Chapter 3

Hypotheses and Objectives

Increased public awareness of protection against the West Nile virus and skin cancer has led to extensive applications of topical insect repellent and sunscreen preparations. Concurrent use of both repellents and sunscreens for summer outdoor activities has now become a common practice by many for the fear of being infected with the dangerous virus, in particular for those living in mosquito-infested regions as well as among special working forces whose job responsibilities demand extensive daily outdoor exposure. Designed as "Topical Use Only" products, active repellent and sunscreen ingredients should remain on the surface of skin with minimal percutaneous penetration and systemic absorption.

DEET (N,N-diethyl-*m*-toluamide) has been one of the effective and long-lasting repellent substances for over five decades. Its effectiveness against a variety of biting insects has been well documented [Fradin, 1998; Moore and Debboun, 2006]. Oxybenzone is one of the primary UVA/UVB sun-blocking agents that is incorporated in the majority of sunscreen preparations. Numerous studies have observed systemic absorption of both compounds after topical skin applications in humans [Selim et al., 1995; Schoenig et al., 1996; Hayden et al., 1997]. Neurological adverse effects of DEET and several other pesticides that are commonly used in military personnel have also been detected when they were used concurrently [Brown and Hebert, 1997; Abdel-Rahman et al., 2004B]. Oxybenzone is a known skin permeation enhancer for other chemicals. In our laboratory, we have observed a synergistic percutaneous permeation between DEET and oxybenzone when the two compounds were applied simultaneously, both *in vitro* and *in vivo* [Gu et al., 2004 & 2005; Kasichayanula et al., 2005 & 2007]. These percutaneous

characteristics are neither desirable nor applicable for these topical consumer care preparations.

Several newer insect repellents have been registered and approved for civil use in Europe and the US since 2000. These include synthetic compounds picaridin (2-(2-hydroxyethyl)-1-piperidinecarboxylic acid 1-methylpropyl ester, KBR3023®, Bayrepel®, Icaridin®) and IR3535® (ethyl butylacetylaminopropionate), and natural repellents lemon eucalyptus oil (active ingredient p-menthane 3,8-diol) and citronella oil. With the availability of these newer, alternative repellents to the general public, as well as the fact that DEET and oxybenzone exert mutual permeation enhancement to each other when used simultaneously, it became beneficial and rational to initiate investigations to characterize percutaneous properties between the repellent picaridin and the sunscreen oxybenzone in order to understand the potential interaction between these two substances and to utilize alternative repellent compounds to their fullest capacity.

The rationale of this thesis was based on the following points,

1) We have found a synergistic transdermal permeation between the repellent DEET and the sunscreen oxybenzone when both were applied concurrently. We hypothesized that there would be a similar interaction between picaridin and oxybenzone when these two substances are used simultaneously;

2) We have proven that percutaneous permeation of DEET and oxybenzone was dependent on the type of preparation, use concentration, and application sequence. We hypothesized that similar properties would be applicable to picaridin and oxybenzone when both are applied concurrently;

3) We have found permeability differences between human skin and artificial membrane models. We hypothesized that picaridin and oxybenzone would demonstrate different permeability between human skin and artificial PDMS membrane.

Therefore, a series of *in vitro* diffusion experiments were designed and carried out to evaluate the permeation characteristics of the repellent picaridin and the sunscreen oxybenzone across human epidermis and artificial PDMS membrane. The experimental setting was identical to that used in previous studies on the repellent DEET and the sunscreen oxybenzone, in order to produce parallel and comparable data. The objectives of this thesis were,

1) To develop and validate an analytical assay to simultaneously measure the concentration of picaridin and oxybenzone from diffusion study samples;

2) To characterize transmembrane properties of picaridin and oxybenzone under a variety of experimental conditions, i.e., applied alone or in combination, different use concentration and sequence, and different preparations;

3) To compare permeability difference between human skin and artificial PDMS to picaridin and oxybenzone;

4) To compare results of picaridin and oxybenzone with those of DEET and oxybenzone obtained from previous studies.

Findings from this thesis would be beneficial in understanding the mechanisms and interactions between active repellent and sunscreen components when they are applied concurrently. They would also be useful in designing and developing specialized repellent and sunscreen preparations that can minimize overall percutaneous permeation and systemic absorption of the active ingredients upon topical skin application.

Chapter 4

Permeation Characterization of Picaridin and Oxybenzone in Ethanolic Solution

4.1. Introduction

Insect repellents and sunscreens are the most practical, cost-effective and well-accepted choice of defence for the mass against vector-borne diseases and skin cancers; they have been extensively used as over-the-counter specialty products by the general public for decades. Before 1999, concurrent application of repellents and sunscreens was rare in North America, because biting insects, such as mosquitoes, are likely to be more active during dawn and dusk when ultraviolet (UV) radiation from the sunlight is still low. The arrival of mosquito-transmitted West Nile virus and its prompt dissemination across the continent have however resulted in the changes with which the general public utilizes repellent and sunscreen products, as the *Culex* mosquitoes that carry and transmit West Nile virus are highly active and aggressive all day long. For the past several years, concurrent use of repellents and sunscreens for summer outdoor activities has become a common practice by many for the fear of being infected with the lethal virus, in particular for those living in mosquito-infested areas and regions as well as special working forces whose job responsibilities require extended daily outdoor exposure.

DEET (N,N-diethyl-*m*-toluamide, OFF®) has been the dominant insect repellent in the market for almost five decades; its repellency against a variety of biting insects has been well documented [Fradin, 1998; Staub et al., 2002; Koren et al., 2003; Katz et al., 2008]. While the repellent mechanism of DEET is still unclear, it is believed that the compound disrupts the receptors in sensing antenna of the insects, subsequently preventing the creatures from locating human subjects. Systemic absorption of DEET through the skin is a known fact [Robbins and Cherniak, 1986; Qiu et al., 1997]; numerous studies have also proven neurological adverse effects of DEET and several

other pesticides that are commonly used in military personnel when used concurrently [Abdel-Rahman et al., 2004A & 2004B]. Since 2000, several newer insect repellents have been registered and approved for civil use in Europe and the US; they include synthetic compounds picaridin (2-(2-hydroxyethyl)-1-piperidinecarboxylic acid 1-methylpropyl ester, KBR3023®, Bayrepel®, Icaridin®) and IR3535® (ethyl butylacetylaminopropionate), and natural repellents lemon eucalyptus oil (active ingredient p-menthane 3,8-diol) and citronella oil [Naucke et al., 2007; Katz et al., 2008; Zhu et al., 2008]. The percutaneous characteristics of these chemicals after topical skin applications are largely unknown to date.

Sunscreen preparations are composed of numerous active sun-blocking ingredients to protect the skin from UV-radiation spectrum. There are both chemical and physical sunscreens present in commercial sunscreen products. Oxybenzone is one of the primary UVA/UVB sun-blocking agents incorporated in a majority of sunscreen preparations [Chatelain et al., 2003; Felton et al., 2004]. Although little investigation has been performed on sunscreen agents, these compounds are generally considered safe in pharmacological and toxicological terms. Nevertheless, oxybenzone is able to permeate across the skin after topical use. Furthermore, oxybenzone is also a skin permeation enhancer to other chemicals [Hayden et al., 1997; Pont et al., 2004].

DEET and oxybenzone are both capable of permeating across the skin after topical skin application. Mixing DEET-based repellents and sunscreens together reduces the sun-protection factor (SPF) of sunscreens due to chemical interactions [Montemarano et al., 1997; Murphy et al., 2000]. In our laboratory, we have reported synergistic percutaneous penetration between DEET and oxybenzone when both compounds were

used simultaneously in a series of studies [Gu et al., 2004 & 2005; Kasichayanula et al., 2007; Wang and Gu, 2007]. These permeation characteristics would be of concern from clinical perspectives, because repellents and sunscreens are designed solely for topical skin protection, and systemic exposure to these active chemical substances is neither productive nor desirable. Furthermore, repellents and sunscreens are generally applied at the discretion of individual users without medically recommended doses; inappropriate use for an extended period of time could inadvertently potentiate adverse effects of the active ingredients in susceptible individuals.

Concurrent use of repellent and sunscreen products is anticipated to become a more prevalent summer routine for health conscious individuals, due primarily to extensive awareness of vector-borne diseases and sun exposure safety. With the availability of newer, alternative repellents to the general public, as well as the fact that DEET and oxybenzone exert mutual permeation enhancement to each other when used simultaneously, it is beneficial and rational to study their percutaneous characteristics in order to utilize these new chemicals to their fullest capacity. In this study, we investigated the permeation characteristics of the insect repellent picaridin across both human epidermis and artificial membrane *in vitro*, with and without the presence of the sunscreen oxybenzone. The objective of the study was to evaluate whether or not similar percutaneous enhancement characteristics would exist between picaridin and oxybenzone, as what had been observed between DEET and oxybenzone. The experimental results were also compared to those from previous studies, with the hope of better understanding the percutaneous permeation of different repellent and sunscreen substances.

4.2. Materials and Methods

4.2.1. Materials

Repellent picaridin was received as a gift from Lanxess Corporation (Pittsburgh, Pennsylvania, USA) and oxybenzone was purchased from Riedel-de Haën GmbH (Seelze, Germany). Ethanol, methanol, potassium phosphate monobasic, and sodium hydroxide were purchased from Fisher Scientific (Fair Lawn, New Jersey, USA). Glacial acetic acid and polyoxyethylene 20-oleyl ether (Brij® 98) were obtained from Mallinckrodt Specialty Chemical Company (Paris, Kentucky, USA) and Sigma-Aldrich Co. (St. Louis, Missouri, USA), respectively. All solvents were HPLC-grade, and other solid chemicals were AC-grade. Deionized HPLC-grade water was obtained from a Milli-Q® Pure Water System (Millipore, Nepean, Ontario, Canada) in the laboratory.

4.2.2. Membrane Models

Excised human epidermis and artificial membrane poly(dimethylsiloxane) (PDMS) were used as the testing models for the diffusion experiments, based on previous experimental protocols in the laboratory [Gu et al., 2004; Wang and Gu, 2007]. Both membrane models have been extensively used in transdermal drug evaluations, and are considered to be representative of percutaneous permeation in real-life situations.

Human skin specimens were obtained from the Department of Surgery, St. Boniface General Hospital of Winnipeg. The collection protocol was approved by the Research Ethics Boards at both the University of Manitoba and St. Boniface General Hospital. The skin samples were kept at –20 °C after being received from the Hospital and thawed at 4 °C overnight prior to the diffusion experiment. On the morning of the

study day, the skin specimen was rinsed with deionized water to remove trace biological fluids, and then dried by gently blotting both surfaces with paper towels. The skin was fully spread on a cutting table and dermatomed to a thickness of 400 μm using an electric dermatome (Padgette Instruments, Kansas City, Missouri, USA). The dermatomed epidermis was further soaked in saline solution to prevent the membrane from dehydrating and shrinking. The integrity of the skin specimens were carefully examined; only undamaged sections with even membrane thickness were selected for the diffusion studies.

PDMS membrane (130 μm in thickness) was obtained from Advanced Biotechnologies Inc. (Silverdale, Washington, USA). Prior to each diffusion study, the membrane was cut into small pieces (2 × 2 cm), and soaked in deionized water for 2 hours before being mounted to the diffusion cells.

4.2.3. Diffusion Studies

All diffusion experiments were performed in an automatic transdermal system (Logan Instruments Corporation, Somerset, New Jersey, USA), which was composed of six vertical Franz-style diffusion cells, a circulating water bath, a magnetic stir console and an automatic sampling collector. The surface area available for drug diffusion in the Franz cells was 0.64 cm^2. Before the membrane was mounted onto the cell, a very thin layer of vacuum grease was applied to the connection surface of the receptor and donor cell to prevent the testing media from leaking. A preheated phosphate buffer (7.0 ml, pH 7.4, 4% Brij® 98, w/v) was then added to the receptor cell. The system was left for 30 minutes at 300 rpm to reach temperature equilibrium (37 ± 0.05 °C).

The testing concentrations were 10% (v/v), 20% and 50% for picaridin and 5, 10 and 20 mg/ml for oxybenzone, either individually or in combination. Picaridin and oxybenzone were accurately measured or weighed, and completely dissolved in 50% ethanol solution. The test sample (1.0 ml) was placed in each donor cell, and the donor cells were covered with a microscope glass cover slip to form an occlusive environment. The applied dose in each study provided an infinite drug amount for the diffusion process.

