IN VITRO EVALUATION OF PERCUTANEOUS CHARACTERIZATION OF CONCURRENT APPLICATION OF INSECT REPELLENTS AND SUNSCREENS

BY

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A Thesis
Submitted to the Faculty of Graduate Studies in Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE

Faculty of Pharmacy
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CANADA

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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List of Abbreviations

DEET:	N,N-diethyl-m-toluamide
DMSO:	dimethyl sulfoxide
EPA:	Environmental Protection Agency
EVA:	ethylene vinyl acetate
FDA:	Food and Drug Administration
GCD:	γ-cyclodextrin
HA:	hyaluronic acid
HDPE:	high density polyethylene
HPDCD:	hydroxypropyl-β-cyclodextrin
HPLC:	high performance liquid chromatography
LDPE:	low density polyethylene
LFC:	low fouling composite
MED:	minimum effective dose
MED:	minimum erythema dose
OMC:	octyl methoxycinnamate
OPP:	overall permeation percentage
OTC:	over the counter
OXBZ:	oxybenzone
PABA:	p-aminobenzoic acid
RED:	Registration Eligibility Decision
SLM:	solid lipid microspheres
SLN:	solid lipid nanoparticles

SPF:	Sun Protection Factor
SSF:	steady-state flux
UV:	ultraviolet
UVR:	ultraviolet radiation

List of Symbols

A: Diffusion area *(cm^2)*

C: Drug concentration *(g/ml)*

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

h: Membrane thickness *(cm)*

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)*

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 for a degree at this or any other university.

Tao Wang

October, 2004

Acknowledgements

My Master's program at the Faculty of Pharmacy, University of Manitoba in the past two years has been a wonderful, challenging and rewarding journey in my career advancement. I received tremendous support and encouragement from the Faculty, my Advisory Committee and my fellow students/friends on a daily basis in both learning experience and daily life.

First of all, I would like to thank my advisor, Dr. Xiaochen Gu, for patiently guiding me throughout my research work as well as for discussing and correcting my thesis.

I would also like to thank the members of my Advisory Committee, Dr. Frank Burczynski of Faculty of Pharmacy and Dr. Jung Han of Department of Food Science, for reviewing and commenting my thesis, in spite of other commitments they had made.

I am grateful to Dr. David Collins, Professor and Dean of Faculty of Pharmacy, for assisting in statistical analysis of the experimental data.

I also would like to thank Dr. Keith Simons, Professor and Associate Dean (Research), for helping me with some research questions.

I would like to thank my fellow students/friends, Ms. Ping Jiang, Mr. Sree Kasichayanula, Mr. Hongtao Li and Mr. Vikram Sarveiya, for supporting and helping me in the common lab works.

I am grateful to Pfizer Company and University of Manitoba for the financial support of my graduate program. Research supports from Canada Foundation for Innovation, Manitoba Institute of Child Health, University of Manitoba and Gattefossé Canada Inc. are also greatly acknowledged.

Finally, I am very grateful to my parents, Ms. Jiaqi Wang and Mr. Rongxia Wang, for their continuing support and encouragement in my study and my life.

Abstract

DEET-based insect repellents and sunscreens have been commercially available as over-the-counter consumer care products for decades. The use of both preparations by the general public has significantly increased over the years, due mainly to health concerns about the West Nile virus and skin cancer as well as the awareness and pursuit of healthy life styles. However, there has been little investigation on the systemic transdermal absorption of the active ingredients from these widely used topical preparations, especially from concurrent application of both insect repellents and sunscreens.

In this thesis, the concurrent application of five commercially available insect repellent and sunscreen preparations was evaluated *in vitro* for their permeation profiles across various membrane models. The influences of formulation type, application amount and application sequence on permeation of the insect repellent DEET and the sunscreen oxybenzone were characterized. The suitability of three artificial membranes for *in vitro* diffusion studies was evaluated and compared. Two formulations were also prepared and tested with the objective of minimizing overall permeation rate and extent of the active repellent and sunscreen ingredients.

It was found from the experiments that the permeation of DEET and oxybenzone was synergistically enhanced when both insect repellent and sunscreen preparations were used concurrently. This enhancement was dependent upon several application parameters. Repellent spray produced less transmembrane permeation than repellent lotion, while combined repellent/sunscreen lotions generally promoted the permeation of DEET and oxybenzone. Depending on the application amount and sequence, premixing repellent and sunscreen preparations enhanced the permeation of DEET and oxybenzone across the membrane models. The use of lipophilic artificial membranes appeared appropriate for *in vitro* diffusion studies of DEET and oxybenzone, which were also lipophilic in nature. The use of hydrophilic membranes produced permeation profiles that significantly deviated from what found in the biological membrane. Modification of formulation properties was able to alter the permeation patterns of DEET and oxybenzone across the membranes. Overall, the interaction between DEET and oxybenzone impacted DEET more than oxybenzone, and the role of oxybenzone as a transdermal absorption enhancer was demonstrated.

DEET and oxybenzone are essential components of insect repellent and sunscreen preparations. The synergistic permeation of both compounds observed from this study would not only compromise the protective efficacy of both products but also potentiate safety or toxicological characteristics of the active ingredients that are intended mainly for localized topical application. The concurrent use of insect repellents and sunscreens therefore warrants further systematical investigations, both *in vitro* and *in vivo*.

Chapter 1. The Skin

1.1. Skin Structure

The skin is the largest organ of the human body. It covers the exterior of the human body, and protects the body from external bacteria invasion, physical and chemical injuries. The skin also keeps internal balance of the fluid and electrolytes, and prevents transdermal water loss to the environment. Even though there are numerous protection mechanisms in the human skin, certain chemical substances can still penetrate across the skin layers to reach the blood circulation and body tissues. Anatomically, the skin is composed of stratum corneum, epidermis, dermis, underlying subdermal tissue, and skin appendages (Figure 1.1). The dead cells in stratum corneum form stratum lucidum and stratum disjunctum. Epidermis and dermis lie in the deeper location of the skin under stratum corneum. A basal membrane separates epidermis and dermis. Epidermis includes four sublayers, beginning from outside to inside, stratum lucidum, stratum granlosum, stratum spinosum, and stratum basale, whereas dermis remains continuous with the subcutaneous and adipose tissues [1].

1.1.1. Stratum Corneum

Stratum corneum, the outer dead layer of epidermis, is also known as horny layer. Essentially it is an intact tissue comprised of flattened, stacked, hexagonal cell building blocks formed from once living cells. Generally, stratum corneum is stacked by 15-25 cells on most body surface [2], which is approximately 10-15μm in thickness and less than one fifth of the thickness of a piece of ordinary paper [2,3].

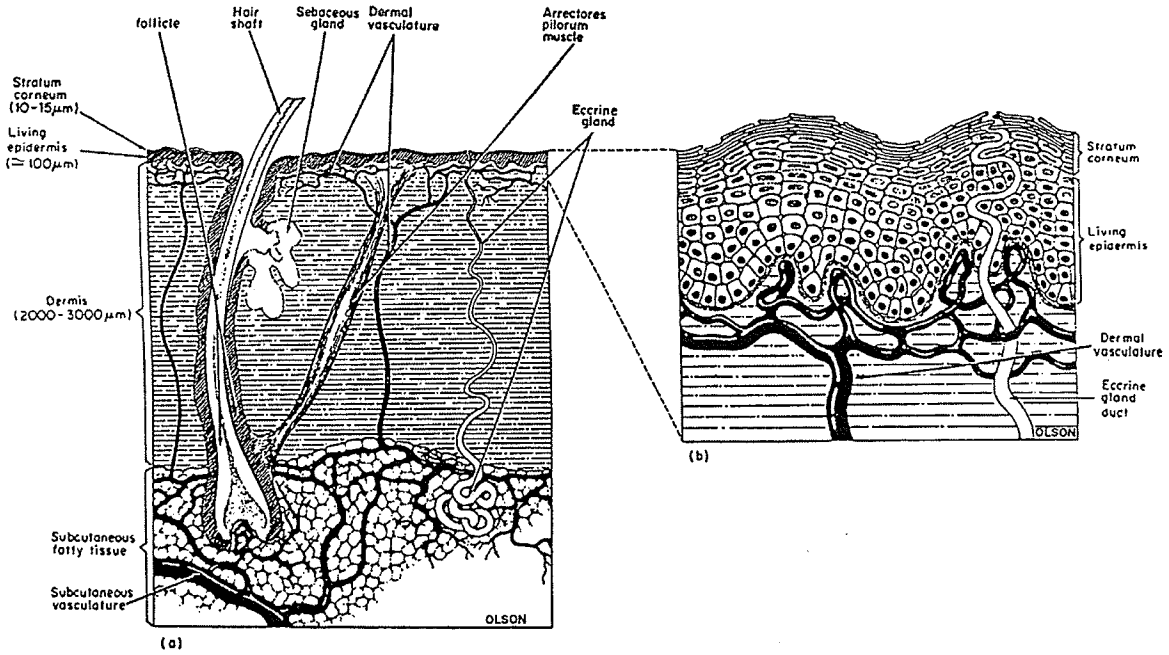


Figure 1.1. The structure of the skin

(Modern Pharmaceutics, Marcel Dekker Inc, 2002)

Stratum corneum is the main element of the skin's permeation barrier, which not only prevents the desiccation of the underlying tissues but also excludes the entry of noxious substances from the environment. Protein and lipid components exist in stratum corneum. Basically, the structure of stratum corneum consists of layers of differentiated keratinocytes embedded in a matrix of lipid bilayers. The structural proteins, keratinocytes, which are composed of semi-crystalline keratin and more amorphous keratin, are packed in the intracellular space of the horny cells. These two keratinocytes are the only visible proteins inside the fully differentiated horny cells by electron microscope. Lipids are also synthesized, collected in epidermis, and finally passed into the intercellular space of stratum corneum [4]. Most of them are present in liquid

crystalline, bilayer assemblage [5]. The densely packed keratin platelets in mix with intercellular lipids make stratum corneum. There are approximately 70-80% proteins (mainly keratin) and 20% water (nomal hydration), with the balance being lipids and a few other substances in stratum corneum, like polysaccharide [2,3].

1.1.2. Epidermis

Under stratum corneum locates epidermis, which is a collection of tightly massed living cells. From bottom to top, they are (a) the basal layer – germinativum, (b) the multicellular spinous layer – spinosum, and (c) the granular layer – gramulosum. Generally, epidermis is 100-250μm in thickness [6]. The principal cells in epidermis are keratinocytes. There are also some other cells that possess various physiological functions. For example, langerhans cells are immunological cells that are white blood cell progeny. The function of langerhans cells is to produce antigens for skin's immunological responses [4]. Melanocytes are the pigment-producing cells of the skin and react to ultraviolet radiation (UVR) of the environment. Their activity is to determine the color of both the hair and the skin. Melanocytes originate in the neural crest and migrate to the basal layer of epidermis and hair matrices during embryogenesis. They are able to supply melanin, which is produced and deposited from melanosome. There are two major forms of melanin produced in epidermis and hair follicles – eumelanin and pheomelanin. Eumelanin is brown to black in color, while pheomelanin is yellow to red in color. UV-induced tanning represents an increase in the content of eumelanin within epidermis and its major purpose is to increase photoprotection [7].

1.1.3. Dermis

Dermis is a layer between epidermis and subcutaneous fat. Its thickness ranges between 1-5mm [8]. Dermis is penetrated by a network of sensory nerves for the sense of pressure, temperature and pain, as well as a lymphatic network [4]. Importantly, the microcirculation is entirely located in dermis. Arteries arising from subcutaneous bottom tissue form a plexus beneath dermis, spread from bottom dermis to upper dermis, and then form subpapillary plexus that bring a blood supply up into the whole dermal region (epidermis is a vascular tissue). The vascular surface, which is able to exchange substances between the blood and local tissues, is approximately 1-2cm^2/cm^2 of the skin. The blood flow is about 0.05ml through the skin per min per gram of tissue at room temperature [3,9].

1.1.4. Skin Appendages

Hair follicles and their associated sebaceous glands, accrine glands and apocrine glands, and finger and toe nails are all considered skin appendages. Hair follicles occupy about 0.1% of the skin surface [6,10]. Every hair follicle has one or more sebaceous glands. The large follicular outlets of sebum that grow microscopic hair are named sebaceous follicles, which also take up 0.1% of the skin surface. Although they occupy only a very little area of the whole skin surface, hair and sebaceous follicles are considered as one of the effective penetrative ways in transdermal drug delivery. They are especially important to some substances during the initiation (lag time) of the transdermal penetration process.

1.2. Routes of Penetration

In stratum corneum, keratinocytes are surrounded by a continuous lamellar lipid phase [11,12]. The water impermeability of the keratin-rich phase and the lipid phase in the intercellular space dictates that the lipophilic compounds tend to permeate stratum corneum. Hydrophilic components, on the other hand, are less permeable to normal stratum corneum.

There are two permeability mechanisms in epidermis according to the cellular structures. The characteristics of the intercellular spaces and cytoplasm are essentially hydrophilic, which becomes a transport barrier to lipophilic substances because lipophilic compounds have low solubility in this environment. On the contrary, cell membranes in epidermis are lipophilic in nature, so there is a low penetration tendency of a hydrophilic substance through the cell membranes. Therefore there are two routes for drug penetration through epidermis, i.e., the paracellular route and the transcellular route (Figure 1.2). A substance can choose any one of these two routes at the same time. Theoretically, a hydrophilic compound primarily chooses the paracellular route, because of the difficulties in penetrating the lipophilic cell membranes. With this route, the main limitations are the tortuous pathways of the intercellular spaces and the limited surface areas. A lipophilic substance, on the other hand, tends to choose the transcellular route by directly penetrating the epithelial cell membranes and transporting across the cytoplasm, because lipophilic compounds have stronger affinity to fluid lipid bilayer of the epithelial cell membranes. The large surface areas and the short transport paths in the transcellular route provide a more favorable environment for the permeation of a compound than in the paracelluar route. In reality, however, stratum corneum and epidermis are very

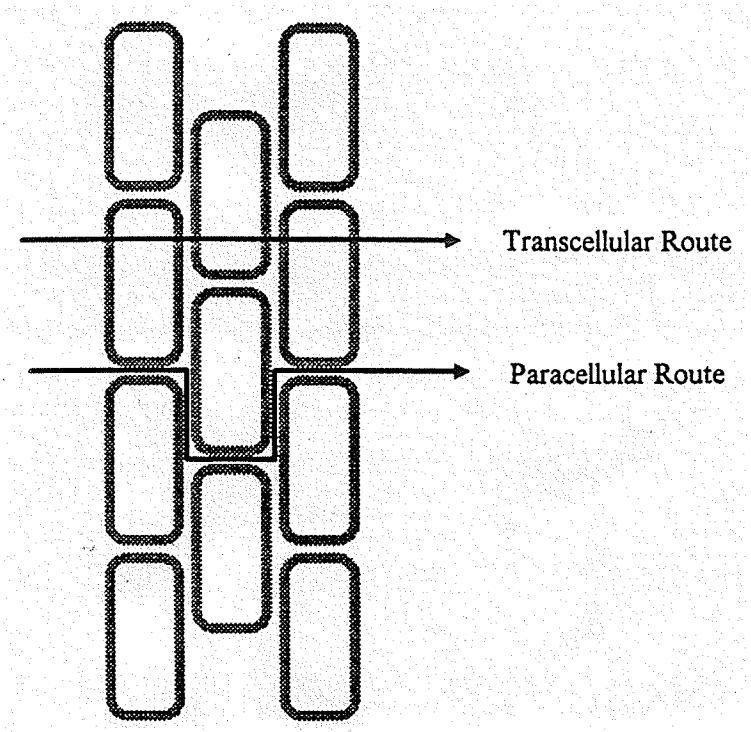


Figure 1.2. Mechanisms of transmembrane drug permeation
(Oral Mucosal Drug Delivery, Marcel Dekker Inc, 1996)

complex structures that pose numerous barrier variables to influence transdermal penetration, which include lipophilic stratum corneum, hydrophilic intercelluar space in epidermis, the fluid lipid bilayer of epithelial cell membranes, and relative hydrophilic cytoplasm. Chemical compounds that have considerable water solubility as well as oil solubility are able to penetrate across the skin and reach the systemic circulation.