An aliquot of receptor medium (100 µl) was collected hourly for six hours during the diffusion study, followed by replenishing the same volume of fresh, preheated receptor medium at each sampling interval. Diffusion experiments were performed in four replicates for human skin specimens and six replicates for PDMS membrane, respectively. Concentrations of picaridin and oxybenzone in the receptor fluid were analyzed using an HPLC assay developed and validated in our laboratory.

4.2.4. HPLC Assay

The HPLC system was comprised of a Waters® Alliance 2690 Solvent Delivery Module, a 996 Photodiode Array Detector, and a µBondapak® C_{18} column (3.9 × 150 mm, 10 µm) (Milford, Massachusetts, USA). The mobile phase was a mixture of methanol and water (pH 3.0, adjusted with glacial acidic acid) (65:35, v:v), delivered at a flow rate of 1.0 ml/min. The detection wavelength was 210 nm for picaridin and 287 nm for oxybenzone, respectively. Under these chromatographic conditions, the retention time was 3.9 minutes for picaridin and 5.8 minutes for oxybenzone; the detection limit was 50 ng for picaridin and 20 ng for oxybenzone. The calibration linearity ($r^2 \geq 0.99$) of picaridin and oxybenzone ranged 100-1500 ng and 25-600 ng, respectively. The collected

diffusion samples were directly injected for analysis without further pretreatment. Concentrations of picaridin and oxybenzone in the receptor media were calculated based on the average calibration curves (n = 6).

4.2.5. Data Analysis

The permeability coefficients (K_p) of picaridin and oxybenzone from the two testing membranes were calculated using the empirical diffusion equations derived from the Fick's First Diffusion Law [Crank, 1975],

$$J_s = K_p C_s$$

where J_s is the steady-state diffusion flux, and C_s is the saturated drug concentration. K_p is often used to compare penetration profiles for solutes examined under different conditions and related to the rate of diffusion of a solute within a membrane adjusted for differences in membrane thickness and solute concentration.

The overall permeation percentages of picaridin and oxybenzone after 6 hours of diffusion were calculated based on the ratio of accumulated permeation amount to the actual application amount of the test products in the donor cells. Statistical analysis was performed using two-way ANOVA and Tukey's test (PC-SAS® 8.02, SAS Institute Inc., Cary, North Carolina, USA). The following statistical analyses of the data were conducted: a) the overall permeation percentages and permeability coefficients of picaridin and oxybenzone between the two testing membrane models; b) the overall permeation percentages of picaridin and oxybenzone among various application concentrations within the same membrane. Differences were considered statistically significant at $p \leq 0.05$.

4.3. Results

4.3.1. Permeation of Picaridin and Oxybenzone across Human Epidermis

The overall permeation percentages of picaridin and oxybenzone across human epidermis after 6 hours are shown in *Figure 4.1* and *Figure 4.2*, respectively. Percutaneous permeation suppression was observed when use concentration was increased and when repellent picaridin and sunscreen oxybenzone were present simultaneously in the donor cell.

Permeation of picaridin across human epidermis ranged between 0.09-0.30% among the study groups. When it was tested separately, the transdermal permeation of picaridin decreased with the concentration by 13-30%. When it was tested together with oxybenzone, the transdermal permeation of picaridin decreased by 10-68%, but statistical significance existed in groups at 50% of picaridin. In comparison to single picaridin group at the same concentration, introduction of oxybenzone into the vehicle resulted in a decrease in permeation of picaridin. The decrease ranged between 4-58% for picaridin; again significant decrease occurred only at high picaridin concentration (*Figure 4.1*).

Permeation of oxybenzone across human epidermis ranged between 0.10-2.00% among the study groups. When it was tested separately, the transdermal permeation of oxybenzone significantly decreased with the concentration by 44-55%. When it was tested together with picaridin, the transdermal permeation of oxybenzone decreased by 3-49%, and statistical significances were observed in all but one study group (10 mg/ml oxybenzone/20% picaridin). In comparison to single oxybenzone group at the same concentration, introduction of picaridin into the donor cell resulted in a decrease in

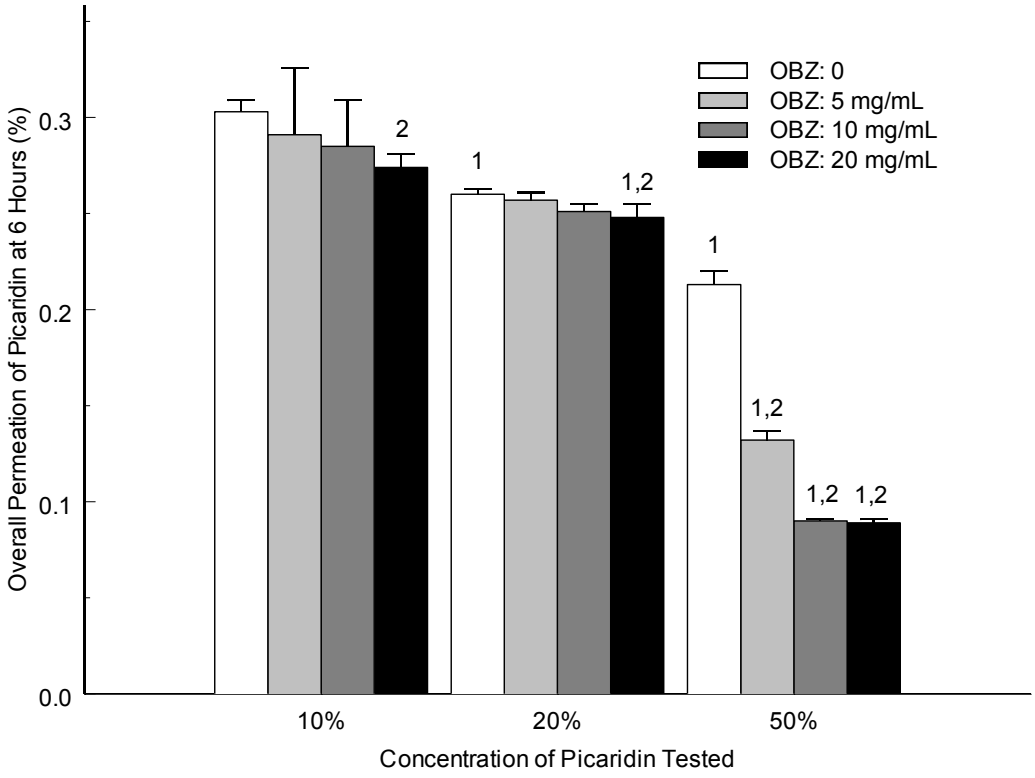

Figure 4.1. Overall permeation percentages of picaridin across human epidermis after 6 hours (1: significant difference from 10% study groups; 2: significant difference from single picaridin groups; $p \leq 0.05$, mean ± SEM, n = 4)

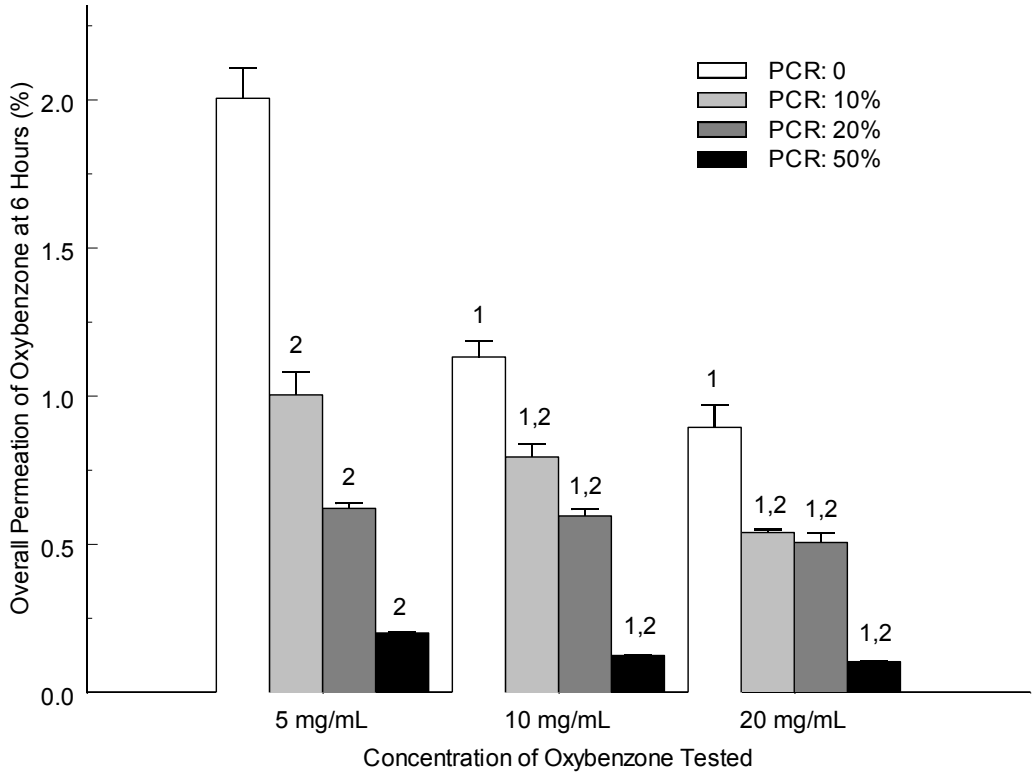

Figure 4.2. Overall permeation percentages of oxybenzone across human epidermis after 6 hours (1: significant difference from 5 mg/ml study groups; 2: significant difference from single oxybenzone groups; $p \leq 0.05$, mean ± SEM, n = 4)

permeation of oxybenzone. The decrease ranged between 30-90%, and all groups showed statistically significant suppression in the permeation of oxybenzone (*Figure 4.2*).

4.3.2. Permeation of Picaridin and Oxybenzone across PDMS Membrane

The overall permeation percentages of picaridin and oxybenzone across artificial PDMS membrane after 6 hours are shown in *Figure 4.3* and *Figure 4.4*, respectively. While permeation percentages of picaridin and oxybenzone decreased with the increase in use concentrations, concurrent use of picaridin and oxybenzone produced mutual permeation suppression only at higher use concentrations.

Permeation of picaridin across PDMS membrane ranged between 1.15-3.05% among the study groups, which was significantly higher than that of human epidermis. When it was tested individually, the transmembrane permeation of picaridin decreased with increasing concentration of picaridin in the donor compartment by 30-53%. When it was tested together with oxybenzone, the transmembrane permeation of picaridin also decreased with the concentration; the decrease ranged between 16-51%, and all differences were statistically significant. In comparison to single picaridin group at the same concentration, the coexistence of oxybenzone at 5 mg/ml would slightly increase the permeation of picaridin. However, the permeation of picaridin decreased when oxybenzone concentration was increased. Statistical significances were observed among various study groups (*Figure 4.3*).