There are two main methods to detect drug transport mechanisms across stratum corneum and epidermis. One approach is direct visualization, which uses sophisticated instruments such as microscopy [13,14], autoradiography [15], and confocal laser scanning microscopy [16] for the detection. The other is permeability measurement, which is based on the partition coefficient of a chemical compound. When a compound penetrates through the paracellular route, the permeability of the compound is

independent of its partition coefficient. In contrast, the permeability of a compound is dependent on its partition coefficient, if the transcelluar route is the main transporting route of the substance [17].

1.3. Percutaneous Absorption Variables

Percutaneous penetration profiles of a substance are influenced by many variables, among which some important parameters include the concentration gradient, the site of application and the solubility of the compound in oil and water.

Drug concentration gradient is an important factor. In general, the amount of a drug percutaneously absorbed per unit of surface area per unit time will increase as the concentration of the active substance in the vehicle is increased. In the study of controlled release of atenolol from an ethylene-vinyl acetate (EVA) matrix, it was reported that the release rate of the drug from EVA matrix increased with drug loading doses [18]. In another study of transdermal testosterone gel in hypogonadal men, it was also found that higher doses of testosterone produced higher serum concentrations of testosterone, and that a 2% testosterone gel was the most successful preparation to maintain serum testosterone concentrations within desirable therapeutic ranges [19].

The site of application significantly impacts on the transdermal drug absorption. Skin surface is neither continuous nor homogeneous. Some regions, such as the fingertips, the palm of the hands and the soles of the feet, display callused or thickened stratum corneum, and render percutaneous drug absorption to negligible levels. Other body surface areas, including the chest, the back of the ears, and the inner side of the thighs, are very tender and smooth; they are regarded as ideal application sites for transdermal

drug delivery systems. In addition, full-term infants have well-developed epidermis and stratum corneum similar to those in children and adults, but premature infants only have one or two cells in epidermis, and no stratum corneum can be detectable. Skin permeability to water [20] and drug substances [21] is higher in premature infants than in full-term counterparts. Therefore, drug administration by transdermal route can be considered an effective alternative in drug therapy for premature infants.

One of the most important parameters in determining the rate and extent of percutaneous drug absorption is the solubility of a compound in both water and oil. In essence, partition coefficient, the ratio of solubility in oil (octanol in most cases) and in water, of a substance strongly influences the rate and extent of drug transport across the skin layers. Stratum corneum provides a rate-limiting step to percutaneous penetration [22]. According to current theories [23,24], a compound diffuses through stratum corneum by the intercellular spaces around corenocytes. As mentioned previously, the intercellular spaces are composed of various structured lipids [25]. A compound with higher partition coefficient is able to form a reservoir in this area [26] and rate-limiting step will transfer to epidermis and dermis [27]. On contrary, compounds with low partition coefficient have rate control in stratum corneum.

Skin hydration is also one of the important factors in enhancing percutaneous drug absorption. Hydration of stratum corneum appears to increase the rate of passage for all substances that penetrate the skin. The increased absorption results probably from the softening of the tissues and consequently the "sponging" effects with an increase in the size of the pores, allowing a greater flow of substances, large and small, through the skin.

Formulation vehicles that increase the amount of moisture imbibed by the skin generally favor the percutaneous absorption of the active ingredients.

Percutaneous absorption enhancers are those chemical compounds that can enhance the penetration of other chemicals through the biological membranes. They are able to facilitate percutaneous drug delivery by increasing the fluidity of the extracellular lipid in stratum corneum, conducting the hydration of stratum corneum, altering the thermodynamic activity of a permeant, or modifying partitioning tendency of a substance [28]. There is no doubt that water in topical formulations, such as creams and lotions, can result in the hydration of the underlying skin and subsequently increase the penetration rate and extent of the active therapeutic agents. Among various other solvents, ethanol has also been shown to have the ability to enhance transdermal penetration of many compounds, since it appears to have the capability of extracting lipid from stratum corneum. In its co-administration with estradiol [29] and nitroglycerin [30], the usefulness of ethanol had been demonstrated as a percutaneous absorption enhancer. Alkyl methyl sulfoxides are another group of commonly-used penetration enhancers. The use of alkyl methyl sulfoxides as penetration enhancers was originated from dimethyl sulfoxide (DMSO), a dipolar aprotic solvent and a common pharmaceutical additive that is miscible with water and other organic solvents. DMSO is able to enhance the penetration of a wide variety of compounds, including steroids [30,31], salicylates [32], and antimycotics [33]. Many mechanisms of DMSO have been proposed, which range from the extraction of lipids and lipoproteins from stratum corneum [34,35], the displacement of bound water and loosening of the polymeric structure in the corneocyte [36,37], to the delamination of stratum corneum [38]. Natural moisturizing compounds

with lower toxicity potential were thought to increase the water binding capacity of stratum corneum, therefore increasing the penetration of the therapeutic agents through the skin [39]. Laurocapram (Azone) is a very effective enhancer of skin permeability, because it was specifically designed and synthesized for this purpose [28]. In the early studies with Azone, it increased the penetration of both hydrophilic and hydrophobic drugs [40,41], including antibiotics, steroids and nucleosides [42-47]. In addition, some miscellaneous solvents are able to increase the percutaneous permeation of substances across the skin, such as acetone [48,49], tetrahydrofurfuryl alcohol [50], and propylene glycol [51,52]. Moreover, surfactants, which can be anionic, cationic, or nonionic, were considered to be enhancers in the transdermal drug delivery systems, because they can lead to the alterations in permeability patterns [53]. In many publications of the biological effects of surfactants, it has been demonstrated that low concentrations of surfactants can significantly enhance membrane transportation, but penetration of substances decreases at higher concentrations, generally above the critical micelle concentration (CMC) of the surfactants [54].

In summary, the skin is a complex organ with many protective mechanisms. Percutaneous penetration of a chemical compound is influenced by a variety of factors, including drug concentration gradient, application site, partition coefficient, skin hydration, and the use of penetration enhancers.

Chapter 2. Skin Transport

2.1. Transport Models

The skin is a complicated organ of the human body. It is composed of several layers, including stratum corneum, epidermis, dermis, and subcutaneous tissues. Even though there are millions of cells tightly stacked together in each skin layer, the skin is regarded as a simple and homogenous membrane as far as transdermal drug delivery is concerned (Figure 2.1). An active ingredient will transfer from the formulation to the surface of the skin, penetrate across the membrane, and then be absorbed into the blood circulation. In most cases, the transport profile of a substance across the skin membrane can be described by the empirical Fick's First Law, which is also the starting basis for all mathematical modeling in transdermal drug development and assessment.

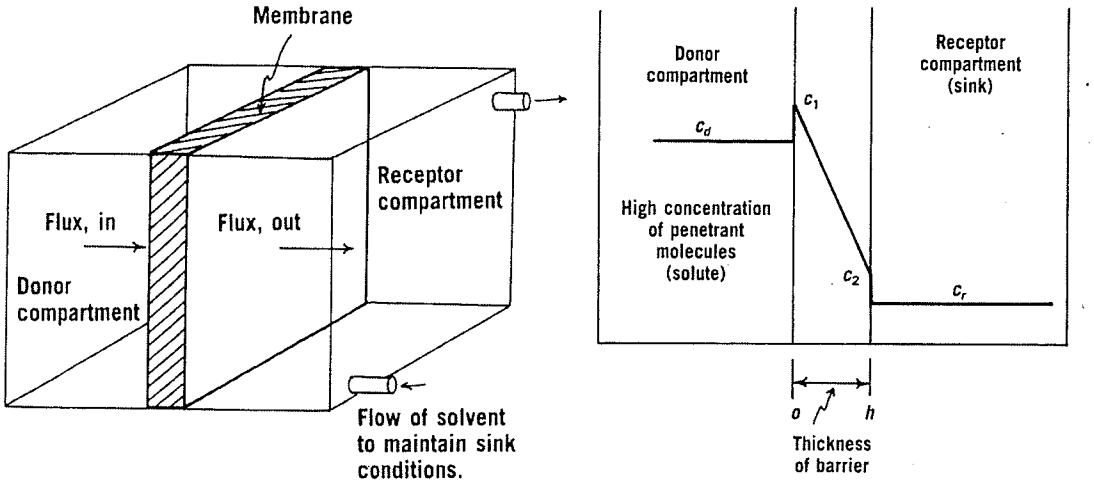


Figure 2.1. Mathematical parameters of transdermal drug delivery
(Physical Pharmacy, Lea & Febiger, 1993)

2.2. The Fick's First Law

The Fick's First Law as one of the fundamental and empirical diffusion theories has been extensively used to interpret drug diffusion across various biological membranes. It is very important for the understanding of the relationship among drug diffusion rate, drug concentration and application surface,

$$J = -D\frac{dC}{dx} \qquad \text{..... (1)}$$

Where J: the flux per area per time

D: the diffusion coefficient

dC/dx: the concentration gradient over the distance x

Since the drug concentration gradient across the membrane model cannot be easily determined, the steady-state transdermal flux, J_s, through the skin barrier is given as,

$$J_s = \frac{KDC_s}{h} \qquad \text{..... (2)}$$

Where K: the partition coefficient

D: the diffusion coefficient

C_s: the saturated reservoir concentration when a sink condition is maintained in the receptor cell

h: the thickness of the membrane

In most *in vitro* diffusion experiments, the measured permeability coefficient (K_p) is the combination of the permeability coefficient of the compound from formulation vehicle to the membrane, the diffusion coefficient of the compound in the membrane and the thickness of the membrane. J_s is therefore simplified to the following equation,

$$J_s = K_p C_s \quad \text{..... (3)}$$

Where K_p: the apparent permeability coefficient

C_s: the saturated reservoir concentration when a sink condition is maintained in the receptor cell

The steady-state flux (J_s) of a substance is often calculated by the accumulative amount of drug permeating through the membrane, area of the application, and the diffusion time. The accumulative amount of drug permeating through membranes (Q_t) is therefore given by,

$$Q_t = J_s A t \quad \text{..... (4)}$$

Where A: the area of application

t: the diffusion time

therefore,

$$J_s = \frac{Q_t}{At} \quad \text{..... (5)}$$

In this thesis, the accumulative drug amount Q_t was measured by the HPLC, the diffusion area of the diffusion cell A was a fixed value, and the experimental diffusion time t was 6 hours. In addition, the percentage of the transmembrane penetration of a compound was calculated by the following equation,

$$P = \frac{Q_t}{C_S V} \qquad \text{..... (6)}$$

Where P: the percentage of transmembrane penetration

C_s: the concentration of test compound in the receptor cell

V: the volume of the formulation applied

2.3. In Vitro Diffusion

The aim of any *in vitro* experimentation in transdermal drug delivery is to understand and predict the delivery and penetration profiles of an active ingredient across the skin membrane in a living object [55]. Generally, there are a variety of diffusion cells and study protocols available to achieve this characterization. The diffusion cells that are commonly used for *in vitro* studies include vertical diffusion cell and horizontal diffusion cell (Figure 2.2), both of which are composed of the donor compartment, in which the test formulation is housed, and the receptor compartment, from which samples are collected for concentration measurement. A membrane model is sandwiched between the two compartments, within which drug diffusion and permeation take place. Both biological membranes from living species and artificial membranes of synthetic nature have been used as membrane models. The temperature of the receptor compartment is well maintained to reduce permeation fluctuation, while the receptor medium is agitated