Permeation of oxybenzone across PDMS membrane ranged between 2.20-16.39% among the study groups, which was again significantly higher than that of human epidermis. When it was tested individually, the transmembrane permeation of

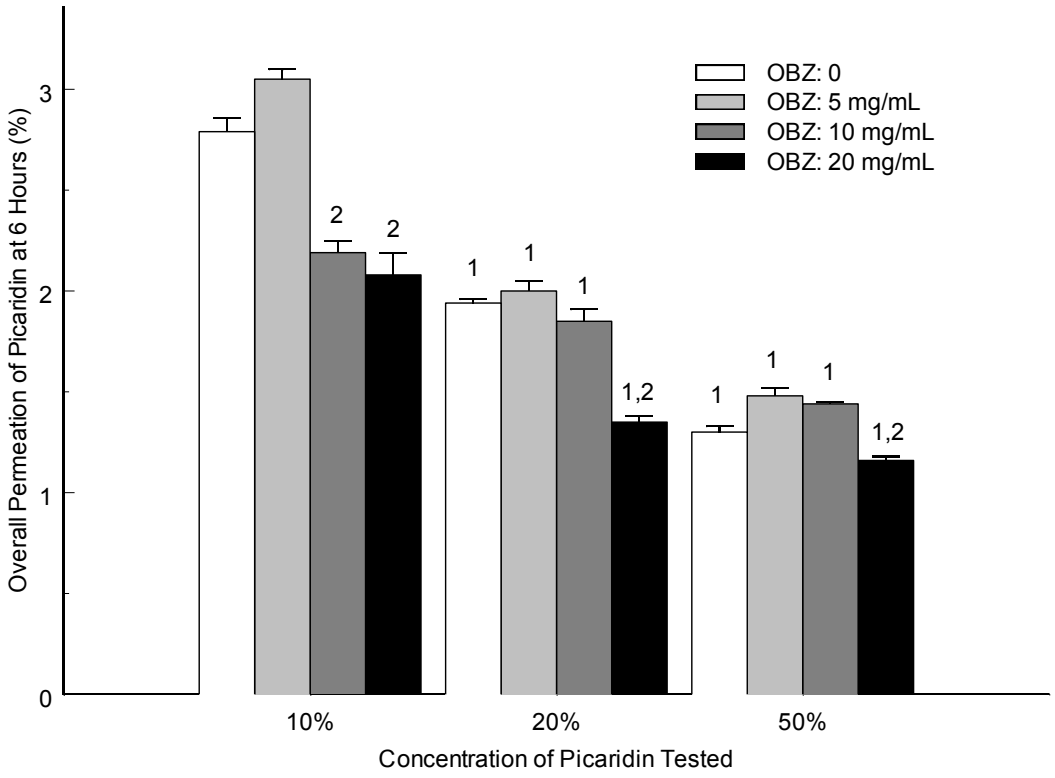

Figure 4.3. Overall permeation percentages of picaridin across PDMS membrane after 6 hours (1: significant difference from 10% study groups; 2: significant difference from single picaridin groups; $p \leq 0.05$, mean ± SEM, n = 6)

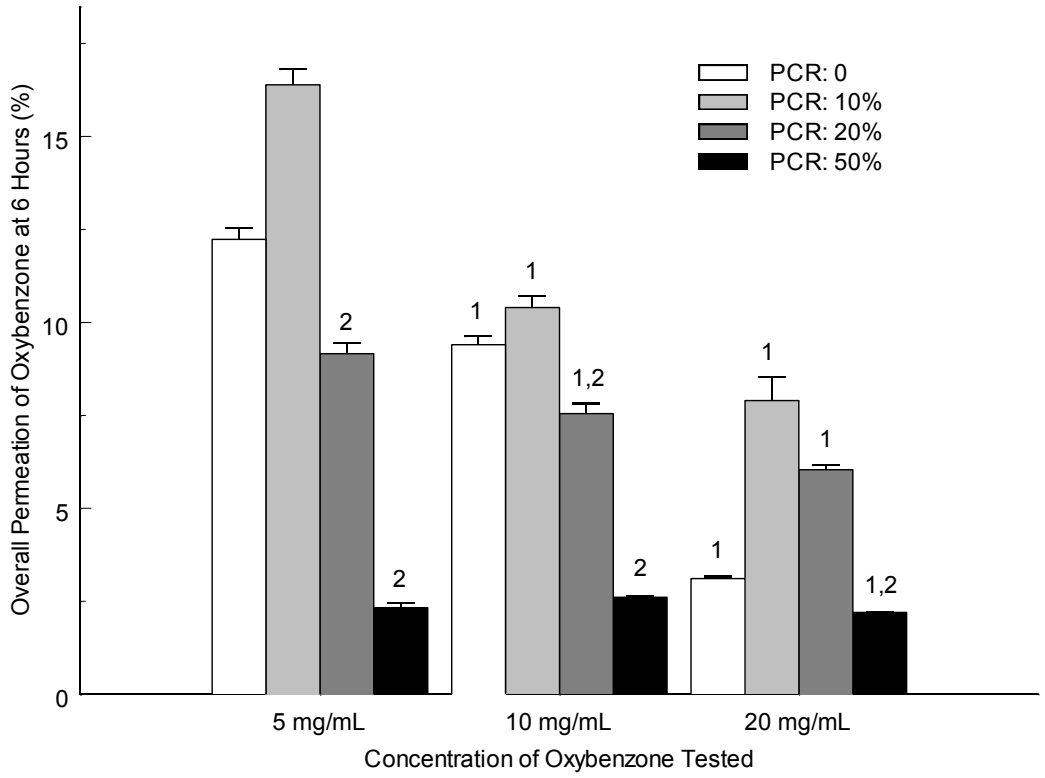

Figure 4.4. Overall permeation percentages of oxybenzone across PDMS membrane after 6 hours (1: significant difference from 5 mg/ml study groups; 2: significant difference from single oxybenzone groups; $p \leq 0.05$, mean ± SEM, n = 6)

oxybenzone decreased with increasing concentration of oxybenzone in the donor compartment by 23-75%. When it was tested together with 10% and 20% of picaridin, the transmembrane permeation of oxybenzone significantly decreased by 18-52%; the permeation of oxybenzone was not changed when picaridin concentration was increased to 50%. In comparison to single oxybenzone group at the same concentration, the co-presence of picaridin at 10% would increase the permeation of oxybenzone. However, the permeation of oxybenzone decreased by 20-81% when picaridin concentration was increased. Statistical significances were observed among various study groups (*Figure 4.4*).

4.4. Discussion

Concurrent use of insect repellents and sunscreens for summer outdoor activities appears to be both necessary and beneficial in protecting individuals from vector-borne diseases and sunburns. It is also prevalent among special working forces such as farmers, postal officers, forest workers and military personnel whose job responsibilities require daily outdoor exposures. For these individuals, concurrent application of insect repellent and sunscreen products on a regular basis may have been well integrated into their daily routines in order for them to maintain healthy conditions and meet working productivity. In addition, applying both repellent and sunscreen concurrently, ideally from one single preparation, would be considered practical and cost-effective for special working forces, since it not only saves time and resource, but also provides application convenience and effectiveness. How to formulate an effective, stable and quality combined

repellent/sunscreen preparation still poses challenges to the manufacturers. Numerous composite repellent/sunscreen products have been commercially available in certain countries. However, the prerequisites for a combined product would be to maintain maximal protection efficacy topically but produce minimal exposure systemically. Therefore, selecting appropriate active ingredients and minimizing overall systemic absorption of the chemicals by novel formulations would be two critical keys. This is of particular importance when repellent and sunscreen products are applied regularly for an extended period of time.

In general, preparations intended for topical skin protection do not require systemic percutaneous absorption of the active ingredients. This is universally applicable to active insect repellent and sunscreen compounds that do not possess any productive pharmacological and therapeutic efficacy *in vivo*. On the contrary, active repellent and sunscreen ingredients should exert minimal systemic disposition, while maintaining maximal protective efficacy on the surface of the skin. It is known that both repellent DEET and sunscreen oxybenzone are percutaneous permeation enhancers, not only to some other chemical substances, but also to each other. This mutual enhancement effect is subsequently undesirable and detrimental to individual users from a clinical perspective. Long-term pharmacological and toxicological effects of active repellent and sunscreen substances on the human body are still generally understudied and not readily understood. An enhanced percutaneous permeation of both ingredients such as DEET and oxybenzone would increase systemic concentration and retention, subsequently potentiating unwanted adverse effects of the chemicals in susceptible subjects. Therefore, it would become necessary and logical to minimize transdermal permeation of the

topically applied dose of DEET and oxybenzone through formulation development or selection of different active chemical compounds.

Permeability is a deciding parameter of the diffusant in terms of the capability of percutaneous diffusion and permeation. *Table 4.1* lists the steady-state permeability coefficients of picaridin and oxybenzone across human skin. The parameters decreased dramatically with increasing the concentrations of both test compounds, and the co-presence of a second component also reduced the permeability coefficient of either picaridin or oxybenzone. In addition, permeability of picaridin was much smaller than that of oxybenzone, indicating its inability to percutaneous diffusion. The tendency in permeability value changes further confirmed that concurrent use of picaridin and oxybenzone suppressed the transdermal permeation of both test compounds, particularly at higher use concentrations. This transmembrane characteristic might be attributed to either a competition for percutaneous permeation between the two test compounds or a saturation of the membrane model by the test substances. No mutual percutaneous enhancement of picaridin and oxybenzone was observed in human epidermis, which was different from what had been observed previously with concurrent use of repellent DEET and sunscreen oxybenzone. This attribute would be clinically desirable, because it might indicate a feasibility of developing a composite repellent/sunscreen preparation including picaridin and oxybenzone as the primary active ingredients.

In comparison to permeability of repellent DEET, picaridin appeared to possess a much lower overall transmembrane permeation. Under identical *in vitro* experimental conditions using human epidermis, DEET demonstrated an overall permeation percentage of 0.5-2.3% after a 6-hour diffusion study, while picaridin showed an overall permeation

Table 4.1. Permeability coefficient of picaridin and oxybenzone across human skin ($\times 10^{-4}$, cm/h, mean ± SEM, n = 4)

Study Code	**Picaridin Concentration**		
	10%	**20%**	**50%**
Without Oxybenzone	7.1 ± 0.2	6.5 ± 0.1	5.7 ± 0.2
5mg/ml Oxybenzone	7.5 ± 1.2	7.2 ± 0.1	3.6 ± 0.1
10mg/ml Oxybenzone	6.8 ± 0.5	6.6 ± 0.1	2.4 ± 0.0
20mg/ml Oxybenzone	6.3 ± 0.2	7.2 ± 0.2	2.4 ± 0.1
Study Code	**Oxybenzone Concentration**		
	5mg/ml	**10mg/ml**	**20mg/ml**
Without Picaridin	53.6 ± 3.5	28.9 ± 1.2	23.7 ± 1.7
10% Picaridin	24.8 ± 2.2	22.9 ± 1.3	14.9 ± 0.2
20% Picaridin	15.0 ± 0.7	15.8 ± 0.7	13.8 ± 0.9
50% Picaridin	2.5 ± 0.1	1.9 ± 0.1	2.3 ± 0.0

percentage of 0.2-0.3% for the same diffusion period [Wang and Gu, 2007]. With concurrent use of sunscreen oxybenzone, the permeation percentages of DEET were increased to 3.6-25.7%, but those of picaridin were not influenced by oxybenzone. A significantly lower percutaneous permeation would result in reduced systemic exposure of the active repellent ingredient, consequently achieving better topical repellent efficiency as well as longer protection duration for the compound. This might also allow for a smaller application dose of the active ingredient, as the chemical would have a lower tendency for loss through systemic percutaneous absorption. From data obtained from this study and other previous studies, picaridin would be considered a better candidate for topical applications, in terms of percutaneous permeation and systemic absorption. If necessary, a much higher application concentration of picaridin could also be applied without compromise in overall transdermal permeation and systemic disposition.

The overall transdermal permeation percentages of sunscreen oxybenzone found from this study were comparable to those of the previous studies, but DEET enhanced the permeation of oxybenzone when applied concurrently [Wang and Gu, 2007]. Overall percutaneous permeation of oxybenzone across excised human epidermis ranged between 0.3-2.0% after a 6-hour diffusion duration from various studies. Systemic absorption of oxybenzone from a single topical skin application in human subjects also amounted to approximately 2% of the applied dose due to transdermal permeation, which was closely comparable to what was observed in this study [Hayden et al., 1997]. The reduced permeation of oxybenzone with the presence of picaridin would be desirable from real-life application perspectives, because this would restrict systemic entry of the sunscreens

into the general circulation across the skin. Even though active sunscreen compounds are generally regarded as safe compounds for humans, it would be appropriate to conclude that these substances do not produce beneficial systemic effects in humans, and therefore their primary role is to defuse UV radiation on the surface of the skin.

Human skin is the first line of defence against foreign assaults of the body from environmental, physical and chemical factors. This type of defence primarily relies on the stratum corneum of the epidermis to restrict the entry of various substances into the human system. Systemic absorption of chemical substances through the skin can take place only after the barrier of stratum corneum has been compromised. There are numerous mechanisms by which chemical molecules permeate across the stratum corneum. Passive transport by concentration gradients would be the principal route of transdermal absorption for topical skin preparations, since the skin application would allow an accumulated amount of chemicals on the surface of the skin. In addition, molecular structures and physicochemical properties of the chemicals would also facilitate skin permeation. In general, lipophilic molecules possess a better chance of skin permeation than hydrophilic molecules due to their interaction with the stratum corneum and other epidermis layers. Once diffusing across the stratum corneum and epidermis and reaching dermis, blood vessels embedded within the skin would transport the absorbents to the general blood circulation, resulting in systemic disposition and metabolism of the chemicals. While transdermal delivery systems require sufficient systemic drug concentrations for certain therapeutic purposes and use various approaches to achieve high drug concentrations in blood, topical skin preparations should possess limited

systemic exposure of the active ingredients in order to maintain localized protective or therapeutic efficacy.