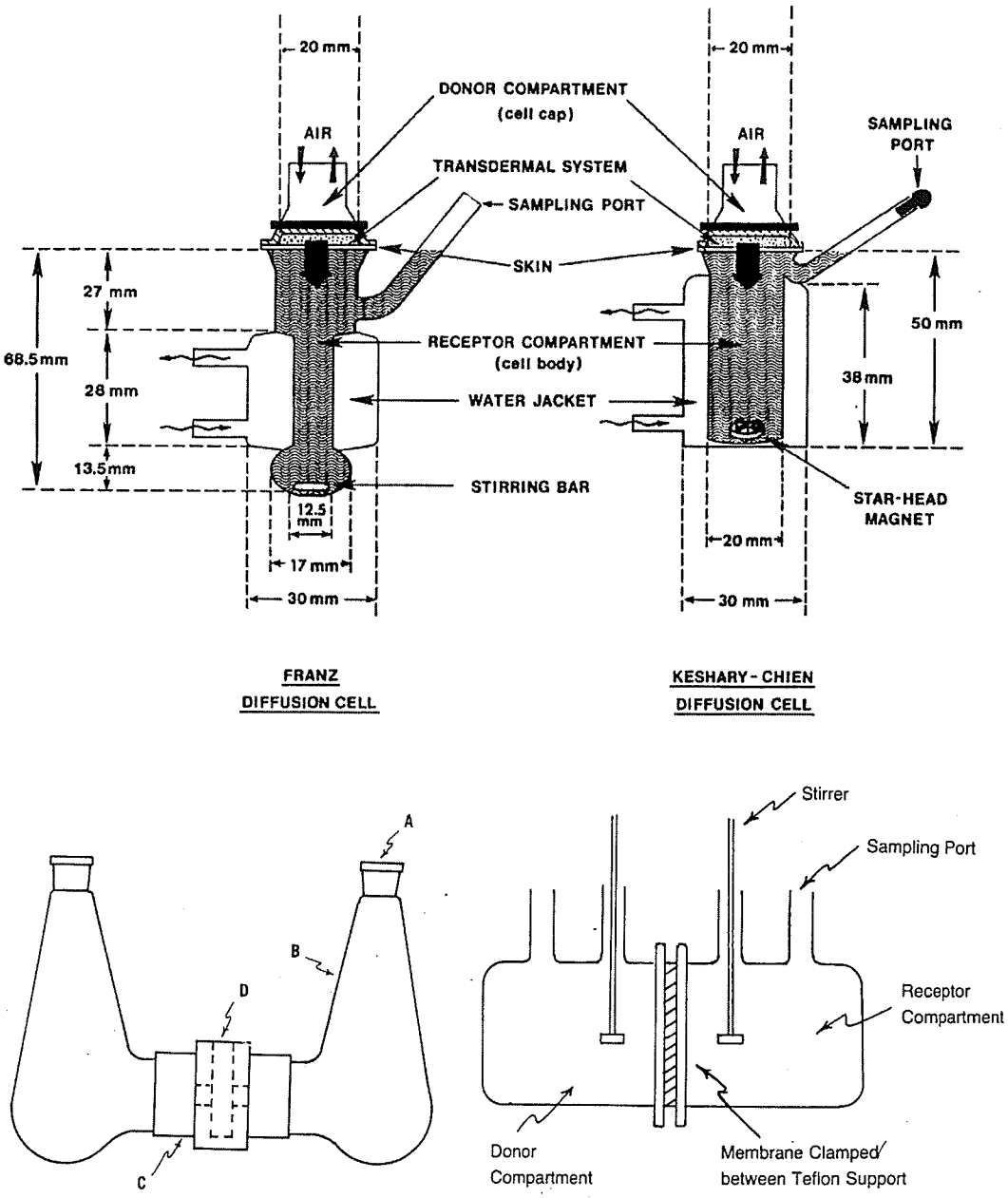


Figure 2.2. Diffusion cells of different designs (top: vertical, bottom: horizontal)
(Physical Pharmacy, Lea & Febiger, 1993)

to ensure uniform concentration distribution. In such an experimental setting, there are many variables that will influence the overall drug diffusion process, among which the design of the diffusion cells, the selection of the membrane model, and the properties of the receptor medium are some significant parameters. It is therefore necessary to control the experimental conditions to achieve reliable and reproducible results from *in vitro* percutaneous characterization.

2.3.1. Diffusion Cell Design

A great deal of attention has been paid to the design of the structure and function of both donor compartment and receptor compartment. Different cell designs are believed to have variable effects on the penetration of test molecules through the membrane model. Vertical diffusion cells have the advantages of easy control over dose application, dose size and application occlusivity. It is possible to adjust the application occlusivity in the donor compartment with a piece of glass on top of the cell. In addition, the applied dose remains static within the donor compartment over the diffusion study, which more realistically simulates the actual topical drug application *in vivo*. Therefore, vertical diffusion cells are more likely to be selected for *in vitro* diffusion experiments. Horizontal diffusion cells, on the other hand, provide an environment in which study temperature is well controlled, and agitation of the applied dose is also achieved [56]. However, it is more difficult to adjust the diffusion environment and dose administration within the donor compartment, because both compartments are completely sealed to form a closed system during diffusion experiments. This experimental setting is also not well representative of the realistic topical drug administration.

The design and function of the receptor compartment have been given some attention in order to maintain uniform agitation and constant temperature, two important variables critical to drug diffusion and penetration. Uneven stirring in the receptor compartment can result in inaccurate experimental results [57], and not all diffusion cells can provide adequate agitation within the receptor phase. In order to find a good diffusion cell with uniform stirring capability, an apparatus was designed, which was the addition of a crystal of potassium permanganate in the receptor fluid. When the magnetic stirrer is switched on, and the whole receptor medium turns mauve within 30 seconds, the diffusion cell is considered capable of providing adequate agitation. In the meantime, the temperature of the receptor compartment is to be controlled around 35-37°C by water bath to simulate *in vivo* biological conditions. Temperature fluctuations can result in change of drug penetration across the membrane, consequently influencing data calculation and interpretation.

2.3.2. Membrane Models

When the objective of the *in vitro* experimentation in transdermal drug delivery is to predict the penetration of the molecules through living skin in humans, human skin is obviously the most ideal and preferable choice for reliable data correlation. Nevertheless, human skin is not readily available in many cases due to a lack of the sources. Even when skin samples are available from various approaches, such as mastectomies, reductions, cadavers and amputations, they are believed to affect the penetration of the test molecule under a variety of conditions [58,59]. Alternative membrane models may include biological membranes from animals and artificial membranes from chemical synthesis.

So far many *in vitro* experiments have been conducted using excised skin from hairless mice. Similarly, skin samples from hairless or fuzzy rats, guinea pigs, rabbits, and piglets are also used. It has been possible to find a predictive and satisfactory relationship between human skin and animal skin in terms of percutaneous characterization [60]. As to artificial membranes, cellulose acetate, silicone [61], nitrocellulose (Mill F-0.025μm membrane) [62], and high density polyethylene (HDPE) [63] are commonly used as substitutes for biological membranes. These synthetic membranes provide simpler application preparation without the need for complicated pretreatment procedures and rigorous storage conditions. The availability of the membranes is also comparatively widespread and inexpensive. Some success has been found from artificial membranes to mimic the penetration profiles of molecules through the biological skin. However, they are only appropriate for the assessment of drug release characteristics from the vehicles or delivery devices, not for the penetration mechanisms of the test molecules across the skin.

2.3.3. Receptor Medium

Two types of receptor medium are usually used for *in vitro* diffusion studies. They are phosphate buffered (pH 7.4) saline with surfactants or bovine serum albumin, and mixture of water and organic solvents in different proportions. Physiological saline with phosphate buffer (pH 7.4) produces a diffusion environment that mimics the realistic *in vivo* biological conditions. However, it has one major disadvantage of hindering the release of lipophilic molecules for the reason of being a simple aqueous medium. The addition of a surfactant or bovine serum albumin could generally provide a simulated

environment to plasma, and at the same time enhance the solubility of lipophilic substances in the receptor medium. For diffusion mediums that are composed of water and organic solvents, they are occasionally used for *in vitro* diffusion studies to evaluate topical drug formulations and transdermal delivery systems. The major deficiency for this type of solvent systems is its difference from realistic *in vivo* biological conditions, which could consequently affect data correlation and interpretation.

There are two types of sampling approaches for *in vitro* experiments. One is aliquot sampling, the other is continuous sampling. Aliquot sampling is a typical protocol for the static diffusion cells. It is simple, straightforward and economical; only a sampling syringe is required. In addition, sampling volume can be easily adjusted and customized according to individual experimental settings and application doses. Continuous sampling is a typical method in flow-through cell designs. Automation is achievable with this approach once equilibrium of the system is established. However, this experimental setting requires large volume of receptor solution and appropriate flow rate to maintain the sink conditions. There is also potential for sample leaking due to multiple injections.

In vitro diffusion study provides a fast and economic method to evaluate percutaneous drug absorption and maintain quality of transdermal drug delivery systems. However, selection of the experimental instrumentation and conditions is still very critical to the correlation and interpretation of *in vitro* data.

Chapter 3. DEET

3.1. Introduction

The full chemical name for DEET is N,N-diethyl-3-methyl-benzamide or N,N-diethyl-m-toluamide ($C_{12}H_{17}NO$). The chemical structure of DEET is shown as following in Figure 3.1. Table 3.1 lists the physical and chemical properties of this chemical insecticide.

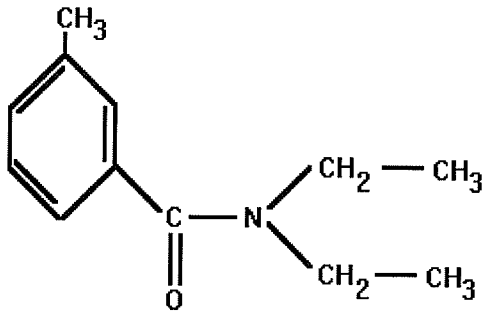


Figure 3.1. Chemical structure of DEET

DEET was initially discovered by scientists at the United States Department of Agriculture, and was subsequently patented by the U.S. Army in 1946 for military use in areas with heavy biting-insect infestation. The history of DEET use for civil purposes started in 1950's, and insect repellents containing DEET have been used for more than 50 years worldwide to repel mosquitoes, ticks, fleas, biting flies and chiggers. DEET possesses a broad repelling spectrum against various insects and is one of the most effective insect repellents commercially available. Effective application of DEET-based repellents is able to reduce vector-human contact and therefore minimize the incidence of disease transmission [64,65].

Table 3.1. Physical and chemical properties of DEET

Parameter	Value / Property
Molecular Weight	191.26
Purity	N, N-diethyl-m-toluamide, 95% Minimum
Specific Gravity (25/25°C)	0.992-0.999
Color/Form	Colorless to off-white, light-yellow, amber liquid
Odor	Nearly odorless
Water Solubility (25°C)	Practically insoluble
Partition Coefficient (K_{ow})	100
Soil Sorption Coefficient (K_{oc})	300
Vapor Pressure (20°C)	5.6×10^{-3}mmHg
EPA Toxicity Classification	Class III
Boiling Point	111°C (1mmHg)
Viscosity (30°C)	13.3cps

* *http://www.rand.org/publications/*

In the early 1980's, the use of DEET-based topical repellent formulations was recommended as the best approach to prevent the transmission of Lyme disease, a disease transmitted through the bite of black-legged ticks (*Ixodes scapularis*) infected with bacterium *Borrelia burgdorferi* [66,67]. Healthcare professionals also strongly recommended the use of insect repellents to prevent malaria for those traveling to the malaria endemic regions such as Sub-Saharan Africa, Southern and Southeast Asia, Mexico, Haiti, the Dominican Republic, Central and South America [68,69]. Moreover,

DEET benefits not only civilians taking part in outdoor recreational activities and workers in the field environments infested with biting insects, but also military personnel in the field of combat and training [70,71]. Particularly, when the troops are performing in the disease-endemic areas, DEET is able to protect soldiers from interruption of the diseases transmitted by biting of mosquitoes to maintain strong fighting capabilities [70].

3.2. Mechanism of DEET

The exact repelling mechanism of DEET against mosquitoes is still not quite clear. The most-acceptable hypothesis is that the compound disturbs the normal functions of receptors in a mosquito's antennae that allow it to locate a living object. Mosquitoes do not have noses; they locate their pray effectively by sniffing around with their antennae. The chemical compounds exuding from the skin and the breath of a living species can land on a mosquito's antennae and consequently direct it to the correct location of the subject. Carbon dioxide from a breathing animal is an attraction to mosquitoes, some of which can detect it as far as a hundred feet away. Heat, lactic acid, and other metabolic byproducts generated by a living animal can also attract mosquitoes through those receptors in their antennae. Insect repellents like DEET evaporate and create a cloud of molecules within about an inch over the skin surface, confusing and numbing a mosquito's ability to detect those ingredients secreted from a human, and subsequently repelling it from biting, even though it can still sometimes locate the subject [72]. DEET as an insecticide does not actually kill mosquitoes.

Among all insect repellents commercially available in the market, DEET showed the longest repelling efficiency in the range of 88-300 minutes (Table 3.2). It was found

from an efficacy test on sixteen commercial products that the time of the first bite in all DEET products was much longer than that of the natural products. In addition, the higher the concentration of DEET applied, the longer the protection lasts. For instance, OFF!® Skintastic containing a relatively small amount of DEET (6.65%) lasted an average of 112.4 minutes [73]. Skin-So-Soft® Bug Guard Plus and Avon® repellents, which contained a new chemical IR3535 (ethyl butylacetylaminopropionate), lasted less than 22.9 minutes on average. Naturally-existing insect repellent products, which were mostly based on plant oils such as eucalyptus oil and soybean oil, did not last as long as DEET either.

The protection duration of DEET is also related to the repelling of different mosquito species. In 1963, Smith *et al* first reported that the application of 10% DEET in ethanol solution could provide 336-372 minutes of protection against *Aedes Aegypti* on the forearm of human volunteers [74]. But in 1969, Altman observed that the protection time with the same amount of DEET was 120-132 minutes against *Anopheles albimanus* [75]. In 1970, Gilbert *et al* tested that in the repelling of *Culex quinquefasciatus* with 10% DEET ethanol solution the protection time was 132 minutes [76]. Nevertheless, DEET is still considered one of the most effective repellents in terms of protection duration.

The minimum effective dose (MED) of DEET indicates a dose of DEET to reduce 95% of insect biting, which is another critical value of DEET and partially related to the mosquito species. Maibach *et al* found that MED was as low as 16μg/cm^2 against *Aedes Aegypti* [77]. However, Gabel *et al* found that MED was 25μg/cm^2 against the same mosquito species [78]. It was reported recently that an estimated dose of DEET to reduce

biting by 95% in *Aedes Aegypti* on human volunteers was 4.4μg/cm^2, whereas an estimated dose for 95% bite reduction of *Anopheles albimanus* was 23μg/cm^2, which was 5 times higher than a dose of DEET against *Aedes Aegypti* [79].

Table 3.2. Comparative efficacy of insect repellents against mosquitoes

Product	Ingredient / Concentration	Protection Time
OFF!® DEEP Woods	DEET / 23.8%	301.5 minutes
Sawyer® Controlled Release	DEET / 20%	234.4 minutes
OFF! ® Skintastic	DEET / 6.65%	112.4 minutes
Bite Blocker for KIDs	Soybean oil / 2%	94.6 minutes
Off! ® Skintastic for KIDs	DEET / 4.75%	88.4 minutes
Skin-So-Soft® Bug Guard P	IR3535 / 7.5%	22.9 minutes
Natrapel®	Citronella / 10%	19.7 minutes
Herbal Armor®	Citronella / 12% Peppermint oil / 2.5% Cedar oil / 2% Lemongrass oil / 1% Geranium oil / 0.05%	18.9 minutes
Skin-So-Soft® Bug Guard	Citronella / 0.1%	10.3 minutes
Gone Plus Repelling Wristband	Citronella / 25%	0.2 minutes

* *N Engl J Med* **347**:13-18 (2002)

3.3. Factors Affecting DEET Efficacy

It is obvious that the intrinsic repellency of DEET is closely associated with not only its physicochemical properties (Table 3.1), such as vapor pressure, boiling point,

partition coefficient, lipophilicity, viscosity, surface tension, and thermodynamic factors [80-82], but also some other environmental conditions, such as air flow, temperature, loss from abrasion, and wash-off after dermal application [83-85].