Human skin specimens are considered realistic and representative testing model for *in vitro* diffusion studies, when samples are adequately available for the experiments. However, skin samples collected from different body sites are believed to affect the transdermal penetration rate and extent of test molecules under a variety of conditions [Feldmann and Maibach, 1967; Scheuplein, 1978]. Artificial membranes like PDMS, on the other hand, are able to minimize experimental variations and simplify application preparation without the need for complicated pretreatment procedures and the rigorous storage conditions that are required for human skin specimens. The use of artificial membranes as a test model would be practical when sufficient amount of human skin samples are unavailable to complete a specific set of diffusion studies. Some success has been achieved from applying artificial membranes to mimic the percutaneous penetration of drug molecules through biological membranes [Jiang et al., 1998; Dias et al., 1999; Stamatialis et al., 2002]. Specifically, artificial membranes are appropriate for assessing drug release characteristics from a formulation vehicle or a delivery system.

From this study, artificial PDMS membrane produced a much higher permeation to both picaridin (more than 10 fold) and oxybenzone (more than 8 fold) than human epidermis. *Table 4.2* lists the steady-state permeability coefficients of picaridin and oxybenzone across the PDMS membrane, which were significantly larger than those of human epidermis, indicating experimental disparity between the two membrane models. PDMS membrane is a polymeric membrane of lipophilic nature. Both picaridin and oxybenzone are lipophilic compounds, and therefore might have produced elevated

Table 4.2. Permeability coefficient of picaridin and oxybenzone across PDMS membrane ($\times 10^{-4}$, cm/h, mean ± SEM, n = 4)

Study Code	**Picaridin Concentration**		
	10%	**20%**	**50%**
Without Oxybenzone	77.4 ± 1.8	50.9 ± 0.4	33.8 ± 0.8
5mg/ml Oxybenzone	83.1 ± 1.5	54.0 ± 1.4	39.1 ± 0.9
10mg/ml Oxybenzone	57.4 ± 1.7	49.6 ± 1.6	38.3 ± 0.3
20mg/ml Oxybenzone	55.5 ± 3.2	35.0 ± 0.8	30.9 ± 0.5
Study Code	**Oxybenzone Concentration**		
	5mg/ml	**10mg/ml**	**20mg/ml**
Without Picaridin	316.4 ± 8.8	247.3 ± 7.8	81.3 ± 1.1
10% Picaridin	440.1 ± 12.6	275.4 ± 8.8	206.8 ± 19.1
20% Picaridin	241.4 ± 9.2	208.1 ± 7.6	161.9 ± 3.6
50% Picaridin	58.3 ± 3.7	68.5 ± 0.9	55.9 ± 0.9

transmembrane permeation across PDMS in comparison to human epidermis. In addition, the thickness of PDMS might also attribute to higher permeability to the two test compounds.

In comparison to similar studies on DEET and oxybenzone, picaridin demonstrated reduced overall transmembrane permeation across PDMS membrane than DEET. The overall permeation of DEET across PDMS for a 6-hour diffusion period ranged between 9.6-16.5% when tested individually and 20.9-30.2% when tested in combination with oxybenzone, respectively [Wissing and Müller, 2002]. Picaridin produced much lower permeation percentages under identical conditions, i.e., 1.3-2.8% when tested separately and 1.2-3.1% when tested in combination with oxybenzone. This pattern suggested a reasonably satisfactory correlation in transmembrane permeation between PDMS and human epidermis. While artificial membranes would still be valuable for *in vitro* diffusion studies, especially when biological membrane models are limited in amount, human skin is considered a more reliable and preferred test model for all transdermal drug evaluations.

Ethanol solution (50%) was used as the dissolving solvent in the study to prepare the diffusion samples. Ethanol is a known facilitator for percutaneous absorption, because it is able to dissolve lipophilic components in the stratum corneum and promote drug permeation through the enlarged pores. In previous studies, ethanol solution produced higher permeation of DEET and oxybenzone than PEG-400 [Gu et al., 2004]. In this study, the effect of the dissolving vehicle on transmembrane permeation of picaridin and oxybenzone seemed to be negligible. Many commercial repellent and sunscreen preparations do use ethanol as one of the primary dissolving vehicles, because it provides

satisfactory dissolution capability to active ingredients and produces quick evaporation of the products once applied. The use of ethanol might be appropriate for picaridin preparations, although further studies are needed to assess its long-term impact on topical skin applications.

Commercial repellent and sunscreen products are generally composed of multiple active ingredients and additives to enhance protection efficacy and application convenience. The presence of these additional components may influence or change the percutaneous permeation of substances like picaridin and oxybenzone across the skin membrane [Wissing and Müller, 2002]. This characteristic was not investigated in this study. In addition, physical properties of a complete liquid or semi-solid product may also alter the transdermal permeation of picaridin and oxybenzone; these properties would include viscosity and formulation type of the preparation, concentration of the active ingredients in the products, and how preparations are applied to the skin surface. Since the general public uses commercial repellent and sunscreen products in real-life situations, it would be more practical and realistic to test actual existing products of picaridin and oxybenzone to further understand and elucidate the percutaneous permeation patterns of picaridin and oxybenzone, both *in vitro* and *in vivo*.

4.5. Conclusion

Percutaneous permeation of the newer insect repellent picaridin and the sunscreen oxybenzone from concurrent applications did not produce enhanced transmembrane permeation, which was different from that of repellent DEET and sunscreen oxybenzone.

Increase in concentration of picaridin and oxybenzone reduced the overall permeation percentages of both compounds, especially at high concentrations and when two compounds were simultaneously present in the experiments. Compared to biological human epidermis, artificial membrane PDMS produced significantly higher permeability coefficients and overall permeation percentages of picaridin and oxybenzone. However, both study membranes exhibited similar permeation patterns of picaridin and oxybenzone, which might be useful and correlative when no sufficient biological membranes are available. In comparison to repellent DEET, picaridin demonstrated significantly lower overall permeation extent across the study membranes, while sunscreen oxybenzone produced comparable permeation results that were further suppressed in the presence of picaridin. Since picaridin is a relatively new repellent agent, its permeation characteristics need to be further investigated, in particular, those from commercially available picaridin products and *in vivo* studies in either animal models or human subjects. Composite repellent/sunscreen preparations are a unique and practical product that possesses advantages in application convenience and cost-effectiveness. With further understanding of percutaneous characteristics of picaridin and other active sunscreen ingredients, it might be possible to develop a combine picaridin/sunscreen preparation that can not only achieve dual protection efficacy but also restrict systemic exposure of the chemicals as topical skin preparations.

Chapter 5

Permeation Characterization of Picaridin and Oxybenzone from Various Formulations

5.1. Introduction

Insect repellents and sunscreens are the most practical, cost-effective and well-accepted choice of defence against vector-borne diseases and skin cancers, and they have been extensively used as over-the-counter specialty products by the general public for decades [Fradin, 1998; Dadlani and Orlow, 2008; 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 permeation of the active ingredients is considered neither productive nor desirable [Robbins and Cherniak, 1986; Koren et al., 2003; Abdel-Rahman et al., 2004A & 2004B; Hexsel et al., 2008]. Previous studies have found systemic absorption of the repellent DEET (N,N-diethyl-*m*-toluamide, OFF®) and the sunscreen oxybenzone from topical skin applications [Hayden et al., 1997; Qiu et al., 1997]. We have reported a synergistic percutaneous enhancement between DEET and oxybenzone in a series of previous 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 [Naucke et al., 2007; Carroll et al., 2008; Katz et al., 2008; Zhu et al., 2008]. Recently we reported 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.

5.2. Materials and Methods

5.2.1. Materials

Pure picaridin standard was received as a free study sample from Lanxess Corporation (Pittsburgh, Pennsylvania, USA), and pure oxybenzone standard was purchased from Riedel-de Haën GmbH (Seelze, Germany). Methanol, phosphoric acid, potassium phosphate monobasic, and sodium hydroxide were purchased from Fisher Scientific (Fair Lawn, New Jersey, USA). Glacial acetic acid and polyoxyethylene 20-oleyl ether (Brij® 98) were obtained from Mallinckrodt Specialty Chemical Company (Paris, Kentucky, USA) and Sigma-Aldrich Co. (St. Louis, Missouri, USA), respectively. All solvents were HPLC-grade, and other chemicals were AC-grade.

Three picaridin-based repellent sprays and one sunscreen lotion were purchased from various pharmacies, and used as obtained without further manipulation. They were

OFF® Skintastic Clean Feel Spray (Product A, 5.0% picaridin, S.C. Johnson and Son Ltd., Racine, Wisconsin, USA), Cutter Advanced Repellent Spray (Product B, 7.0% picaridin, Spectrum, Division of United Industries Corporation, St. Louis, Missouri, USA), Repel Sportsman Formula Spray (Product C, 20.0% picaridin, WPC Brands, Inc., Bridgeton, Missouri, USA), and Coppertone® Oil Free Sunblock Lotion (Product D, 4.0% oxybenzone, SPF 30, Schering-Plough HealthCare Products, Point-Claire, Quebec, Canada). To maintain the comparability between this study and previous results of repellent DEET and sunscreen oxybenzone [Wang and Gu, 2007], identical application approaches were employed. *Table 5.1* lists the study design of the diffusion experiments where these four preparations were tested either individually or in combination.

5.2.2. Diffusion Studies

In vitro diffusion experiments were performed in an automatic transdermal system (Logan Instruments Corporation, Somerset, New Jersey, USA), which was composed of six vertical Franz-style diffusion cells, a circulating water bath, a magnetic stir console, and an automatic sampling collector. The surface area available for drug diffusion in the Franz cells was 0.64 cm^2. The receptor fluid used 7.0 ml phosphate buffer solution that contained 4% Brij® 98 (w:v) with pH adjusted to 7.4 using concentrated phosphoric acid.

Full human skin specimens were obtained from the Department of Surgery, St. Boniface General Hospital of Winnipeg. The collection protocol was approved by the Research Ethics Boards at both the University of Manitoba and St. Boniface General Hospital. Prior to each experiment, the skin samples were dermatomed to a thickness of

Table 5.1. *In vitro* diffusion study settings and study preparations

Study Code	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Picaridin	1[A]	1[B]	1[C]	---	0.5[A]	0.5[B]	0.5[C]	0.3[B]	0.6[B]	0.5[B]	0.5[B]
Oxybenzone	---	---	---	1[D]	0.5	0.5	0.5	0.6	0.3	0.5	0.5
Application Method	Direct application	Direct application	Direct application	Direct application	Mixing	Mixing	Mixing	Mixing	Mixing	B:bottom, No mixing	B:top, No mixing

A: Product A, 5.0% picaridin, B: Product B, 7.0% picaridin,
C: Product C, 20.0% picaridin, D: Product D, 4.0% oxybenzone

400 μm according to standard preparation procedures previously established [Wang and Gu, 2007; Gu and Chen, 2009]. The specimen was carefully examined and undamaged epidermis was selected for the diffusion experiment.

The diffusion studies were carried out at 37 ± 0.05 °C and 300 rpm. The test sample (1.0 ml) was placed in each donor cell, and the donor cells were covered with a microscope cover slip to form an occlusive environment. The applied dose in each study provided an infinite drug amount for the diffusion process. An aliquot of receptor medium (100 μl) was collected hourly for six hours during the diffusion study, followed by replenishing with the same volume of fresh, preheated receptor medium. Diffusion experiment was performed in four replicates for each study group. Concentrations of picaridin and oxybenzone in the receptor fluid were analyzed using an HPLC assay developed and validated in our laboratory.

5.2.3. HPLC Assay

The HPLC system was comprised of a Waters® Alliance 2690 Solvent Delivery Module, a 996 Photodiode Array Detector, and a μBondapak® C_{18} column (3.9 × 150 mm, 10 μm) (Milford, Massachusetts, USA). The mobile phase was a mixture of methanol and water (pH 3.0, adjusted with glacial acidic acid) (65:35, v:v), delivered at a flow rate of 1.0 ml/min. Picaridin and oxybenzone were detected at 210 nm and 287 nm, respectively. The detection limit was 50 ng for picaridin and 20 ng for oxybenzone. Diffusion samples were directly injected for analysis without further pretreatment. Concentrations of picaridin and oxybenzone in the receptor media were then calculated based on the average calibration curves (n = 6).

5.2.4. Data Analysis

Overall permeation percentages of picaridin and oxybenzone after 6 hours of diffusion were calculated for each study group based on the ratio of accumulated permeation amount in the receiver cell to the application amount of test product in the donor cell. Results of this study were compared to those of DEET and oxybenzone obtained from a previous study under identical experimental conditions [Wang and Gu, 2007]. Statistical analyses were performed using one-way ANOVA (PC-SAS® 8.02, SAS Institute Inc., Cary, North Carolina, USA) to compare: a) overall permeation percentages of picaridin and oxybenzone among various study groups; b) overall permeation percentages of picaridin, DEET and oxybenzone under similar experimental conditions. Differences were considered statistically significant at $p \leq 0.05$.

5.3. Results

5.3.1. Permeation of Picaridin

Figure 5.1 shows the accumulated permeation percentage of picaridin across human epidermis from three repellent sprays after 6 hours. Adding sunscreen oxybenzone to Product A (5%) and Product B (7%) did not significantly alter permeation characteristics of picaridin. However, Product C (20%) 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.

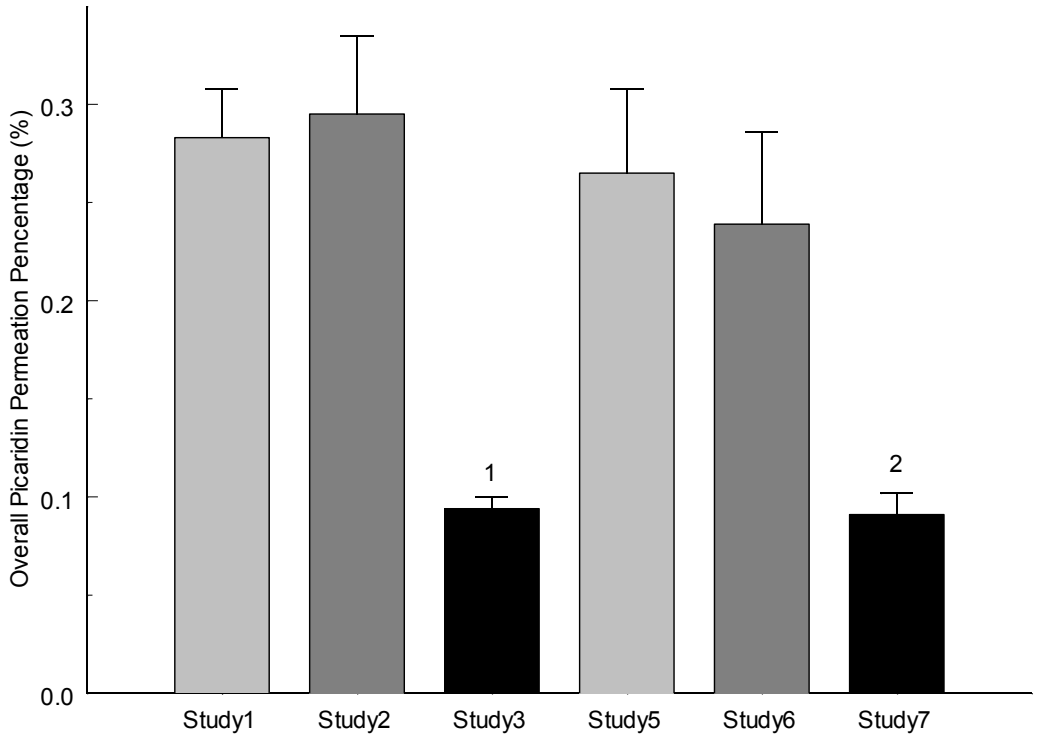

Figure 5.1. Accumulated permeation percentages of picaridin from 3 repellent sprays across human epidermis after 6 hours
(1: significant difference from studies 1 and 2; 2: significant difference from studies 5 and 6; $p \leq 0.05$, mean ± SEM, n = 4)

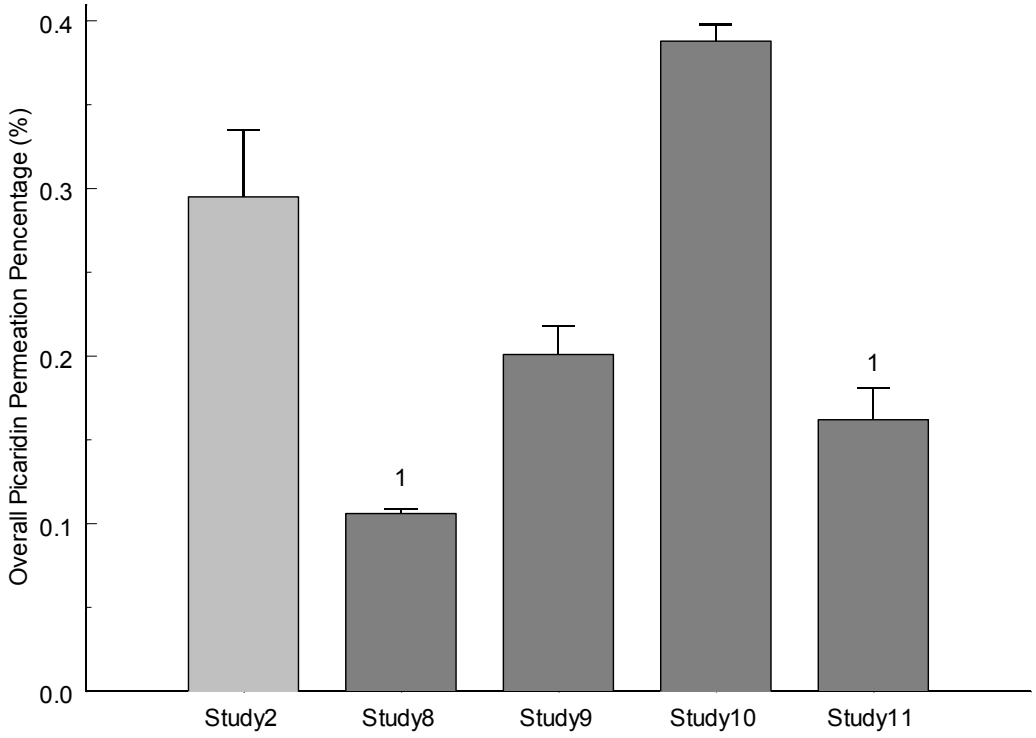

Figure 5.2. Accumulated permeation percentages of picaridin from 4 application approaches across human epidermis after 6 hours
(1: significant difference from study 2; $p \leq 0.05$, mean ± SEM, n = 4)

Figure 5.2 shows the accumulated permeation percentage of picaridin (Product B only) across human epidermis from different use proportions and application sequences after 6 hours. When picaridin and sunscreen was mixed at a ratio of 1:2 (w:w), suppression of picaridin permeation was statistically significant at 178%. Placing picaridin spray on top of sunscreen without premixing also significantly reduced the permeation by 82%. This was mainly attributed to a physical barrier of sunscreen 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.

5.3.2. Permeation of Oxybenzone

Figure 5.3 shows the accumulated permeation of oxybenzone across human epidermis after 6 hours, for all test groups. No significant change in oxybenzone permeation occurred when the sunscreen was mixed with picaridin spray of lower concentration (Products A and B). However, permeation of oxybenzone was significantly suppressed by 34% when picaridin concentration was increased to 20%. Significant suppression of oxybenzone permeation was also observed when picaridin and oxybenzone 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.

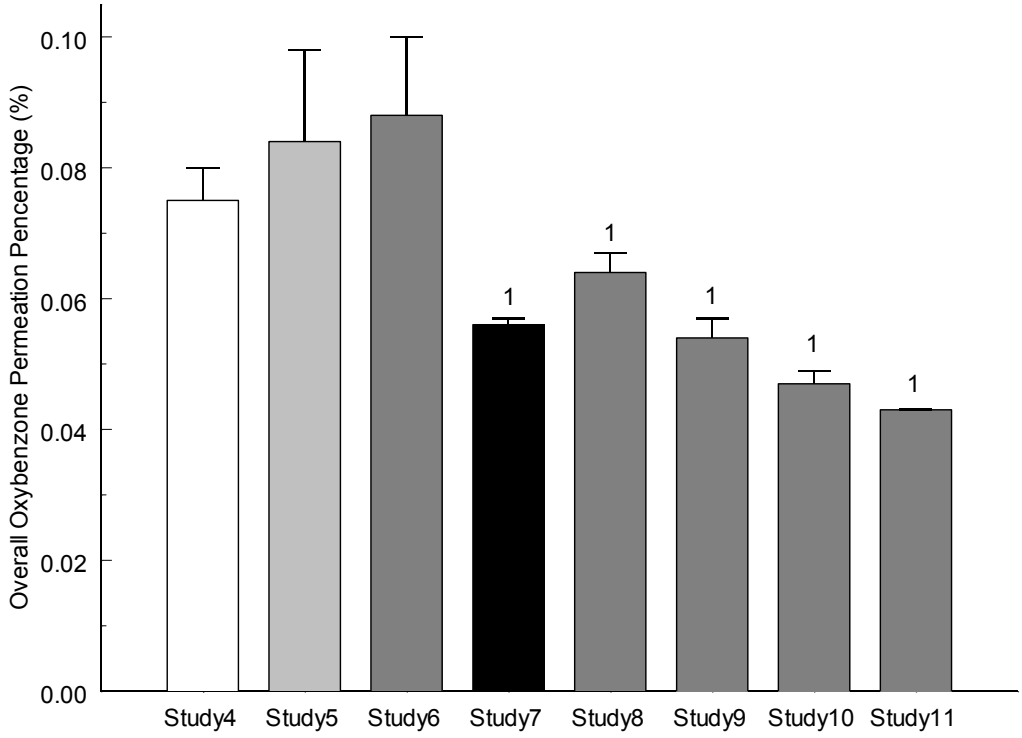

Figure 5.3. Accumulated permeation percentages of oxybenzone from all study groups across human epidermis after 6 hours
(1: significant difference from study 4; $p \leq 0.05$, mean ± SEM, n = 4)

Transdermal suppression from these four experiments ranged 17-74% in comparison to single sunscreen use.

5.3.3. Comparison of Picaridin and DEET

One of the primary objectives of the study was to characterize concurrent use of commercial repellent and sunscreen products. Because concurrent use of repellent DEET and sunscreen oxybenzone produced synergistic percutaneous permeation of both compounds from commercially available preparations [Gu et al., 2005; Wang and Gu, 2007], results obtained from this study were compared to those of DEET and oxybenzone under identical experimental conditions.

Figure 5.4 shows the comparative results of picaridin and DEET in association with oxybenzone from 5 different study groups. Permeation of DEET was significantly greater than that of picaridin in all study groups; the increments ranged between 3-66 fold. 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 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 condition. Mixing DEET and oxybenzone at 2:1 (w:w) also produced a 36-fold permeation increase than mixing picaridin and oxybenzone.