In 1973, Khan *et al* investigated the effects of temperature within the range of 26-50°C on the efficacy of insect repellent, and reported that increasing 10°C would decrease the protection time of DEET by 50% [86,87]. In addition, rising air currents also resulted in decrease in protection time of DEET. For example, applying DEET on the surface of the skin at a dose of 0.16mg/cm^2 with static air currents at 26°C allowed the protection time of DEET to last for 200 minutes. In comparison, airflow at 192m/min would reduce protection time of DEET to 73 minutes. DEET also has excellent resistance to sweating and wash-off, which has been demonstrated in a series of tests with several species of mosquitoes [88]. Wash-off resistance offers greater protection to swimmers and fishermen who frequently come in contact with water. While DEET continues to repel biting insects after many wipes of the skin, other repellents are commonly rubbed off easily after half the number of wipes.

Skin lipids and sweating conditions can also alter the protection time of DEET formulations. Skinner found that lipid from the skin of forearm and foreheads in human volunteers exerted satisfactory effect on the repellent against *Aedes Aegypti* [89], which was attributed to the unique hydrocarbon fraction of the lipid molecule that is derived from unsaturated volatile fatty acids and also exhibits a degree of repellency [90,91]. Sweat is believed to effect the protection time of DEET formulation too. Components from human sweat are able to attract mosquitoes. Skinner investigated and demonstrated that *Aedes Aegypti* was attracted by the diluted solution of lyophilized human sweat.

Sometimes, sweat could dilute and remove DEET from the skin surface, especially under hot and humid conditions.

Extensive transdermal penetration has a significant effect on the protection efficacy and duration of DEET formulations. It was reported that up to 16.7% of DEET applied topically was absorbed systemically over a period of 5 days in human volunteers [92]. The amount of DEET applied on cattle was transdermally absorbed as high as 72.9% within the first 14 hours. The loss through systemic percutaneous absorption would reduce protection efficacy of DEET, and at the same time, increase *in vivo* distribution and accumulation of DEET that is intended mainly for localized topical protective actions.

3.4. Pharmacokinetics

Even though DEET-based insect repellents have been used extensively for decades, few pharmacokinetic studies have been conducted in humans. The main reason is perhaps attributed to the potential toxicity of DEET as an insecticide in humans. Studies have demonstrated that the penetration of DEET through human skin is extensive and rapid both *in vitro* and *in vivo*. The average transdermal absorption of DEET in two human volunteers was 11%, 11%, 38% and 54% in the second hour after an ethanol solution of DEET was applied to the forearm at the dosage of 1.86, 0.93, 0.16 and 0.08mg/cm^2 [74]. Penetration of DEET from *in vitro* studies was found to be 50.8% in human skin within the first hour after an ethanol DEET solution was applied at the dose of 25mg/cm^2 [94]. The transdermal bioavailability of DEET was determined by Selim *et al* in the complete investigation of DEET in absorption, distribution, metabolism, and

excretion [95]. Bioavailability value of DEET was considered as the ratio of the total C_{14}-radioactivity recovered in urine and feces to the dermal C_{14}-radioactivity dose in human volunteers. The result of its bioavailability was 5.6% and 8.4% when technical DEET (100% DEET) and 15% DEET ethanol solution were applied respectively at the doses of 0.625mg/cm^2 and 0.5mg/cm^2, which was lower than that reported by Feldmann and Maibach in 1970 (16.7%) [58].

It is evident that DEET is able to penetrate rapidly into the systemic circulation by transdermal route. Measured with C_{14}-radioisotopic technique, the average hourly absorption of DEET in the first 12 hour was 0.773% after DEET was applied on the ventral surface of the forearm at 4μg/cm^2 in humans [92]. The peak time for plasma radioactivity was achieved at about 6 hours, and 73-75% of DEET was absorbed through the skin surface within the first 12 hours [95]. Transdermal profile of DEET from repeated applications is different from that of a single application. Investigation conducted in a small number of National Park employees with repeated dermal applications of DEET indicated that DEET concentrations in urine ranged from 0.58-5.69μg/ml within the first two hours after repeated applications of a lotion containing 71% of DEET [96]. In other investigations of transdermal absorption of various insect repellents in human volunteers, the results were consistent with those observed by Dremova *et al* in 1971 [97]. The maximum blood concentration of DEET was approximately 20μg/ml, detected within 2 hours after dermal application of 1g DEET cream on the top of hands, feet and back of the volunteers [97]. There is little information available on the distribution of DEET in humans. In the limited cases of suicide caused by ingestion of DEET, DEET was found to have the highest tissue concentrations in

blood, liver and urine [98]. Recent pharmacokinetic studies found that C-14 radioactivity existed at various levels in the following tissues, brain, heart, lungs, kidneys, muscle, stomach, small and large intestines, cecum, fat, bone, spleen, spinal cord, sciatic nerve, and blood in rats that had received oral or dermal administration of C_{14}-labeled DEET [99].

Extensive metabolism takes place in humans before DEET is excreted in urine. At least six metabolites were identified in the urine samples, when human volunteers were treated with 12-15mg DEET on the forearm [95]. No unchanged DEET was detected by HPLC, but there were high levels of two identified metabolites, N,N-diethyl-3-carboxylbenzamide and N-ethyl-3-carboxybenzamide. Investigations of DEET metabolism in rats revealed similar metabolic patterns, i.e., there was no parent compound found in the urine, and N,N-diethyl-3-carboxylbenzamide and N-ethyl-3-carboxylbenzamide were considered the major metabolites, after rats were treated with C_{14}-labeled DEET by oral and dermal administration. The elimination of DEET is rapid and complete in humans, and *in vivo* bioaccumulation is therefore unlikely. Study by Selim *et al* demonstrated that 99% absorbed radioactivity was eliminated from urine after technical grade DEET and 15% ethanol DEET solution were applied on the surface of the forearm in human volunteers [95]. At the same time, less than 0.1% of DEET treated topically was found to be eliminated in the feces. In addition, rapid elimination of DEET in urine also occurred in rats, dogs and cattle, as indicated by various pharmacokinetic parameters [95].

3.5. Pharmacology and Toxicology

DEET has undergone extensive toxicological and dermatological testing. Its safety for use as an insect repellent has been demonstrated by the results of numerous studies. However, incidents of serious side effects associated with the use of DEET have been increasing in recent year [98]. The number of calls related to the exposure to DEET escalated 103% from 35.8 per million packages sold in 1958 to 72.8 in 1995 according to information from the Poison Control Centers [100]. Recently, DEET products have also been hypothesized to contribute to "Gulf War Syndrome", in which neurological toxicity was the major symptoms. In addition, health threats from the West Nile virus are also prompting the general public to apply insect repellents more frequently than before, due mainly to the lack of an effective human vaccine against the deadly virus. Reevaluation of DEET is subsequently becoming a critical and essential task to ensure the safety and effectiveness of DEET-based products.

The reported side effects of DEET include neurotoxicity, cardiovascular toxicity, acute manic psychosis, immunologic responses such as anaphylaxis, and even death. Three deaths were reported to be associated with the dermal use of DEET formulations in young children. One of them was a 17-month old girl who died of acute encephalopathy after repeated applications of DEET lotion for three days [101]. Other cases of systemic adverse effects such as acute manic psychosis, cardiovascular toxicity, and anaphylaxis have also been attributed to topical use of DEET products. One clinical report cited a unique case in which a healthy 61 year old woman suffered a short hypotension with her systolic blood pressure dropping to 80mmHg, who also experienced nausea, vomiting, and diarrhea after she applied both sunscreen and DEET spray. The patient was

completely asymptomatic and had a stable blood pressure several hours after admitted into a hospital [102]. Dermatitis is another major local side effect of DEET from dermal application. In 1982, ten male soldiers ranging between 18-20 years old developed acute dermatitis in the antecubital fossae several hours after using a 50% DEET formulation. Clinic symptoms included a burning sensation, erythema, and blisters at the application site, followed by ulceration and scarring in some individuals [103]. The report suggested that precautions should be advised in the use of insect repellents containing DEET. In another clinical case, DEET was found to cause immediate-type hypersensitivity. A paper reported that several military personnel sporadically developed a baffling bullous eruption in the antecubital fossae after they applied a DEET formulation before bedtime. The skin eruption healed afterwards with scarring [104]. In another clinical study, no skin irritation appeared on the arms of the human volunteers, who were supplied with an isopropanol solution of 50% DEET on the arm and face for 5 days. But they all felt a slight tingling sensation, dryness and astringency [105]. All these incidence reports may indicate the role of transdermal absorption of DEET in its adverse effects, which are still mostly unclear to date.

The toxicity of DEET has been investigated only in the animal models. In 1978 and 1986, Rutledge and Robbins respectively analyzed the literatures on the toxicity of DEET and published the critical review and bibliography of DEET [106,107]. Recently, more reports have focused on the teratological and developmental toxicity, neurotoxicity and cardiovascular toxicity to update knowledge on the safety of DEET. Some important results on DEET toxicity in animals are listed in Table 3.3. Nevertheless, its status as an insecticide highly restricts the possibilities of conducting clinical trials in humans

because of ethics and toxicological concerns. Toxicological correlations between humans and small animals are not satisfactory, especially in dermal or topical drug applications where drug absorption may differentiate significantly among species. Most currently available DEET information in humans was actually based largely on previous clinical reports that were published several decades ago.

Table 3.3. Toxicity of DEET in animal models

ACUTE TOXICITY		
Rats	Oral administration	LD_{50} 2.0g/kg
Rabbits	Dermal administration	LD_{50} 10g/kg
SUBACUTE ORAL TOXICITY		
Rats and Dogs	300mg/kg (daily for 90 days)	No evidence of gross or microscopic damage
SUBACUTE DERMAL TOXICITY		
Rabbits	1000mg/kg (daily for 90 days)	Mild skin irritation
Dogs	300mg/kg (daily for 90 days)	Mild skin irritation

* *http://www.reillyind.com/our-products/deet1.htm*

3.6. Current Application Status

For effective repellency application, DEET has been formulated into numerous preparations, including pump spray, aerosol spray, lotion, gel, soap, and impregnated towelette [108]. Topical DEET formulations are all classified as over-the-counter (OTC)

products and are commercially available to the general public across North America [100]. Formulations registered for direct application to human skin contain from 4% to 100% DEET. In the United States, the two insect repellent formulations registered for military use contain 75% DEET in ethanol and 33.3% DEET in a lotion [109]. In Canada, Health Canada recommends that all DEET-based products contain no more than 30% DEET concentration, which should normally provide adults with sufficient protection for daily outdoor routines. The concentration of DEET in products that are intended for children between 2-12 years of age should be less than 10%. In 2003, Health Canada also advised the once-daily-dose of DEET for infants between 6-24 months old. This recommendation was solely based on previous literature information on DEET, and no new clinical studies were conducted to support this recommendation.

The relationship between application dose of DEET and repellency protection persistence has been explored [110,111]. There is no significant evidence to demonstrate that higher concentrations of DEET would produce incremental repelling benefits [112]. However, higher concentrations of DEET do provide longer protection duration, which may be convenient for those involved in outdoor work settings [64].

Even though DEET is the best broad-spectrum insect repellent currently available for public consumption according to the Registration Eligibility Decision (RED) for the chemical DEET recently issued by the U. S. Environmental Protection Agency (EPA), its short protection time and potential toxicity have been regarded as major deficiencies for this compound. In the hope of improving its protection efficacy and duration, DEET has been formulated into various modern preparations using sustained-release technology and new polymers, which include hydrogel emulsions, lipospheres, microcapsules, and β-

cyclodextrin complex. Qiu *et al* developed a hydrogel emulsion system with polyethylene glycol 400, polyacrylic acid polymer Carbopol 940NF, and Pemulen TR-2, which was effective in reducing DEET skin permeation [98]. The steady-state flux of DEET from this new formulation system decreased by up to 61.7% compared to a brand commercial DEET cream containing the same concentration of DEET *in vitro*. Transdermal DEET bioavailability of this formulation in beagle dogs was reduced by 23% in comparison with the commercial DEET cream. It also demonstrated better protection against *Aedes Aegypti* in laboratory testing for a period of 6 hours [113].

A liposphere system was developed for DEET formulation to decrease percutaneous penetration and provide effective repellent protection. Domb *et al* reported that a liposphere system containing 10% DEET reduced the transdermal bioavailability by 29% in rabbits compared to a 10% DEET ethanol solution. Another liposphere formulation composed of solid hydrophobic triglycerides and 20% DEET could provide six hour protection against *Aedes Aegypti* and *Anopheles stephensi* compared with other DEET formulations [114,115].

A study of the sub-micron encapsulated Sawyer Controlled Release formula published in November 1999 reported that the protection time of this DEET formulation in four volunteers at the dosage of 1.5g/600cm^2 could last the full 20 hours against *Culex quinquefasciatus* – a mosquito species that transmits the West Nile virus. The absorption rate of ^{14}C-DEET as a microencapsulated product and dissolved in ethanol was conducted in the shaved abdominal skin (20cm^2) of 6 male rhesus monkeys. Formulations remained in contact with the skin for 8 hours. Based on urinary and fecal excretion of the radioactivity, approximately 6.6% of the microencapsulated dose was absorbed, which

was equivalent to approximately 16.3% of the dose absorbed from the ethanol preparation. This difference was statistically significant [116].

Cyclodextrin incorporation is another alternative to formulate topical preparations for DEET. DEET formulations were prepared with γ-cyclodextrin (GCD, 20% DEET w/w) and hydroxypropyl-β-cyclodextrin (HPDCD, 30% DEET w/w). The release of DEET from these vehicles was compared with an ethanol-containing DEET formulation. The cyclodextrins produced a significant decrease in DEET permeation compared to the ethanol solution, which also indicated satisfactory repellency and good formulation stability [117].

In summary, the efficacy and protection duration of DEET-based formulations are not only associated with its physicochemical properties, such as boiling point, vapor pressure, partition coefficient, and surface tension, but also influenced by the environmental factors, such as temperature, humidity, airflow condition, loss from the skin surface, and formulation types. Changes in any one of these parameters could subsequently affect the protection performance of DEET as an effective insect repellent.

Chapter 4. Sunscreens

4.1. History

Prior to the 1920's, fair skin and light complexion were perceived as a higher social and financial status in European and Eastern Asian countries. However, this public perception changed in 1920, when a famous French fashion designer developed a suntan skin color during her cruising trip from Paris to Cannes, which subsequently stirred up a brand new trend in the fashion world. In the meantime, Josephine Baker, a caramel-complexioned pop singer, was becoming one of the rising top stars in Paris. The general public gradually began to regard tanned skin color as a fashion and purposely achieved tanning by exposing to sunbath and other outdoor activities. Zinc cream was used initially to protect the skin from sunburns.