Figure 5.5 shows the comparative results of oxybenzone permeation in association with either picaridin or DEET spray. Permeation of oxybenzone with DEET

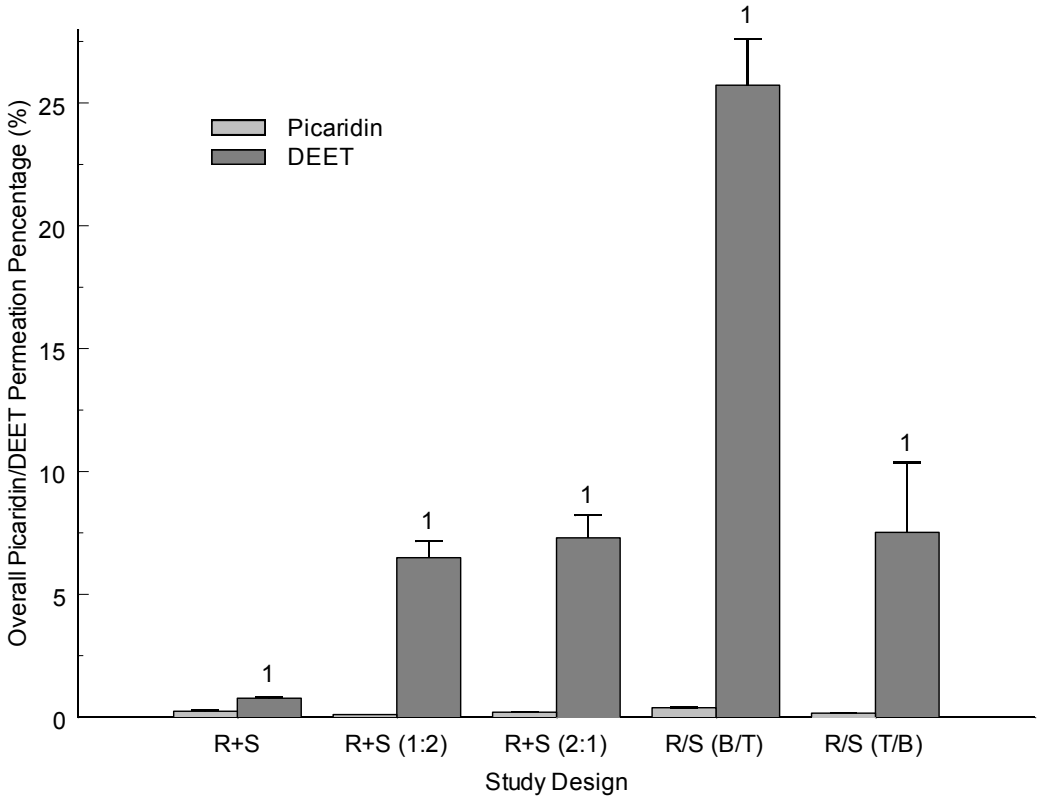

Figure 5.4. Comparative permeation percentages of picaridin and DEET in association with oxybenzone across human epidermis after 6 hours (1: significant difference from corresponding picaridin study groups; $p \leq 0.05$, mean ± SEM, n = 4; R: repellent, S: sunscreen, B: bottom, T: top; DEET/oxybenzone data from Wang and Gu, 2007)

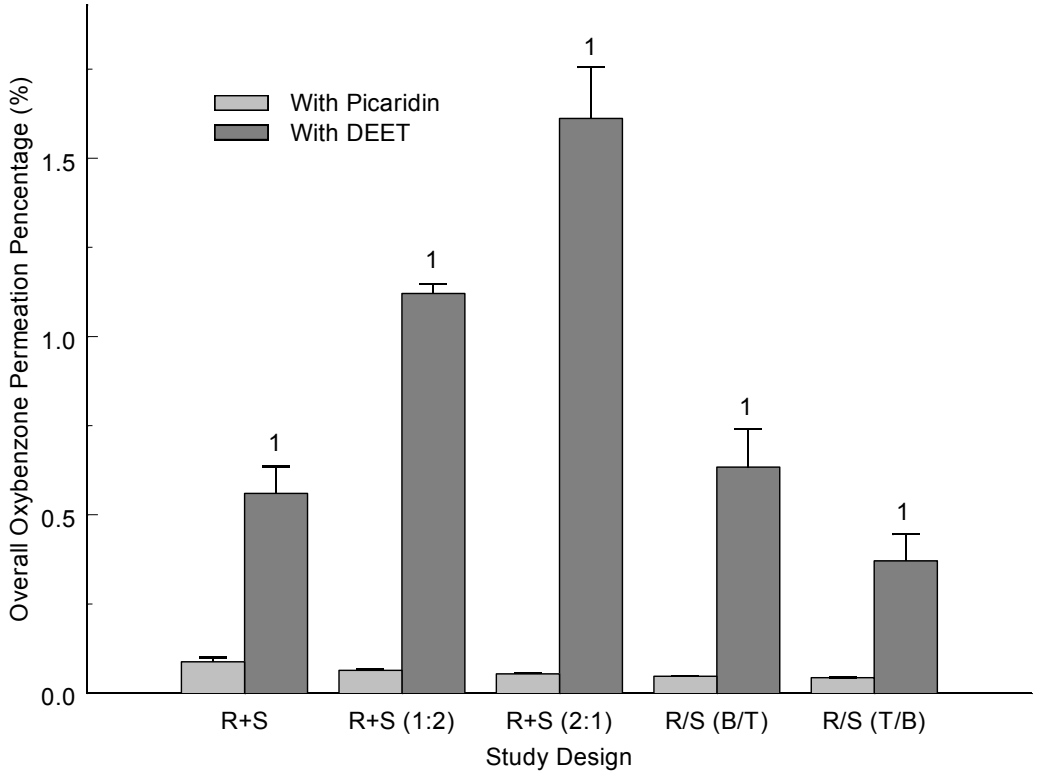

Figure 5.5. Comparative permeation percentages of oxybenzone in association with picaridin or DEET across human epidermis after 6 hours (1: significant difference from corresponding picaridin study groups; $p \leq 0.05$, mean ± SEM, n = 4; R: repellent, S: sunscreen, B: bottom, T: top; DEET/oxybenzone data from Wang and Gu, 2007)

was significantly larger than that of oxybenzone with picaridin; the overall permeation increments ranged between 6-30 fold. 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 (increment of 18 and 30 fold, respectively), and when DEET spray was placed under sunscreen lotion (a 14-fold increment). On the other hand, mixing picaridin with sunscreen generally showed no change or even suppression of oxybenzone permeation from these study groups.

5.4. Discussion

Human skin is the first line of defence against foreign assaults of the body from environmental, physical and chemical factors. This type of defence primarily relies on the stratum corneum of the epidermis to restrict entry of various substances into human system. In the meantime, the skin also offers a convenient access to drug delivery, enabling both localized and systemic drug effects. In general, it is undesirable for active ingredients in cosmetic or consumer care products to permeate into deeper skin layers or to be systemically absorbed. Percutaneous absorption from topical preparations such as insect repellents and sunscreens may sometimes lead to unwarranted pharmacological and toxicological consequences in susceptible subjects [Koren et al., 2003; Hexsel et al., 2008].

Concurrent use of insect repellents and sunscreens during summer months has become both necessary and beneficial in protecting individuals from vector-borne diseases and sunburns. It is particularly prevalent among special working forces such as

farmers, postal officers, forest workers and military personnel whose job responsibilities require extensive outdoor exposures. To maintain productivity as well as personal health conditions, these individuals have to apply repellent and sunscreen products regularly. Applying repellent and sunscreen, ideally from one single preparation, would be both practical and cost-effective, because this not only facilitates use convenience but also saves time and cost. For recreational outdoor activities, a composite repellent/sunscreen product might also be useful and cost-effective. The availability of a properly-designed preparation would be attractive to individuals who are conscious of protection against vector-borne diseases and skin cancers.

DEET and picaridin are effective insect repellents that have demonstrated long-lasting repellence against a variety of biting insects [Katz et al., 2008]. However, the two compounds display different skin permeation properties when combined with sunscreen oxybenzone. DEET and oxybenzone produced a synergistic percutaneous permeation across the skin when used simultaneously [Kasichayanula et al., 2007; Wang and Gu, 2007]. Picaridin, on the other hand, either was unaffected by or produced a suppressive skin permeation when used concurrently with sunscreen oxybenzone. The comparative data shown in *Figure 5.4* and *Figure 5.5* demonstrated the differences of the three test compounds in terms of the transdermal permeation. Repellent and sunscreen products are widely available to the general public. Concurrent use of both preparations is largely under the discretion of individual users, with no medically recommended dose and application approach. From perspective of systemic exposure to the chemicals, picaridin would be a more appropriate choice for concurrent application. This would be

particularly applicable to those who use both products for extended period of time on a regular basis.

Percutaneous permeation of picaridin, DEET and oxybenzone was also dependent upon several other parameters including preparation type, use concentration, and application sequence. It was found from previous studies that lotion preparations produced enhanced percutaneous permeation due to prolonged contact of the formulation with the skin [Gu et al., 2005; Wang and Gu, 2007]. Liquid preparations such as spray possessed lower permeation. Under real-life use conditions, liquid vehicles evaporate relatively quickly at ambient temperature, and subsequently reduce potential for the chemicals to interact with the skin membrane. High use dose may also saturate the skin surface, limiting further diffusion and permeation of the substance into deeper skin layers. Application sequence is particularly important to repellent and sunscreen products, as misuse could 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 use approach, because sunscreens are designed for skin protection while repellents are intended for averting insect bites. Applying repellent underneath sunscreen would diminish the repellence efficacy and promote the transdermal absorption, which has been proven for both picaridin and DEET. It would therefore be useful to illustrate the correct application methods on repellent and sunscreen product labels so that consumers can follow these directions safely and effectively.

Commercial repellent and sunscreen products are composed of multiple active and auxiliary compounds to produce stable and useful preparations. The presence of

excipients may contribute to characteristics of partition and diffusion of the active compounds within the skin membrane. Mixing DEET-based repellents and sunscreens reduced the Sun Protection Factor (SPF) of sunscreen preparation but not the repellent efficacy of DEET [Montemarano et al., 1997; Murphy et al., 2000]. It would be useful to assess whether or not there is a similar pattern between picaridin and sunscreen preparations. Health Canada decided to discontinue composite DEET/sunscreen products in the Canadian market several years ago. Combined products, however, are still commercially available in other countries [Hexsel et al., 2008]. In the light of findings from this study and comparison between picaridin and DEET, a combined repellent/sunscreen preparation would be more appropriate and desirable by combining picaridin and various sunscreen components, in order to reduce overall systemic absorption of the active ingredients and provide dual protection of the products.

5.5. Conclusion

Percutaneous permeation characteristics of the newer repellent picaridin in association with the 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 incorporated. Permeability of oxybenzone was generally reduced when mixed with commercially available picaridin products. It was therefore concluded that picaridin would be a better candidate for concurrent use with sunscreen preparations in terms of percutaneous permeation. This would 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.

Chapter 6

Conclusion and Future Studies

Appropriate application of insect repellents and sunscreens has been one of the most effective, practical and inexpensive means for the general public in preventing vector-borne diseases and sunburns while enjoying healthy lifestyle and outdoor activities at the same time. Applying active repellent and sunscreen ingredients, ideally from one single product, would be both convenient and cost-effective. The availability of a properly-designed preparation is particularly attractive to specialty workforces including military, forest, farming, and postal personnel whose job responsibilities require of extensive outdoor activities, as well as to those individuals who are willing to apply both products for summer recreational activities due to health concerns from vector-borne diseases and skin cancer. The selection of appropriate active repellent and sunscreen ingredients and the novel formulation of the components into a stable and acceptable product would be two important keys in maximizing the protection efficacy of the composite product and minimizing the overall systemic absorption of the active ingredients from topical skin application.

It has been known that the repellent DEET and the sunscreen oxybenzone are percutaneous permeation enhancers, not only to some other chemical compounds but also to each other. This characteristic of mutual permeation enhancement is neither desirable nor productive from a clinical perspective, because repellent and sunscreens preparations are indicated for external protection, and there is no need for the active ingredients to be absorbed systemically from a topical skin application. Moreover, repellent and sunscreen products are generally applied at individual discretion without medically recommended dose and application frequency, exposure to these topical preparations for a prolonged

period of time could subsequently increase systemic accumulation of the active ingredients in the body and potentiate unwarranted side effects in susceptible subjects.

Results from this thesis indicated that the newer insect repellent picaridin possessed a significantly lower transmembrane permeation than DEET *in vitro*. In addition, concurrent application of picaridin and the sunscreen oxybenzone did not produce synergistic permeation of the compounds as what had been observed between DEET and oxybenzone. On the contrary, picaridin either was unaffected by or produced a suppressive permeation when used with oxybenzone concurrently. Increase in use concentration of picaridin and oxybenzone also reduced the overall permeation of both compounds, especially at high concentrations and when the two substances were present in the same medium. This character of transmembrane permeation would be considered desirable and beneficial for topical preparations, as the protection efficacy of repellent and sunscreen products is intended for external environment.