The first sunscreen preparation was not documented until 1928 when benzyl salicylate and benzyl cinnamate were formulated into an emulsion in the United States. In the 1930's, 10% salol (phenyl salicylate) [118], quinine oleate and quinine bisulfate [119] were also used as protective sunscreen agents in Australia and the US. In 1936, Eugène Schueller, the founder of French cosmetic giant L'Oréal, introduced the first commercial sunscreen product [120]. During the 1940's, 2-5% p-aminobenzoic acid (PABA) was prescribed to patients in either aqueous cream or 70% alcohol solution as sunscreens. At the same time, physical sunscreens such as zinc oxide and titanium dioxide were investigated for their sun-blocking properties [121]. Commercial sunscreen products gradually took shape and the market soon developed and grew. Today, sunscreens have become a line of major consumer-care products around the world.

Allergic reactions to sunscreen compounds such as PABA had been recorded during the 1960's. The Food and Drug Administration (FDA) changed the category of the

sunscreens from cosmetics to over-the-counter (OTC) drugs in 1972. At the same time, FDA declared that sunscreens were safe and effective in the prevention of sunburn and skin cancer and alleviation of the skin aging process [122]. The Sun Protection Factor (SPF) system was introduced to measure the protective effectiveness of sunscreens [123]. Before the 1980's, most sunscreens were designed to block only the UVB. Today, almost all sunscreen products have the capability of blocking against both the UVB and the UVA. Due to the dramatic growing tendency in skin cancer, dermatologists emphasize regular application of the sunscreens, not only in adults but also in children. There have been numerous commercial sunscreen products available in the market, designed for either daily routine applications or special outdoor activities. Sun safety programs have also been well established in many developed countries to encourage regular sunscreen use to minimize skin cancer incidences.

4.2. Skin Cancer

Skin cancer is one of the most common forms of cancer in humans, in which cancer cells are found in the outer layers of the skin. It is estimated that over 1 million new cases of skin cancer occur annually in the United States [124]. The annual rate of all forms of skin cancer is increasing each year, representing growing public concerns and healthcare costs associated with the diagnosis and the treatment. The causes of skin cancer may stem from several factors in combination, the most important among them are the "global warming" and depletion of the "ozone layer", improvement in living standards and healthy lifestyles, and early diagnosis and treatment of the skin cancer.

There are several different types of cancer that originate in the skin. The most common ones include basal cell cancer and squamous cell cancer. Basal cell cancer is the most common skin cancer and accounts for more than 90% of all skin cancer diagnosed in North America. Both basal and squamous cancer do not spread (metastasize) to other parts of the body. They can, however, cause skin damage by growing and invading the surrounding tissues. The most common sign of these two types of skin cancer is a pathological change on the skin, such as a growth or a sore that would not heal. It may also form a small lump or a flat red spot that is rough or scaly in shape [125].

Melanoma is a more serious type of skin cancer than basal cell cancer or squamous cell cancer. In melanoma, the cancer (malignant) cells are found in the cells that color the skin (melanocytes). Melanoma can metastasize quickly to other parts of the body through the lymph system or through the blood circulation. Melanoma can occur on any skin surface. In general, it is often found on the trunk (the area from the shoulders to the hips) or the head and neck in men. In women, melanoma often develops on the lower legs or the trunk. Melanoma is rare in black people and people with dark skin. When it does develop in dark-skinned people, it tends to occur under the fingernails or toenails, or on the palms or soles [126].

The cause of skin cancer is affected by many factors, which include family history, sun exposure, and living environment. Family history is a genetic parameter, in which children with parents who have skin cancer tend to have a higher risk for developing skin cancer than those with healthy parents. In addition, it is more common for people with light skin color to develop skin cancer. Sun exposure is another important factor in the development of skin cancer. Skin cancer has been mostly found in body parts that have

been exposed to more sunlight, such as the face, neck, hands, and arms. Scientific evidences have well proven the relationship between UV radiation from the sun and the skin cancer. Artificial sources of UV radiation, such as sunlamps and tanning booths, are also capable of inducing skin cancer. The risk of developing skin cancer is apparently related to the geological location as well. People who live in areas that receive higher levels of UV radiation from the sun are more likely to develop skin cancer. In the United States, for example, skin cancer is more common in Texas than it is in Minnesota, where the sun is not as strong. Worldwide, the highest rate of skin cancer is found in South Africa and Australia, where people receive the highest amounts of UV radiation. In addition, skin cancer is related to lifetime exposure to UV radiation. While skin cancer appears commonly in the fifties, the damaging effects such as acute sunburn and skin discomfort, chronic changes of premalignant, and immunologic suppression begin actually at a much earlier age, particularly during childhood and adolescence. Reports have indicated that severe acute sunburns in childhood or adolescence could increase the incidence of malignant neoplasms of the skin in adulthood by 75% [127-129]. Therefore, protection should start as early as possible, best in childhood, in order to minimize skin cancer later in life [130]. Regular application of sunscreens plays a critical role in the prevention of skin cancer, especially for children and young adults.

4.3. Protection Mechanisms of Sunscreens

UV radiation from the sun is composed of segments of a broad spectrum, with wavelength ranging from 220nm to 400nm. The shorter wavelengths of UV are known as UVB and UVC. UVC (220-290nm) is extremely harmful to human skin, but is also

totally absorbed by the ozone layer. The wavelength of UVB ranges between 290-320nm; it also damages the skin, and can only be partially absorbed by the ozone layer. Studies have indicated that UVB is the major cause to the formation of the skin cancer and photoaging. Some early commercial sunscreen products particularly focused on the protection against UVB [131]. UVA, whose wavelength is between 320-400nm, is the other UV source and presents in as much as 1000 times the intensity of UVB. It cannot be absorbed by the ozone layer, so it has a greater chance to impact human skin for a lifetime span. Even though UVA is not as harmful as UVB, protection against it is still necessary to compensate for its longer exposure time. In addition, UVA enhances the acute and chronic effects of UVB [132]. Protection against UVA has become one of the important design goals for all modern sunscreen products.

Sunscreen compounds are categorized into chemical and physical ingredients, which utilize different mechanisms to protect skin against UV radiation. Chemical sunscreens such as oxybenzone and octyl methoxycinnamate (OMC) can produce photoprotection to the skin because of their special chemical structures. Molecules of chemical sunscreens contain a number of double bonds in a benzene ring or linear configuration. The electrons in these double bonds locate in the low-energy orbitals. By absorbing energy, such as that from UV radiation, electrons are excited to higher energy orbitals [133]. The wavelengths of UV radiation from which the molecules of chemical sunscreens absorb are directly proportional to the numbers of the double bonds in the structures [134]. Compounds (e.g., t-butylmethoxydibenzoylmethane) that absorb UVA (320-400nm) have much more double bonds than those (e.g., PABA) that absorb UVB (290-320nm).

Physical sunscreens are generally opaque substances that contain high amount of minute particles. Those minute particles can scatter and reflect UV radiation, thus minimizing penetration of UV light into the epidermis. Common physical sunscreens include talc ($MgSiO_2$), titanium dioxide (TiO_2), zinc oxide (ZnO), calamine (FeO_2), and red petrolatum [135]. Table 4.1 and Figure 4.1 list some common sunscreen ingredients that are approved for use in commercial sunscreen products.

Table 4.1. FDA-approved category 1 sunscreens

UVA Absorbers	Butylmethoxydibenzoylmethane Oxybenzone Sulisobenzone Dioxybenzone
UVB Absorbers	p-aminobenzoic acid 2-ethoxyethyl p-methoxycinnamate Diethanolamine p-methoxycinnamate Digalloyl trioleate Ethyl 4-bis (hydroxypropyl) aminobenzoate 2-ethylhexyl-2-cyano-3,3-diphenylacrylate Ethylhexyl p-methoxycinnamate 2-ethylhexyl p-methoxycinnamate 2-ethylhexyl salicylate Gylceryl aminobenzoate Homomenthyl salicylate Lawsone with dihydroxyacetone Octyl-dimethyl PABA 2-phenylbenzimidazole-5-sulfonic acid Tnethanolamine salicylate
Physical Blockers	Red petrolatum Titanium dioxide Zinc oxide Talc

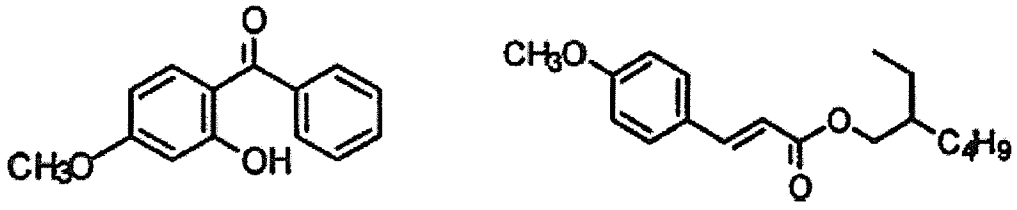


Figure 4.1. Chemical structures of selected sunscreens (oxybenzone and OMC)

The photoprotection effectiveness of a sunscreen preparation is defined by its SPF value, the ratio between Minimum Erythema Dose (MED) on sunscreen-protected skin and Minimum Erythema Dose (MED) on unprotected skin [134],

$$SPF = MED_{(\text{sunscreen-protected skin})} / MED_{(\text{unprotected skin})}$$

Generally an SPF 15 sunscreen blocks 93% possible UV damage, and an SPF 30 sunscreen blocks 97% possible UV damage. FDA strongly suggests the use of sunscreens with an SPF 15 or more and "broad spectrum" products, which means that they can protect against both UVA and UVB radiation. In addition, sunscreen use should be "regular, frequent and ample", once every two hours is strongly recommended, especially during the day or when in contact with water.

4.4. Transdermal Absorption of Sunscreens

Sunscreens are applied on the surface of the skin to provide effective protection against the UV radiation for the human skin. Same as all medications intended for topical application, active sunscreen ingredients should retain maximal local protection with minimal transdermal systemic absorption. Little information has been available in the

literature in terms of transdermal absorption, distribution, and metabolism of topically applied active sunscreen ingredients. It is therefore essential to determine the risk of skin absorption of the sunscreen products, in order to apply these OTC products safely and effectively.

In vitro diffusion studies are appropriate as a simulated model to assess profiles of transdermal absorption of the active sunscreen ingredients across the skin membrane. The correct selection of a membrane model exerts significant effects on the validity of the diffusion studies. Differences have been found among different membranes, such as those between biologic membranes and artificial membranes [136]. The choice of the receptor solution is also important for *in vitro* diffusion. The receptor solution that is appropriate for this study should provide adequate sink condition for sunscreen ingredients, most of which are lipophilic compounds and generally have low solubility in the aqueous environment (saline solution for example). In order to increase the solubility of these lipophilic compounds in the aqueous solution, surfactant and ethanol were commonly added to the receptor solution to establish an adequate sink condition [137]. In addition, the vehicle in which the sunscreens are dispersed is another important factor influencing the penetration of the compounds. Formulation vehicles not only change the partition coefficient of a compound, but also dictate the affinity between excipients and the active ingredient, subsequently controlling the rate and extent of diffusion of the compound across the membrane model.

In vivo systemic absorption of oxybenzone after topical application has been proven; metabolites of oxybenzone were also found in the urinary excretion 48 hours after topical use of sunscreens [138]. Feldman and Maibach reported that 28% of PABA

dose was detected in the urine after human volunteers applied the sunscreen topically for 5 days [139]. Absorption profiles of PABA from three different formulations were also evaluated by Arancibia and coworkers, in which no discernible differences of transdermal absorption of the substance through the skin were found among three formulations (hydroalcoholic gel, oil-in-water emulsion/pH 4.2, and oil-in-water emulsion/pH 6.5) [140]. Results from these studies indicated a need for further investigation of the percutaneous absorption of sunscreens in humans.

Sunscreen agents are able to induce contact skin irritation, phototoxicity, and photoallergy. In terms of mutagenic and carcinogenic potentials of sunscreens, however, little information has been published so far. The US National Cancer Institute emphasized this deficiency in 1989 [141]. The agency suggested that certain sunscreen agents might cause mutagenic effects if they were absorbed into epidermis of the skin and beyond [139]. Even though toxicological profiles of sunscreens are still being evaluated in both laboratory and clinic, the best practical approach is to minimize the overall penetration of sunscreens to the viable tissues through formulation modifications.

Active ingredients in sunscreens can not only possess potential toxicity to humans, but also enhance the penetration of other chemical substances. In the study of *in vitro* permeation of DEET and the sunscreen oxybenzone, Gu *et al* reported that the absorption of DEET across pigskin was enhanced by 289%, 243%, and 112% when both DEET and oxybenzone were concurrently present in propylene glycol, ethanol, and poly(ethylene glycol), respectively [142]. It was also reported that commercial sunscreen products enhanced the percutaneous penetration of the herbicide 2,4-dichlorophenoxyacetic acid. In this study, six out of nine commercially available

sunscreen products led to significant penetration enhancement of the herbicide, with level as high as 81% [143]. In addition, five of the six active sunscreen ingredients tested (7.5% octyl methoxycinnamate, 7% octocrylene, 0.6% oxybenzone, 5% octyl salicylate, 8% padimate-o, 10% sulisobenzone) produced significant enhancement effects on penetration of the herbicide compared to the control, except for octocrylene [144].

Different sunscreen formulations could produce different transdermal absorption characteristics across the skin. Traditional preparations of sunscreens are mostly liquid or semi-solid formulations such as spray, cream, lotion, and gel. It has been demonstrated that active sunscreen ingredients in all these formulations can be absorbed into the systemic circulation through the skin. In order to reduce percutaneous absorption and potential toxicity of the sunscreens in humans, attempts have been made to develop new sunscreen formulations. For example, microparticles and nanoparticles are used to incorporate sunscreen components to formulate sunscreen preparations. Yener and coworkers prepared solid lipid microspheres (SLM) of octyl methoxycinnamate (OMC) to reduce penetration of this UV absorber across the skin and to improve its photostability. They found that the rate of percutaneous penetration was dependent upon the preparations and could be significantly decreased by up to 77% through SLM formulations. Compared to different topical vehicles such as cream, hydrogel, and o/w emulsion, OMC was released and penetrated into rat skin more slowly from SLM preparation. In addition, photostability was improved in SLM form [145]. It was also found that solid lipid nanoparticles (SLN) decreased *in vitro* release rate of sunscreen oxybenzone by 50% in comparison to conventional o/w emulsion, whereas *in vivo* release rate of oxybenzone from SLN preparation into stratum corneum was reduced by 30-60%

[146]. Semi-synthetic hyaluronic acid (HA) benzyl ester and a synthetic polymer were used to prepare sunscreen microcapsules, which were characterized by satisfactory preparation stability, good spread ability, low toxicity, better tolerability and formulation convenience [147].

Chapter 5. Hypotheses and Aims

5.1. Hypotheses

Mosquito infestation during summer months has always been a widespread problem in the province of Manitoba. With the arrival of the West Nile virus in North America and its widespread dissemination by mosquitoes, mosquito infestation has now become an imminent health hazard for the general public. Vaccines against the West Nile virus for human usage are still being developed and tested; however, they are not expected to be clinically available for mass vaccination in foreseeable future. Therefore, the application of topical mosquito repellents has been recommended as an essential health preventive measure for all those involved in outdoor activities.

Skin cancer is another common disease closely associated with summer outdoor activities. It is currently increasing at an epidemic rate of approximately 5% each year. UV radiation from sunlight has been identified as causing more than 90% of all skin cancers. The application of sunscreen products has been widely accepted by the general public as an effective and practical approach to minimize sunburn and skin damage, especially in children and young adults.

Concurrent application of repellents and sunscreens is gradually becoming a summer routine in many regions across the continent, especially in places where mosquitoes that carry the West Nile virus are highly active and aggressive during day light. Intended as "Topical-Use Only" preparations, active ingredients from DEET-based insect repellents and sunscreens should remain on the surface of the skin and produce minimal percutaneous and systemic absorption. Commercially available repellent and sunscreen products are commonly composed of multiple active ingredients and excipients to maximize application efficacy, to optimize preparation elegancy and stability, and to

facilitate application convenience. In addition, repellents and sunscreens as OTC products are applied without fixed dose according to each individual's preference. It is therefore necessary and important to investigate the transdermal profiles of active repellent and sunscreen ingredients from a concurrent application of commercially available DEET-based repellent and sunscreen preparations.

It was hence hypothesized with this thesis that,

1). Concurrent application of commercially available DEET-based insect repellent and sunscreen products would produce synergistic percutaneous penetration of the active ingredients *in vitro*;

2). Percutaneous penetration profiles of the test substances would depend upon formulation types, application amounts and application sequences;

3). Appropriate formulation modifications would minimize the overall transdermal penetration of the test compounds from concurrent application of repellent and sunscreen preparations.

5.2. Aims

The objectives of this thesis were,

1). To evaluate *in vitro* transdermal penetration of concurrent application of commercially available DEET-based insect repellent and sunscreen products, using traditional diffusion study setting, and DEET and oxybenzone as the model compounds;

2). To compare *in vitro* transmembrane profiles of DEET and oxybenzone through both biological membrane and synthetic membranes;

3). To define the roles of physical and chemical parameters of the test compounds

in transdermal penetration and interaction;

4). To optimize formulation parameters for the benefit of reducing the overall transdermal penetration of DEET and oxybenzone.

Chapter 6. Materials

6.1. Chemicals

Following chemical substances were used in the experiments of diffusion studies, HPLC analysis and formulation preparations.

1. Acetonitrile (HPLC Grade) – Fisher Scientific, Fair Lawn, New Jersey, USA
2. Apifil Pastilles (PEG-8 Beeswax) – Gattefossé Canada Inc., Mississauga, Ontario, Canada
3. Brij® 20 Oleyl Ether (Brij 98) – Sigma-Aldrich Co., St. Louis, Missouri, USA
4. Castor Oil – Fisher Scientific, Fair Lawn, New Jersey, USA
5. Cetyl Alcohol – Medisca Pharmaceutique Inc., Montreal, Quebec, Canada
6. DEET (N,N-Diethyl-3-Methyl-Benzamide) – Fluka Chemika GmbH, Buchs, Switzerland
7. Emulium 22 Pasitilles (Tribehenin PEG-20 Esters) – Gattefossé Canada, Inc., Mississauga, Ontario, Canada
8. Ethanol (HPLC Grade) – Fisher Scientific, Fair Lawn, New Jersey, USA
9. Geleol Pastilles (Glycerly Stearate) – Gattefossé Canada, Inc., Mississauga, Ontario, Canada
10. Glacial Acetic Acid – Mallinckrodt Specialty Chemical Company, Paris, Kentucky, USA
11. Glycerin – Mallinckrodt Specialty Chemical Company, Paris, Kentucky, USA
12. Labrafac PG (Propylene Glycol Dicaprylate/Dicaprate) – Gattefossé Canada Inc., Mississauga, Ontario, Canada
13. Labrafil M 1944 CS (Apricot Kernel Oil PEG-6 Esters) – Gattefossé Canada Inc., Mississauga, Ontario, Canada

14. Methanol (HPLC Grade) – Fisher Scientific, Fair Lawn, New Jersey, USA
15. MOD (Octyldodecyl Myristate) – Gattefossé Canada Inc., Mississauga, Ontario, Canada
16. Oxybenzone (OXBZ) – Riedel-de Har GmbH, Seelze, Germany
17. Potassium Phosphate Monobasic – Fisher Scientific, Fair Lawn, New Jersey, USA
18. Precirol Ato 5 (Tripalmitin Tristearin) – Gattefossé Canada Inc., Mississauga, Ontario, Canada
19. Sodium Hydroxide – Fisher Scientific, Fair Lawn, New Jersey, USA
20. Xanthan Gum – Medisca Pharmaceutique Inc., Montreal, Quebec, Canada

6.2. Insect Repellent and Sunscreen Products

Five commercially available insect repellent and sunscreen products were purchased from a local pharmacy. They were used as obtained without further manipulation. The names and major active ingredients are listed as follows.

1. OFF!® Skintastic Insect Repellent Spray (*Product 1*)
   [S.C. Johnson and Son, Ltd., Brantford, Ontario, Canada]
   Active ingredient: DEET 7.0%

2. OFF!® Skintastic Insect Repellent Lotion (*Product 2*)
   [S.C. Johnson and Son, Ltd., Brantford, Ontario, Canada]
   Active ingredient: DEET 7.5%

3. Coppertone® UVA/UVB Protection Sunscreen Lotion (SPF30) (*Product 3*)

   [Schering-Plough Healthcare Products Canada, Mississauga, Ontario, Canada]

   Active ingredients: Ethylhexyl p-Methoxycinnamate 7.5%

   2-Ethylhexyl Salicylate 5.0%

   Homosalate 8.0%

   Oxybenxone 5.0%

4. OFF!® Skintastic Insect Repellent with Sunscreen Lotion (SPF 15) (*Product 4*)

   [S.C. Johnson and Son, Ltd., Brantford, Ontario, Canada]

   Active ingredients: DEET 7.5%

   2-Ethylhexyl Salicylate 5.0%

   Octyl Methoxycinnamate 7.5%

   Oxybenzone 5.0%

5. Muskol® Insect Repellent with Sunblock Lotion (SPF 15) (*Product 5*)

   [Schering-Plough Healthcare Products Canada, Mississauga, Ontario, Canada]

   Active ingredients: DEET 10.0%

   Homosalate 5.0%

   Octyl Methoxycinnamate 7.5%

   Octyl Salicylate 5.0%

   Oxybenzone 4.0%

6.3. Instrumentation

Two main instruments were used for the experiments, i.e., diffusion system for diffusion studies and HPLC system for concentration analysis.

1. Diffusion System – Logan Instrument Corporation, Somerset, New Jersey, USA

The diffusion system was composed of 6 vertical Franz-style diffusion cells, water bath, stirring station and automatic sampling module. The volume of donor compartment was 1ml and the volume of receptor compartment 7ml. The diffusion surface was $0.64cm^2$.

2. HPLC System – Waters Corporation, Milford, Massachusetts, USA

The HPLC system was composed of Alliance® solvent delivery module, photodiode array detector, and a computer. The system was operated with software Millennium® 32. The column used was a Nova-Pak® C_{18} column (3.9×150mm, 4μm).

3. Electric Dermatome – Padgette Instruments, Kansas City, Missouri, USA
4. Milli-Q Pure Water System – Millipore, Nepean, Ontario, Canada

6.4. Membrane Models

Both biological and artificial membranes were used in the experiments to assess the suitability of various membrane models for *in vitro* transdermal evaluation. There were one biological membrane and three synthetic membranes. The selection and use of three artificial membranes in the thesis were based on information available from previous references.

1. Piglet skin – Glenlee Research Station, University of Manitoba, Winnipeg, Manitoba, Canada

The full-thickness skin of 3 week old piglets was obtained from the Swine Unit, Glenlea Research Station of the University of Manitoba. The Animal User Protocol was approved by the University of Manitoba Fort Garry Campus Protocol Management and Review Committee, and conducted according to current guidelines published by the Canadian Council on Animal Care (CCAC). The freshly excised skin samples were kept at –20C° after the collection, and thawed at 4°C overnight before each study.

2. LDPE (Low-Density Polyethylene) Membrane – Key Container, Winnipeg, Manitoba, Canada

This membrane was made of low-density polyethylene resin, and possessed lipophilic properties in nature. The technical parameters of the membrane are listed in Table 6.1.

3. LFC1 (Low Fouling Composite) Membrane – Hydranautics, Oceanside, California, USA

This membrane was made of polysulfone membrane, polyester fabric, and polyamide membrane. A polysulfone membrane was cast on the top of polyester fabric, which was further coated with a thin layer of polyamide membrane. The ultimate membrane was described as a thin film composite that could be visualized as a piece of skin with different layers with hydrophilic properties. The membrane was applied in the diffusion experiments with polyester fabric facing the donor compartment. The technical parameters of the membrane are listed in Table 6.2.

4. Mill-F (Nitrocellulose) Membrane – Millipore, Bedford, Massachusetts, USA

This cellulose membrane had hydrophilic properties and its technical parameters are listed in Table 6.3.

Table 6.1. Specification of LDPE membrane

Parameter	Value / Property
Melt Index (g/min)	0.08
Density (g/cc)	0.921
Softening Point (°C)	92
Ultimate Tensile (Resin, kg/cm^2)	145
Ultimate Elongation (Resin, %)	710
Membrane Thickness (mm)	0.05
Ultimate Tensile (Film, Machine/Cross, kg/cm^2)	270/250
Tensile Tear (Film, Machine/Cross, kg/cm)	100/95
Ultimate Elongation (Film, Machine/Cross, %)	400/500
Dart Impact (g)	120
Gloss 45° (%)	70
Haze (%)	6.0
Heat Seal Range (°C)	120-180

Table 6.2. Specification of LFC1 membrane

Parameter	Value / Property
NaCl Solution (PPM)	1500
Applied Pressure (psi/MPa)	225/1.55
Operating Temperature (°C/°F)	25/77
Permeate Recovery (%)	15
pH Range	6.5-7.0
Maximum Applied Pressure (psi/MPa)	600/4.14
Maximum Feed Flow (GPM/m^3/h)	75/17
Maximum Operating Temperature (°C/°F)	45/113
Feedwater pH Range	3.0-10.0
Maximum Feedwater Turbidity (NTU)	1.0
Maximum Feedwater SDI (15min)	5.0
Maximum Chlorine Concentration (PPM)	<0.1
Maximum Ratio of Concentrate to Permeate Flow	5:1
Maximum Pressure Drop for Each Element (psi)	10
Thickness (μm)	150

Table 6.3. Specification of Mill-F membrane

Parameter	Value / Property
Bubble Point at 23°C (bar)	≥ 21.1
Air Flow Rate (l/min/cm^2)	0.15
Filter Code	VSWP
Gravimetric Extractables (%)	1.5
Porosity (%)	70
Refractive Index	1.5
Filter Diameter (mm)	25
Filter Surface	Plain
Max Operating Temperature (°C)	75
Filter Color	White
Filter Material	Mixed Cellulose Esters
Filter Type	Screen Filter
Filter Pore Size (µm)	0.025
Wettability	Hydrophilic
Water Flow Rate (ml/min/cm^2)	0.15
Thickness (µm)	130

Chapter 7. Methods

7.1. In Vitro Diffusion Studies

In vitro diffusion study was the main experimental method for the evaluation of transmembrane profiles of the repellent DEET and the sunscreen oxybenzone. The concentrations of the test compounds in the receptor medium were analyzed using a high-performance liquid chromatographic assay. The rate and amount of the substances that permeated across the membrane were therefore calculated and compared.

7.1.1. Application Approaches

The five commercially available repellent and sunscreen products that were tested using four membrane models included OFF!® Skintastic Insect Repellent Spray (Product 1), OFF!® Skintastic Insect Repellent Lotion (Product 2), Coppertone® UVA/UVB Protection Sunscreen Lotion (Product 3), OFF!® Skintastic Insect Repellent with Sunscreen Lotion (Product 4) and Muskol® Insect Repellent with Sunblock Lotion (Product 5). They were applied to the donor cell with different application amount and application sequence as what it would be encountered in "real-life" situations. All preparations were used directly as obtained without further manipulation or pretreatment. Table 7.1 lists 11 different application approaches used in the experiments.

7.1.2. Pretreatment of the Pigskin

The full-thickness skin of 3-week old piglets was obtained from the Swine Unit, Glenlea Research Station, the University of Manitoba. All skin samples were kept at –20°C prior to studies. Prior to each study day, the skin sample was taken out from the freezer and thawed at the room temperature. After it was thawed, the pigskin was rinsed

Table 7.1. Experimental design of the in vitro diffusion study

Product	Study Code										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Product 1 (ml)	1.0			0.5 (T)	0.5 (B)	0.5		0.25	0.5		
Product 2 (g)		1.0					0.5				
Product 3 (g)			1.0	0.5 (B)	0.5 (T)	0.5	0.5	0.5	0.25		
Product 4 (g)										0.5	
Product 5 (g)											0.5
Application Method	DA	DA	DA	NM	NM	PM	PM	PM	PM	DA	DA

DA: Direct application
NM: No mixing
PM: Prior mixing
T: Top
B: Bottom

with deionized water and dried with paper towels. The hair was removed with a razor to provide a smooth skin surface, and the fat and underlayer skin tissues were separated with a scalpel. The skin was then placed on a plastic cutting board, and the epidermis (380μm in thickness) was dermotomed from one end to the other by an electric dermatome. The integrity of the excised epidermis was carefully examined and only undamaged section was selected. The epidermis was further cut into small pieces (2×2cm). The prepared samples were then soaked in saline solution, and stored at 4°C for the diffusion experiments.

7.1.3. Artificial membranes

No special pretreatment was used for all three artificial membranes used in the studies. Prior to each diffusion study, the membrane was cut into small pieces (2×2cm). They were soaked in deionized water for 2 hours before being mounted to diffusion cells.