Commercially available repellent and sunscreen preparations are composed of multiple auxiliary components to aid in producing stability and acceptance of the final products. The presence of these non-medicinal excipients may also contribute to altering the characteristics of partition and diffusion of the active ingredients within the skin membrane. Results from this thesis using various commercially available picaridin sprays and sunscreen lotion demonstrated similar percutaneous patterns that were observed from using ethanolic solution of picaridin and oxybenzone. Picaridin possessed much smaller permeability across human epidermis than DEET, and its permeation was not overly affected when sunscreen lotion was incorporated simultaneously. The permeability of oxybenzone was generally reduced when mixed concurrently with commercial picaridin

spray preparations, due mainly to a saturated skin surface for further diffusion and permeation.

It was concluded from this thesis that picaridin would be a better candidate for concurrent use with sunscreen preparations in terms of percutaneous permeation. Because picaridin is still a relatively new repellent agent, its permeation characteristics need to be further investigated and illustrated. Future studies could therefore be focused on understanding the mechanisms of its transdermal diffusion and permeation and its interaction with oxybenzone and other sunscreen ingredients after topical skin application. *In vivo* studies in either animal models or human subjects are also required to characterize and elucidate the suppressive permeation between the two compounds that had been observed *in vitro*.

Developing and evaluating composite repellent/sunscreen preparations would be the ultimate goal of the research, as they not only offer application convenience, but also are in high demand by the specialty workforces. In the findings from this thesis and the comparison between picaridin and DEET, a combined picaridin/sunscreen preparation would be more appropriate and feasible, because it is possible to minimize overall systemic absorption of the active ingredients and provide dual repellent/sun-blocking efficacy by novel formulation strategies. With the availability of these innovative preparations, it will provide product choice to both the professionals and the general public in using repellent and sunscreen products safely and effectively and improving the work productivity and the quality of life at the same time.

References

Abdel-Rahman, A., Abou-Donia, S.M., El-Masry, E.M., Shetty, A.K., Abou-Donia, M.B., 2004A. Stress and combined exposure to low doses of pyridostigmine bromide, DEET, and permethrin produce neurochemical and neuropathological alterations in cerebral cortex, hippocampus, and cerebellum. *J. Toxicol. Environ. Health, Part A*. 67, 163-192.

Abdel-Rahman, A., Dechkovskaias, A.M., Goldstein, L.B., Bullman, S.H., Khan, W., El Masry, E.M., Abou-Donia, M.B., 2004B. Neurologivcal deficits induced by malathion, DEET, and permethrin, alone or in combination in adult rats. *J. Toxicol.Environ. Health, Part A*. 67, 331-356.

Barnard, D.R., Bernier, U.R., Posey, K.H., Xue, R.D., 2002. Repellency of IR 3535, KBR 3023, *para*-menthane-3,8-diol, and deet to black salt marsh mosquitoes (Diptera: Culicidae) in the Everglades National Park. *J. Med. Entomol.* 39, 895-899.

Barry, B.W., 2002. Drug delivery routes in skin: a novel approach. *Adv. Drug Delivery Rev.* 54(sp1), S31-S40.

Berti, J.J., Lipsky, J.J., 1995. Transcutaneous Drug Delivery: A Practical Review. *Mayo Clin. Proc.* 70, 581-586.

Blank, I.H., 1953. Further observations on factors which influence the water content of the stratum corneum. *J. Invest. Dermatol.* 21, 259-271.

Brain, K.R., Walters, K.A., Watkinson, A.C. Methods for studying percutaneous absorption. In: Walter, K.A. (ed.), 2002. Dermatological and Transdermal Formulations. *Marcel Dekker Inc. New York.* pp.197-269.

Bronaugh, R.L., Stewart, R.F., Simon, M., 1986. Methods for in-vitro percutaneous absorption studies. VII: use of excised human skin. *J. Pharm. Sci.* 75, 1094-1097.

Brown, M., Hebert, A.A., 1997. Insect repellent: an overview. *J. Am. Acad. Dermatol.* 36, 243-249.

Carroll, J.F., Benante, J.P., Klun, J.A., White, C.E., Debboun, M., Pound, J.M., Dheranetra, W., 2008. Twelve-hour duration testing of cream formulations of three repellents against Amblyomma americanum. *Med. Vet. Entomol.* 22(2), 144-151.

Caspers, P.J., Lucassen, G.W., Carter, E.A., Bruining, H.A., Puppels, G.J., 2001. In vivo confocal raman microspectroscopy of the skin: noninvasive determination of molecular concentration profiles. *J. Invest. Dermatol.* 116(3), 434-442.

Chatelain, E., Gabard, B., Surber, C., 2003. Skin penetration and sun protection factor of five UV filters: effect of the vehicle. *Skin Pharmacol. Appl. Skin Physiol*. 16, 28-35.

Clowes, H.M., Scott, R.C., Heylings, J.R., 1994. Skin absorption: flow-through or static diffusion cells? *Toxicol. in Vitro* 8, 827-830.

Collins, P., Ferguson, J., 1994. Photoallergic contact dermatitis to oxybenzone. *Br. J. Dermatol.* 131(1), 124-129.

Cosmetic Ingredient Review, 1983. Final report on the safety of benzophenone-1, -3, -4, -5, -9, and -11. *J. Am. Coll. Toxicol.* 2, 35-77.

Crank, J., 1975. The Mathematics of Diffusion, 2nd ed. *Oxford University Press, New York.*

Cross, S.E., Anissimov, Y.G., Magnusson, B.M., Roberts, M.S., 2003. Bovine-serum-albumin-containing receptor phase better predicts transdermal absorption parameters for lipophilic compounds. *J. Invest. Dermatol.* 120(4), 589-591.

Curtis, C.F., 1992. Personal protection methods against vectors of disease. *Rev. Med. Vet. Entomol*. 80:543-553.

Dadlani, C., Orlow, S.J., 2008. Planning for a brighter future: a review of sun protection and barriers to behavioural change in children and adolescents. *Dermatol. Online J.* 14(9), 1.

Daniels, R., 2004. Strategies for skin penetration enhancement. *scf-online.com. Issue 37.*

Debboun, M., Strickman, D.A., Klun, J.A., 2005. Repellents and the military: our first line of defense. *J. Am. Mosq. Control Assoc.* 21(sp1), 4-6.

Dennis, L.K., Beane Freeman, L.E., VanBeek, M.J., 2003. Sunscreen use and the risk for Melanoma: A Quantitative Review. *Ann. Intern. Med.* 139(12), 966-978.

Dias, M., Farinha, A., Faustino, E., Hadgraft, J., Pais, J., Toscano, C., 1999. Topical delivery of caffeine from some commercial formulations. *Int. J. Pharm*. 182, 41-47.

Eckert, R.L., 1989. Structure, function, and differentiation of the keratinocyte. *Physiol. Rev.* 69, 1316-1346.

Elias, P.M., 1981. Epidermis lipids, membranes and keratinisation. *Int. J. Dermatol.* 20, 1-9.

Elias, P.M., 1983. Epidermis lipids, barrier function, and desquamation. *J. Invest. Dermatol*. 80(spl), 44-49.

Elias, P.M., 1991. Epidermal barrier function: intercellular lamellar lipid structures, origin, composition and metabolism. *J. Controlled Release* 15, 199-208.

Fartasch, M., Bassukas, I.D., Dipegen, T.L., 1993. Structural relationship between epidermal lipid lamellae, lamellar bodies and desmosomes in human epidermis. An ultrastructural study. *Br. J. Dermatol.* 128, 1-9.

Feldmann, R.J., Maibach, H.I., 1967. Regional variation in percutaneous penetration of ^{14}C cortisol in man. *J. Invest. Dermatol*. 48, 181-183.

Felton, L.A., Wiley, C.J., Godwin, D.A., 2004. Influence of cyclodextrin complexation on the in vivo photoprotective effects of oxybenzone. *Drug Dev. Ind. Pharm.*30, 95-102.

Flynn, G.L. Topical drug absorption and topical pharmaceutical systems. In: Banker, G.S., Rhodes, C.T. (eds.), 1990. Modem Pharmaceutics. *Marcel Dekker, New York.* pp.263-325.

Flynn, G., Yalkowsky, S.H., Roseman, T.J., 1974. Mass transport phenomena and models. *J. Pharm. Sci.* 63, 479-510.

Fradin, M.S., 1998. Mosquitoes and mosquito repellents: A clinician's guide. *Ann. Intern. Med.* 128(11), 931-940.

Frances, S.P., Dung, N.V., Beebe, N.W., Debboun, M., 2002. Field evaluation of repellent formulations against daytime and nighttime biting mosquitoes in a tropical rainforest in northern Australia. *J. Med. Entomol.* 39, 541-544.

Frances, S.P., Picaridin. In: Debboun, M., Frances, S.P., Strickman, D. (eds.), 2006. Insect Repellents: Principles, Methods, and Uses. *CRC Press. Boca Raton, FL.* pp337-341.

Friend, D.R., 1992. In vitro skin permeation techniques. *J. Controlled Release* 18, 235-248.

Garmendia, A.E., VanKruiningen, H.J., French, R.A., 2001. The West Nile virus: its recent emergence in North America. *Microbes Infect.* 3, 223-229.

Garson, L.R., Winnike, M.E., 1968. Relationships between insect repellency and chemical and physical parameters: a review. *J. Med. Entomol.* 5, 339-352.

Gonzalez, H.G., Farbrot, A., Larkö, O., 2002. Percutaneous absorption of benzophenone-3, a common component of topical sunscreens. *Clin. Exp. Dermatol.* 27(8), 691-694.

Gonzalez, H.G., Farbrot, A., Larkö, O., Wennberg, A-M., 2006. Percutaneous absorption of the sunscreen benzophenone-3 after repeated whole-body applications, with and without ultraviolet irradiation. *Br. J. Dermatol.* 154(2), 337-340.

Grubauer, G., Feingold, K.R., Harris, R.M., Elias, P.M., 1989. Lipid content and lipid type as determinants of the epidermis permeability barrier. *J. Lipid Res.* 24, 120-130.

Gu, X., Chen, T., 2009. In vitro permeation characterization of repellent picaridin and sunscreen oxybenzone. *Pharm. Dev. Technol*. 14, 332-340.

Gu, X., Kasichayanula, S., Fediuk, D.J., Burczynski, F.J., 2004. In-vitro permeation of the insect repellent N,N-diethyl-m-toluamide (DEET) and the sunscreen oxybenzone. *J. Pharm. Pharmacol.* 56(5), 621-628.

Gu, X., Wang, T., Collins, D.M., Kasichayanula, S., Burczynski, F.J., 2005. In vitro evaluation of concurrent use of commercially available insect repellent and sunscreen preparations. *Br. J. Dermatol.* 152, 1263-1267.

Gubler, D.J., 2007. Emerging infections: the continuing spread of West Nile Virus in the Western Hemisphere. *Clin. Infect. Dis.* 45, 1039-1046.

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

Hayes, C., 1989. West Nile fever. In: Monath, T.P. (ed.), The Arboviruses: Epidemiology and Ecology. *CRC Press. Boca Raton, FL.* pp. 59-88.

Hexsel, C.L., Bangert, S.D., Hebert, A.A., Lim, H.W., 2008. Current sunscreen issues: 2007 Food and Drug Administration sunscreen labelling recommendations and combination sunscreen/repellent products. *J. Am. Acad. Dermatol.* 59, 316-327.

Hikima, T., Maibach, H., 2006. Skin penetration flux and lag-time of steroids across hydrated and dehydrated human skin in vitro. *Biol. Pharm. Bull.* 29(11), 2270-2273.

Houk, J., Guy, R.H., 1988. Membrane Models for Skin Penetration Studies. *Chem. Rev.* 88(3), 455-471.

Hueber, F., Schaefer, H., Wepierre, J., 1994. Role of transepidermal and transfollicular routes in percutaneous absorption of steroids: in vitro studies on human skin. *Skin Pharmacol.* 7, 237-244.

Jacobi, U., Kaiser, M., Toll, R., Mangelsdorf, S., Audring, H., Otberg, N., Sterry, W., Lademann, J., 2007. Porcine ear skin: an in vitro model for human skin. *Skin Res. Technol.* 13(1), 19-24.