7.1.4. Diffusion Study

The Logan Diffusion System used for all diffusion studies was composed of six vertical Franz-style diffusion cells. Each cell consisted of a 1ml donor compartment and a 7ml receptor compartment, with a 0.64cm^2 surface area available for diffusion process. Before each study, a very thin layer of vacuum grease was applied to the connection area of both the donor and receptor to prevent test samples from leaking. Approximately 6ml of phosphate buffer (pH 7.4 with 4% Brij 98) was added to the receptor compartment. The prepared membrane was then mounted between the two compartments, and fixed with a metal clap. Once the membrane was mounted, the remaining 1ml phosphate buffer

was added to the receptor compartment through the sampling port. The receptor medium had a direct and intimate contact with the membrane without overflowing from the receptor compartment. The water bath was then turned on in circulation to warm up the receptor medium to 37°C. The magnetic stir bar in each diffusion cell was also set at 300rmp to maintain uniform agitation. After approximately 30 minutes to reach temperature equilibrium, the test samples were carefully added to the donor compartment as described in Table 7.1. The sample was then checked to ensure a complete coverage of the membrane surface. 100μl of receptor fluid was collected at 1, 2, 3, 4, 5 and 6 hour, followed by the replenishment of 100μl fresh phosphate buffer after each sampling. All samples were directly analyzed by HPLC for concentration of DEET and oxybenzone.

7.2. HPLC Assay

The concentrations of DEET and oxybenzone in all diffusion samples were analyzed simultaneously by an HPLC assay developed in the laboratory. The HPLC conditions were listed as following,

Mobile phase: Acetonitrile (65): Methanol (20): Water (15) (pH 3.0, acetic acid)

Flow rate: 1ml/min

Detection wavelength: DEET 254nm, Oxybenzone 287nm

Detection limit: DEET 20ng, Oxybenzone 5ng

Retention time: DEET 1.49 minutes, Oxybenzone 1.98 minutes

Calibration range: DEET 50-2000ng, Oxybenzone 8-500ng

The method was calibrated and validated before and during the experiments. Calibration curves were obtained from six replicates. The concentrations of DEET and

oxybenzone in samples were calculated based on the average calibration curves.

7.3. Formulations of DEET and Oxybenzone

Two oil-in-water emulsion bases were prepared in the laboratory, and DEET and oxybenzone were incorporated into these two formulations at the same concentrations as what would found in the commercially available products. Similar diffusion study was carried out to assess the transmembrane characteristics of DEET and oxybenzone from the developed preparations.

7.3.1. Preparation of Formulations

Formulation A

Emulium 22	6g
Geleol Pastilles	3g
MOD	5g
Apricot Kernel Oil	5g
Precirol Ato 5	4g
Glycerin	12g
Deionized Water	65g
Total	**100g**

Weigh the ingredients of the oil phase, Emulium 22, Geleol Pastilles, MOD, Apricot Kernel Oil, and Precirol Ato 5, add them to a beaker and heat the substances to 65°C under agitation. The water phase, glycerin and deionized water, was put in another

beaker and heated to 65°C under agitation. After both the oil phase and the water phase reached 65°C, the oil phase was slowly poured into the water phase under agitation. The mixture was stirred continuously for 30 minutes, and allowed to cool down to the room temperature. The prepared water-in-oil emulsion base was separated into three parts; 7% DEET, 5% oxybenzone, and 7% DEET + 5% oxybenzone were added into the bases to make three different preparations.

Formulation B

Apifil	6g
Cetyl Alcohol	4g
Labrafac PG	10g
Geleol Pastilles	10g
Castor Oil	5g
Hydrogenated Polydecene	5g
Xanthan Gum	0.4g
Deionized Water	60g
Total	**100g**

Weigh xanthan gum and add it to a beaker. Add approximately 10ml of deionized water, and allow the gum to stand at the room temperature for 4 hours. After xanthan gum was completely swelled, the remaining water was added to the beaker and the materials were heated to 65°C. Weigh the ingredients of the oil phase, Apifil, Cetyl Alcohol, Labrafac PG, Geleol Pastilles, Hydrogenated Polydencene, and Castor Oil, add them to

another beaker, and heat the substances to 65°C under agitation. After both the oil phase and the water phase reached 65°C, the oil phase was slowly poured into the water phase under agitation. The mixture was stirred continuously for 30 minutes, and allowed to cool down to the room temperature. The prepared water-in-oil emulsion base was separated into three parts; 7% DEET, 5% oxybenzone, and 7% DEET + 5% oxybenzone were added into the bases to make three different preparations.

7.3.2. Diffusion Study

The six formulations prepared in the laboratory were tested using similar diffusion settings as described in 7.1. 1 gram of each sample was applied to the donor compartment, using the same four membrane models. Sampling times and concentration analysis were the same as what described previously.

7.4. Data Analysis

The overall permeation percentage of DEET and oxybenzone after 6 hour diffusion study was calculated from the accumulated amount of the two compounds found in the receptor medium. The steady-state flux of DEET and oxybenzone was calculated as the ratio of the permeation amount against the diffusion time and area according to the Fick's Law. Statistical analyses were conducted to compare the differences among each application approach and among different membrane model used, using two-way ANOVA and Tukey's test. Differences were considered statistically significant at $P \leq 0.05$.

Chapter 8. Results and Discussion

8.1. HPLC Assay

An HPLC assay was developed in the laboratory to simultaneously measure the concentrations of DEET and oxybenzone in all test samples. The detection wavelength was set at 254nm for DEET and 287nm for oxybenzone respectively. The mobile phase was composed of acetonitrile, methanol, and water (pH 3.0, adjusted with glacial acetic acid) (65:20:15). When the flow rate was set at 1ml/min, the retention time for DEET was 1.49 minutes and the retention time for oxybenzone was 1.98 minutes (Figure 8.1). No other ingredients or excipients from the commercial preparations interfered with the measurement of DEET and oxybenzone. The detection limit of the assay was approximately 20ng for DEET and 5ng for oxybenzone. The calibration linearity ranged between 50-2000ng for DEET and 8-500ng for oxybenzone respectively (Figure 8.2 and Figure 8.3). Concentrations of DEET and oxybenzone in samples were calculated based on their respective calibration curves.

This HPLC assay for the simultaneous analysis of DEET and oxybenzone was fast, specific, selective and reproducible. It did not require any special chemical reagents, pretreatment or extraction procedures for the test samples. Samples were either directly injected to the HPLC or subject to simple dilution for concentration measurement. DEET and oxybenzone were well separated and the chromatographic run time was 5 minutes, permitting a fast throughput of a large number of test samples. The assay was used for analysis of all samples in this thesis.

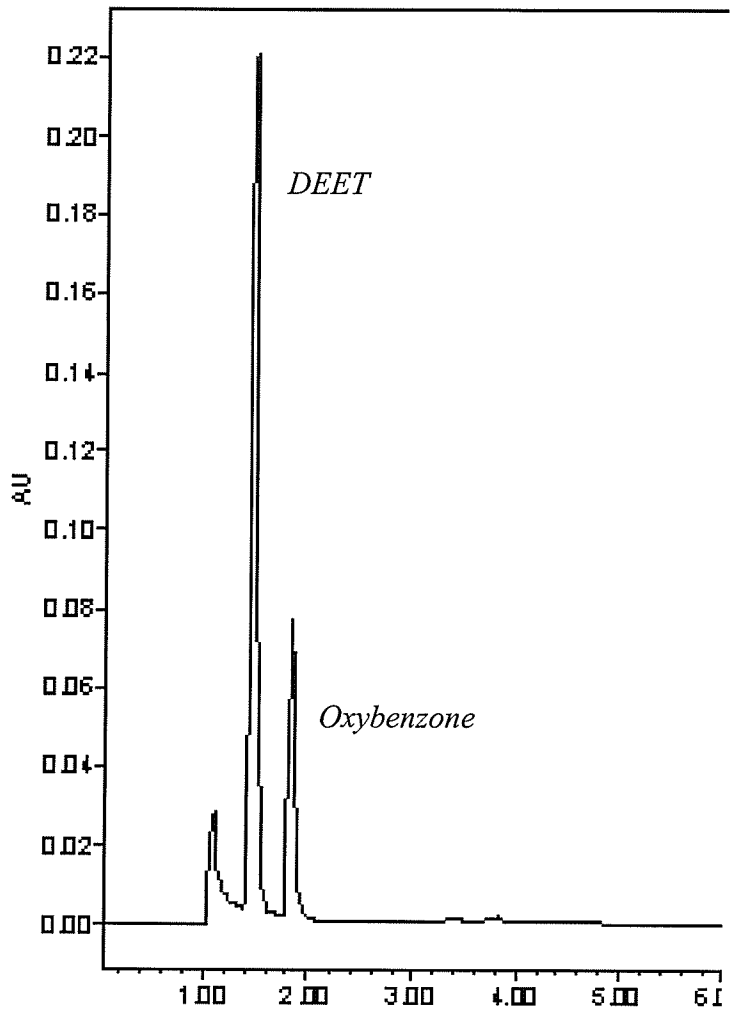
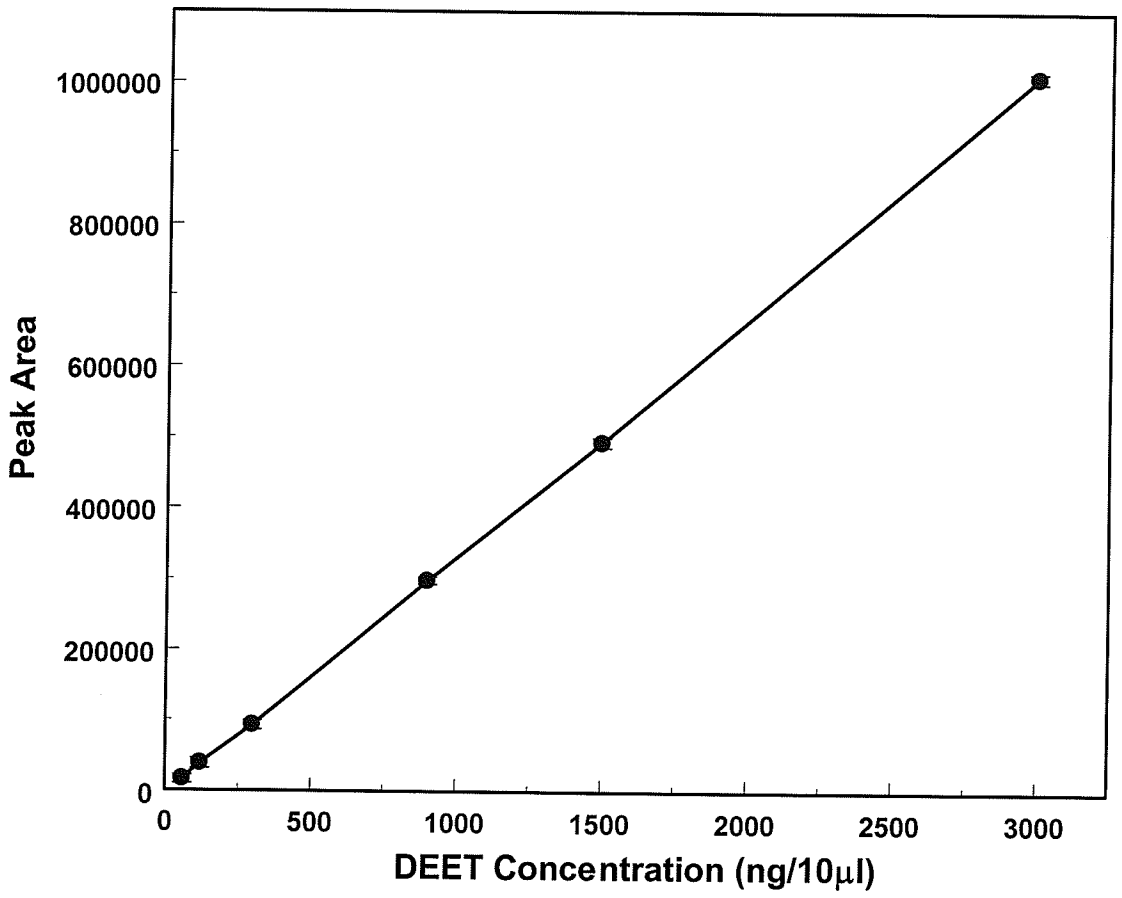
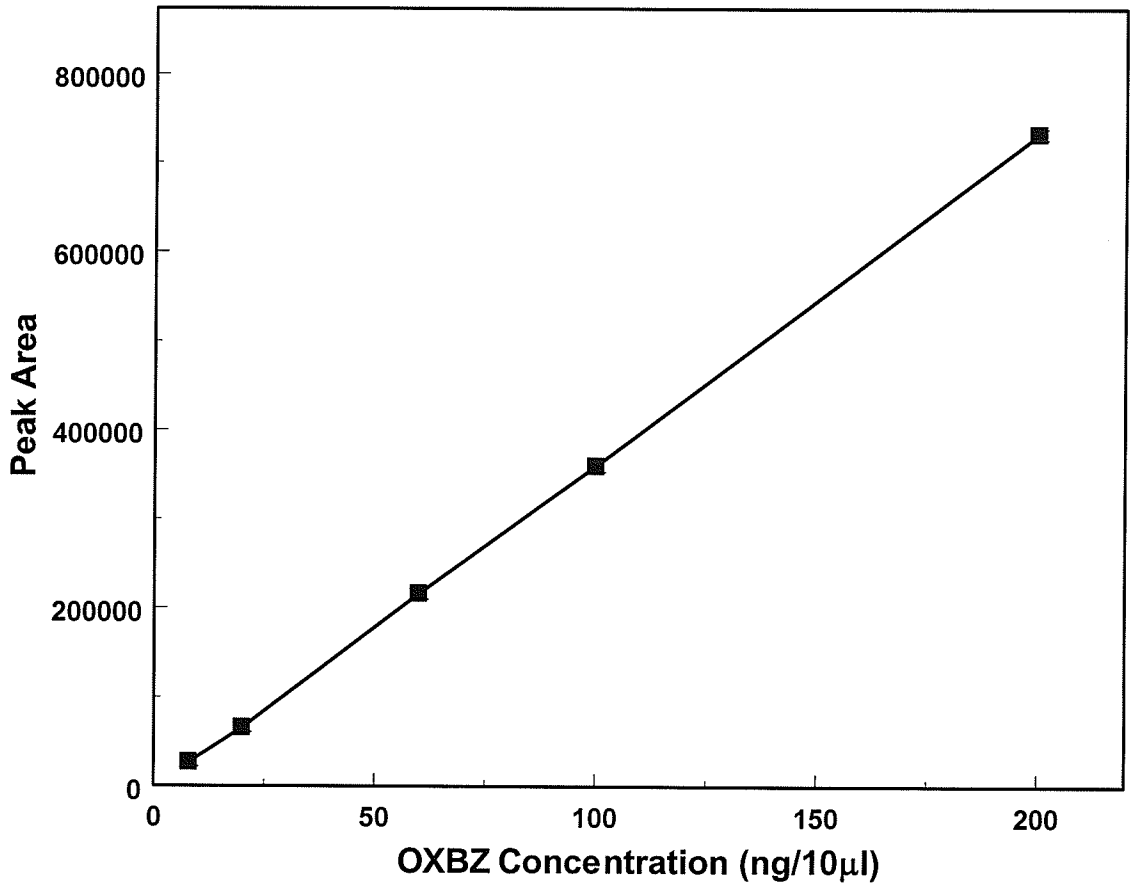


Figure 8.1. Chromatogram of DEET and oxybenzone



Peak Area = 337 × (DEET Amount in ng) − 6090
(r = 0.9999, n = 6)

Figure 8.2. Calibration curve of DEET



Peak Area = 3680 × (Oxybenzone Amount in ng) – 5910
(r = 0.9999, n = 6)

Figure 8.3. Calibration curve of oxybenzone

8.2. Pigskin

Five commercially available products were tested in this thesis, which included two DEET-based insect repellents (one liquid spray and one lotion), one sunscreen lotion, and two combined repellent/sunscreen lotions. Lotion and spray are two most popular formulation types for commercially available repellent and sunscreen products, because they are user-friendly, economic, stable and versatile. Over 90% of repellent and sunscreen products are categorized under these two formulation types. Combined insect repellent/sunscreen products had been discontinued by Health Canada by the end of 2003. However, these products are still commercially available in the US market.