Janjua, N.R., Mogensen, B., Andersson, A-M., Petersen, J.H., Henriksen, M., Skakkebæk, N.E., Wulf, H.C., 2004. Systemic absorption of the sunscreens benzophenone-3, octyl-methoxycinnamate, and 3-(4-methyl-benzylidene) camphor after whole-body topical application and reproductive hormone levels in humans. *J. Invest. Dermatol.* 123, 57-61.

Janjua, N.R., Kongshoj, B., Andersson, A-M., Wulf, H.C., 2008. Sunscreens in human plasma and urine after repeated whole-body topical application. *J. Eur. Acad. Dermatol. Venereol.* 22(4), 456-461.

Jiang, R., Benson, H.A., Cross, S.E., Roberts, M.S., 1998. In vitro human epidermal and polyethylene membrane penetration and retention of the sunscreen benzophenone 3 from a range of solvents. *Pharm. Res*. 15, 1863-1868.

Kasichayanula, S., House, J.D., Wang, T., Gu, X., 2005. Simultaneous analysis of insect repellent DEET, sunscreen oxybenzone and five relevant metabolites by reversed-phase HPLC with UV detection: application to an in vivo study in a piglet model. *J. Chromatogr. B.* 822, 271-277.

Kasichayanula, S., House, J.D., Wang, T., Gu, X., 2007. Percutaneous characterization of the insect repellent DEET and the sunscreen oxybenzone from topical skin application. *Toxicol. App. Pharmacol*. 223, 187-194.

Katz, M., Poulsen, B.J., 1971. Absorption of drugs through the skin. In: Brodie, B.B., Gillette, J.R., (eds). Handbook of Experimental Pharmacology: Concepts in Biochemical Pharmacology. *Springer-Verlag, New York.* pp. 103-174.

Katz, T.M., Miller, J.H., Hebert, A.A., 2008. Insect repellents: Historical perspectives and new developments. *J. Am. Acad. Dermatol.* 58(5), 865-71.

Keeney, S., McKenna, H., Fleming, P., Mcllfatrick, S., 2009. Attitudes, knowledge and behaviours with regard to skin cancer: a literature review. *Eur. J. Oncol. Nurs*. 13(1), 29-35.

Khan, A.A., Maibach, H.I., Skidmore, D.L., 1973. A study of insect repellents: II. Effect of temperature on protection time. *J. Econ. Entomol*. 66, 437-438.

Knobler, E., Almeida, L., Ruzkowski, A.M., Held, J., Harber, L., Deleo, V., 1989. Photoallergy to benzophenone. *Arch. Dermatol.* 125(6), 801-804.

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.

Lampe, M.A., Burlinghame, A.L., Whitney, J., Williams, M.L., Brown, B.E., Roitman, E., Elias, P.M., 1983. Human stratum corneum lipids: characterisation and regional variation. *J. Lipid Res.* 24, 120-130.

Lenique, P., Machet, L., Vaillant, L., Bensaid, P., Muller, C., Khallouf, R., Lorette, G., 1992. Contact and photocontact allergy to oxybenzone. *Contact Dermatitis* 26(3), 177-181.

Maibach, H.I., Khan, A.A., Akers, W., 1974. Use of insect repellents for maximum efficacy. *Arch. Dermatol.* 109(1), 32-35.

Marjukka, S.T., Joke, A. Bouwstra, A.U., 1999. Chemical enhancement of percutaneous absorption in relation to stratum corneum structural alterations. *J. Controlled Release* 59, 149-161.

Martin, A., 1993. Physical Pharmacy. *Lea & Febiger, Philadelphia, PA.*

Matoltsy, A.G., Downes, A.M., Sweeney, T.M., 1968. Studies of the epidermal water barrier. Part II. Investigation of the chemical nature of the water barrier. *J. Invest. Dermatol.* 50, 19-26.

Moloney, F.J., Collins, S., Murphy, G.M., 2002. Sunscreens safety, efficacy and appropriate use. *Am. J. Clin. Dermatol.* 3(3), 185-191.

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

Moore, S.J., Debboun, M. History of Insect Repellents. In: Debboun, M., Frances, S.P., Strickman, D. (eds.), 2006. Insect Repellents: Principles, Methods, and Uses. *CRC Press. Boca Raton, FL*. pp. 3-30.

Morimoto, Y., Sugibarashi, K., Natsume, H., 1994. The Transdermal Drug Delivery System and Transcutaneous Absorption. *Acta Derm.-Venereol., Suppl.*185,15-17.

Mulder, M. (ed.), 1996. Basic principles of membrane technology. 2nd ed. *Kluwer Academic Publishers. Netherlands.*

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.

Naucke, T.J., Kröpke, R., Benner, G., Schulz, J., Wittern, K.P., Rose, A., Kröckel, U., Grünewald, H.W., 2007. Field evaluation of the efficacy of proprietary repellent formulations with IR3535 and picaridin against Aedes aegypti. *Parasitol. Res.* 101, 169-177.

Perkins, N.C., Heard, C.M., 1999. In vitro dermal and transdermal delivery of doxycycline from ethanol/migliol 840 vehicles. *Int. J. Pharm.* 190, 155-164.

Peterson, C., Coats, J., 2001. Insect repellents – past, present and future. *Pestic. Outlook* 12, 154-158.

Pont, A.R., Charron, A.R., Brand, R.M., 2004. Active ingredients act as topical penetration enhancers for the herbicide 2,4-dichlorophenoxyacetic acid. *Toxicol. Appl. Pharmacol*. 195, 348-354.

Potts, R.O., Francoeur, M.L., 1991. The Influence of Stratum Corneum Morphology on Water Permeability. *J. Invest. Dermatol.* 96(4), 495-499.

Potts, R.O., Bommannan, D.B., Guy, R.H., 1992. Percutaneous absorption. In: Mukhtar, H. (ed.), 1992. Pharmacology of the skin. *CRC Press. Boca Raton, FL*. pp.12-27.

Pustišek, N., Lipozencić, J., Ljubojević, S., 2005. A review of sunscreens and their adverse reactions. *Acta Dermatovenerol Croat* 13(1), 28-35.

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.

Riviere, J.E., Heit, M.C., 1997. Electrically-assisted transdermal drug delivery. *Pharma. Res.* 14(6), 687-697.

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.

Saladi, R.N., Persaud, A.N., 2005. The causes of skin cancer: a comprehensive review. *Drugs Today* 41(1), 37-53.

Schaefer, H., Redelmeier, T.E. (eds), 1996. Skin Barrier: Principles of percutaneous absorption. *Karger AG, Basel.*

Scheuplein, R.J., 1965. Mechanism of percutaneous absorption: I. Routes of penetration and the influence of solubility. *J. Invest. Dermatol.* 45, 334-346.

Scheuplein, R.J. The physiology and pathophysiology of the skin. In: Jarrett, A. (ed.), 1978. Site Variations in Diffusion and Permeability, *Academic Press, New York*. pp. 1731-1752.

Scheuplein, R.J., Blank, I.H., 1971. Permeability of the skin. *Physiol. Rev.* 51, 702-747.

Schoenig, G.P., Hartnagel, R.E., Osimitz, T.G., Lianso, S., 1996. Absorption, distribution, metabolism, and excretion of N,N-diethyl-m-toluamide in the rat. *Drug Metab. Dispos.* 24(2), 156-163.

Schurer, N.Y., Elias, P.M., 1991. The biochemistry and function of stratum corneum lipids. *Adv. Lipid Res*. 24, 27-56.

Selim, S., Hartnagel, R.E., Osimitz, T.G., Gabriel, K.L., Schoenig, G.P., 1995. Absorption, metabolism, and excretion of N,N-diethyl-m-toluamide following dermal application to human volunteers. *Fundam. Appl. Toxicol.* 25(1), 95-100.

Šitum, M., Buljan, M., Bulat, V., Mihić, L.L., Bolanča, Ž., Šimić, D., 2008. The role of UV radiation in the development of basal cell carcinoma. *Coll. Antropol.* 32(sp2), 167-170.

Skelly, J.P., Shah, V.P., Maibach, H.I., Guy, R.H., Wester, R.C., Flynn, G., Yacobi, A., 1987. FDA and AAPS report of the workshop on principles and practices of in vitro percutaneous penetration studies: relevance to bioavailability and bioequivalence. *Pharma. Res.* 4(3), 265-267.

Squier, C.A., Cox, P., Wertz, P.W., 1991. Lipid content and water permeability of skin and oral mucosa. *J. Invest. Dermatol.* 96, 123-126.

Stamatialis, D.F., Rolevink, H.H.M., Koops, G.H., 2002. Controlled transport of timolol maleate through artificial membranes under passive and iontophoretic conditions. *J. Controlled Release* 81, 335-345.

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(1), 12-20.

Stenberg, C., Larkö, O., 1985. Sunscreen application and its importance for the sun protection factor. *Arch. Dermatol.* 121(11), 1400-1402.

Surber, C., Wilhelm, K-P. Hori, M., Maibach, H.I., Guy, R.H., 1990. Optimization of topical therapy: partitioning of drugs into stratum corneum. *Pharm. Res.* 7(12), 1320-1324.

Sweeney, T.M., Downing, D.T., 1970. The role of lipids in the epidermal barrier to water diffusion. *J. Invest. Dermatol.* 55, 135-140.

Thomas, B.J., Finnin, B.C., 2004. The transdermal revolution. Drug Discovery Today 9(16), 697-703.

Walker, R.B., Smith, E.W., 1996. The role of percutaneous penetration enhancers. *Adv. Drug Delivery Rev.* 18(3), 295-301.

Wang, T., Gu, X., 2007. In vitro percutaneous permeation of the repellent DEET and the sunscreen oxybenzone across human skin. *J. Pharm. Pharm. Sci.* 10(1), 17-25.

Wertz, P.W., 2000. Lipids and barrier function of the skin. *Acta. Derm.-Venereol., Suppl.* 208, 7-11.

Wertz, P.W., Downing, D.T. Stratum corneum: biological and biochemical considerations. In : Hadgraft, J., Guy, R.H. (eds.), 1989. Transdermal Drug Delivery : Development Issues and Research Initiatives, *Marcel Dekker, New York*, pp.1-22.

WHO Specifications and Evaluations for Public Health Pesticides – Icaridin, 2004. World Health Organization: Geneva, Switzerland.

Wiechers, J.W., 1989. The barrier function of the skin in relation to percutaneous absorption of drugs. *Pharm. Weekbl., Sci. Ed.* 11(6): 185-198.

Wildnauer, R.H., Miller, D.L., Humphries, W.T., 1975. A physicochemical approach to the Characterization of stratum corneum. *Adv. Chem.* 145, 74-124.

Wilkes, G.L., Brown, I.A., Wildnauer, R.H., 1973. The biomechanical properties of skin. CRC *Crit. Rev. Bioeng.* 1(4), 453-495.

Williams, A. Structure and function of human skin. In: Williams, A., 2003. Transdermal and Topical Drug Delivery. *Pharma. Press.* pp. 1-25.

Williams, A.C., Cornwell, P.A., Barry, B.W., 1992. On the non-gaussian distribution of human skin permeabilities. *Int. J. Pharm.* 86, 69-77.

Wissing, S.A., Müller, R.H., 2002. Solid lipid nanoparticles as carrier for sunscreens: in vitro release and in vivo skin penetration. *J. Control Release* 81, 225-233.

Yap, H.H., Jahangir, A., Chong, S.C., Adanan, C.R., Chong, N.L., Malik, Y.A., Rohaizat, B., 1998. Field efficacy of a new repellent, KBR3023, against *Aedes albopictus* (Skuse) and *Culex quinquefasciatus* (Say) in a tropical environment. *J. Vec. Ecol.* 23, 62-68.

Yap, H.H., Jahangir, K., Zairi, J., 2000. Field efficacy of four insect repellent products against vector mosquitoes in a tropical rainforest. *J. Am. Mosq. Control Assoc.* 16, 241-244.

Zhu, J., Zeng, X., O'Neal, M., Schultz, G., Tucker, B., Coats, J., Bartholomay, L., Xue, R.D., 2008. Mosquito larvicidal activity of botanical-based mosquito repellents. *J. Am. Mosq. Control Assoc*. 24, 161-168.

http://www.healthtype.com/supposedly-safe-sunscreen-ingredients.html

http://www.picaridin.info/

http://www.spectrumchemical.com/