Epidermis from piglets was dermatomed to be used as a biological membrane model in the study. In a living species, the blood vessels are normally located in the dermis and beyond. After a substance penetrates across epidermis, it is absorbed further through the vascular tissues in dermis to reach the systemic circulation. Therefore, the structure of the pigskin is an important factor in deciding the penetration profile of the test compounds. Pig skin has been used extensively in transdermal drug delivery and dermatology because of its physiological and anatomical similarities to human skin. The use of piglet skin in this thesis would provide detailed information on transdermal characterization of the repellent and sunscreen preparation, which could be subsequently correlated to *in vivo* application in humans. The epidermis that was dermatomed was composed of two layers, i.e., stratum corneum that was a layer of differentiated keratinocytes embedded in a matrix of lipid bilayers, and epidermis that consisted of the hydrophilic intercellular spaces, cytoplasm and lipophilic membrane. Compounds with considerable oil and water solubility were able to percutaneously penetrate epidermis. In

addition, some non-medicinal ingredients in the formulations, which have high affinity to lipid components in epidermis, could influence the penetration of the active compounds.

8.2.1. Penetration of DEET

Figure 8.4 shows the overall permeation percentage (OPP) of DEET through piglet epidermis after 6 hours for all test groups. OPP of DEET in No.1 and No.2 were 0.56±0.01% and 4.05±0.18%, respectively. No.2 produced 600% higher penetration through piglet skin than No.1. The OPP of DEET in No.4 was 0%, while that of DEET in No.5 was 2.32±0.10%. No.5 had a 314% increase in DEET permeation compared to No.1. In No.6, the OPP of DEET was 9.70±0.36%, while that of DEET in No.7 was 15.46±0.39%. No.7 increased by 59% compared to No.6 (a significant difference), while No.6 produced 1634% higher permeation than No.1 and No.7 was 282% higher than No.2. In No.8 and No.9, the OPP of DEET were 3.48±0.40% and 2.12±0.08%. No.8 was 64% higher than No.9 (a significant difference), while No.8 and No.9 were 521% and 280% higher in DEET permeation than No.1, respectively. The OPP of DEET in No.10 and No.11 were 6.20±0.06% and 7.23±0.39%, respectively, which were 53% and 79% higher than No.2 (significant differences). The largest increment of DEET was found in the mixture of repellent spray and sunscreen lotion (1:1), which was 1600% higher in DEET penetration than DEET spray control. However, the highest overall permeation percentage of DEET (15.46±0.39%) was produced by the mixture of repellent lotion and sunscreen lotion (1:1). Table 8.1 and Table 8.2 list the overall permeation percentage of DEET and increase percentage respectively.

Steady-state flux is a parameter commonly used to predict the rate of

percutaneous absorption of a substance. The higher the steady-state flux of a compound, the faster the compound is able to penetrate across the membrane, and subsequently the more the compound permeates percutaneously. Table 8.3 lists the steady-state flux (SSF) of DEET through pigskin in 6 hours for all test groups. The SSF in No.1 and No.2 were 12.52±0.35$\mu g/cm^2h$ and 104.83±5.29$\mu g/cm^2h$, respectively. Flux of DEET in No.2 was 737% higher than that in No.1, which also reflected from the overall penetration percentages between the two formulations. There was no flux of DEET in No.4, since no DEET permeated across the skin. The SSF of DEET in No.5 was 57.75±2.90$\mu g/cm^2h$. Flux of DEET in No.5 was 360% higher than that in No.1. In No.6 and No.7, the SSF of DEET were 90.08±3.99$\mu g/cm^2h$ and 199.20±6.59$\mu g/cm^2h$, respectively. Values in No.6 and No.7 were 619% and 90% higher than those in No.1 and No.2, respectively. In No.8 and No.9, the SSF of DEET were 23.78±3.20$\mu g/cm^2h$ and 36.44±1.56$\mu g/cm^2h$, respectively. They were 90% and 190% higher than the SSF in No.1. The SSF of DEET in No.10 and No.11 were 118.70±4.24$\mu g/cm^2h$ and 192.30±14.61$\mu g/cm^2h$, respectively. They also increased by 848% and 1436% respectively compared to the SSF in No.1. These values increased by 13% and 83% respectively compared to No.2. Again, the mixture of repellent lotion and sunscreen lotion (1:1) produced the highest value of the SSF of DEET (199.20±6.59$\mu g/cm^2h$). Table 8.4 lists the results of the statistical comparison.

In summary, except for No.4, in which repellent spray was applied on the top of sunscreen lotion without prior mixing, all other test groups produced significant increase in percutaneous penetration of DEET across pigskin compared to DEET control groups.

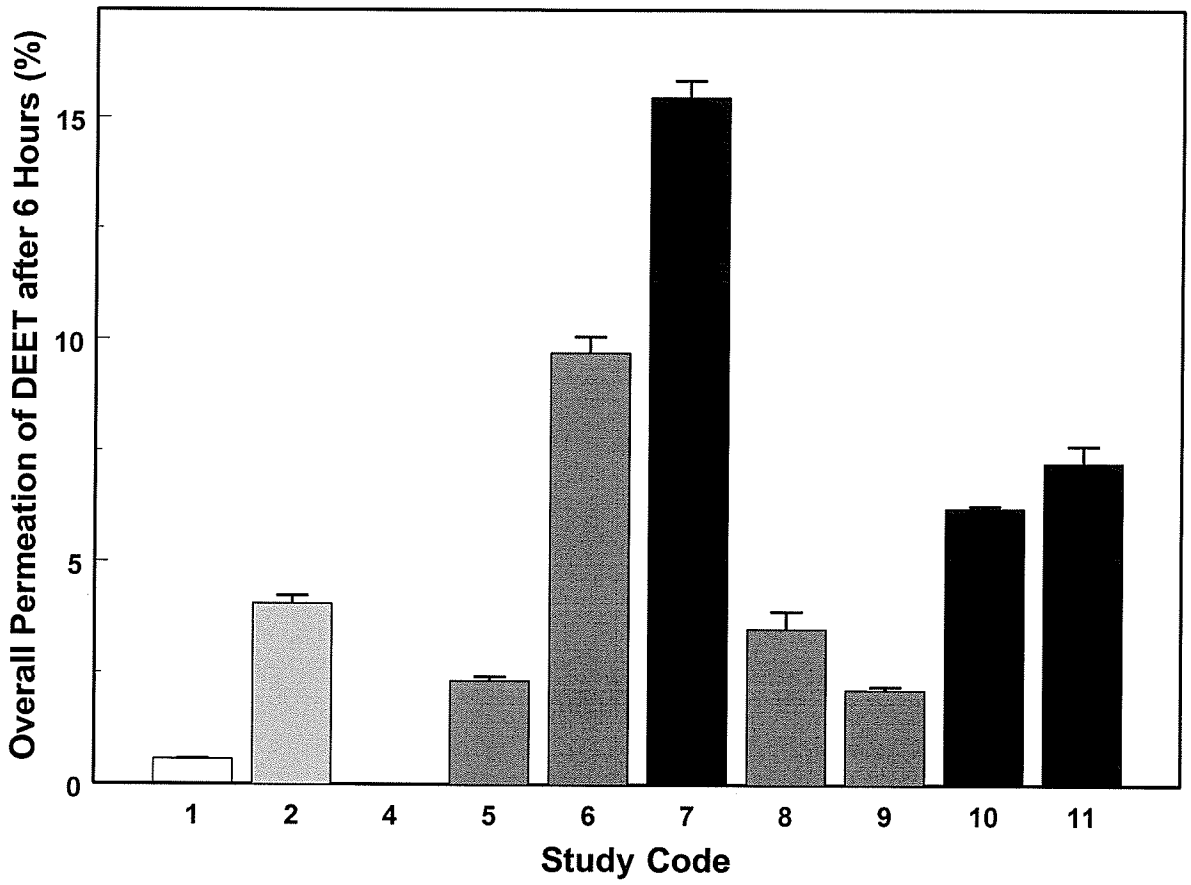


Figure 8.4. Overall permeation percentage of DEET through pigskin after 6 hours

Table 8.1. Overall permeation percentage of DEET through pigskin after 6 hours (%)

No.	Study Code									
	1	**2**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
1	0.57	3.90	0.00	2.34	9.77	15.45	2.56	2.18	6.32	6.16
2	0.56	3.34	0.00	2.31	8.33	14.75	2.91	2.18	6.21	7.56
3	0.60	3.91	0.00	2.08	9.34	15.70	2.71	2.24	5.94	6.44
4	0.57	4.57	0.00	2.06	9.78	14.03	3.59	1.76	6.39	7.96
5	0.53	4.43	0.00	2.69	9.95	16.58	4.05	2.25	6.13	6.67
6	0.51	4.14	0.00	2.45	11.00	16.23	5.09	2.10	6.22	8.57
Mean	0.56	4.05	0.00	2.32	9.70	15.46	3.48	2.12	6.20	7.23
SEM	0.01	0.18	0.00	0.10	0.36	0.39	0.40	0.08	0.06	0.39

Table 8.2. Comparison of percutaneous permeation of DEET through pigskin (%)

DEET	5	6	7	8	9	10	11
1	314*	1634*		521*	280*		
2			282*			53*	79*
6			59*				
9				64*			

** Significantly different ($p \leq 0.05$)*

Table 8.3. Steady-state flux of DEET through pigskin ($\mu g/cm^2 h$)

No.	Study Code									
	1	2	4	5	6	7	8	9	10	11
1	13.08	101.25	0.00	58.67	92.94	199.83	16.92	38.13	132.73	160.61
2	12.51	83.70	0.00	58.45	70.93	190.07	17.03	38.71	108.54	173.24
3	13.78	101.45	0.00	50.51	90.04	200.19	17.89	39.79	104.61	164.49
4	12.62	120.09	0.00	49.55	93.61	173.50	25.22	29.38	120.56	238.25
5	11.72	116.40	0.00	68.89	94.53	218.36	30.08	37.62	124.01	180.15
6	11.41	106.08	0.00	60.44	98.41	213.01	35.53	35.01	121.56	237.04
Mean	12.52	104.83	0.00	57.75	90.08	199.16	23.78	36.44	118.67	192.30
SEM	0.35	5.29	0.00	2.90	3.99	6.59	3.20	1.56	4.24	14.61

Table 8.4. Comparison of steady-state flux of DEET through pigskin (%)

DEET	2	5	6	7	8	9	10	11
1	737*	360*	619*		90*	190*	848*	1436*
2				90*			13	83*

** Significantly different ($p \leq 0.05$)*

8.2.2. Penetration of Oxybenzone

Figure 8.5 shows the OPP of oxybenzone through piglet epidermis after 6 hours for all test groups. The OPP of oxybenzone in No.3, No.4, and No.5 were 0.33±0.01%, 0.61±0.02%, and 0.21±0.01%, respectively. The value in No.4 was 190% higher than that in No.5, indicating a direct effect of oxybenzone on the skin surface. In addition, compared to No.3, the OPP in No.5 was 36% lower while that in No.4 was 85% higher. In No.6 and No.7, the OPP of oxybenzone were 0.93±0.06% and 1.23±0.11% respectively. The value in No.7 was 32% higher than that in No.6 (a significant difference). The OPP in No.6 and No.7 increased by 181% and 272% respectively compared to the OPP in No.3. The OPP of oxybenzone in No.8 and No.9 were 0.40±0.01% and 1.16±0.11%, respectively. The value in No.9 increased by 190% compared to No.8, while No.9 and No.8 were 252% and 21% higher than No.3 (significant differences). The values of OPP of oxybenzone in No.10 and No.11 were 1.04±0.01% and 1.28±0.03%, which were 215% and 288% higher than that in No.3, respectively. The highest OPP of oxybenzone was found in one of the combined repellent/sunscreen lotions (Muskol® lotion), but there was no statistically significant difference between the two combined products. The mixture of repellent lotion and sunscreen lotion also produced higher oxybenzone penetration than the mixtures of repellent spray and sunscreen lotion. Table 8.5 and Table 8.6 list the overall permeation percentage of oxybenzone and increase percentage change respectively.

The SSF of oxybenzone in No.3 was 4.19±0.21$\mu g/cm^2h$ (Table 8.7). The SSF of oxybenzone in No.4 and No.5 were 7.93±0.39$\mu g/cm^2h$ and 2.59±0.18$\mu g/cm^2h$, respectively. The flux in No.5 decreased by 38% compared to that of No.3, but the flux in

No.4 increased by 90% compared to the same test control. The SSF of oxybenzone in No.6 and No.7 were 1.88±0.16$\mu g/cm^2h$ and 3.89±0.23$\mu g/cm^2h$, respectively. The flux in No.6 and No.7 decreased by 55% and 7% compared to that in No.3. In No.8 and No.9, the SSF of oxybenzone were 3.84±0.16$\mu g/cm^2h$ and 5.29±0.25$\mu g/cm^2h$, respectively. No.8 and No.9 respectively decreased by 8% and increased by 26% when compared to No.3. The SSF of oxybenzone in No.10 and No.11 were 14.46±0.25$\mu g/cm^2h$ and 13.81±0.37$\mu g/cm^2h$ respectively, which were 245% and 230% higher than No.3. No.10 and No.11 had higher values in the SSF of oxybenzone than any other mixtures of repellent/sunscreen products. Table 8.8 lists the statistical comparisons of the steady-state flux among all test groups.

In summary, except for No.5, in which sunscreen lotion was applied on the top of DEET spray without prior mixing, all other test groups produced significant percutaneous penetration enhancement of oxybenzone through pigskin compared to the sunscreen lotion control. Overall, the penetration percentages of oxybenzone from these test groups were much lower than those of DEET. The effect of oxybenzone on the transdermal permeation of DEET was therefore higher than the effect of DEET on the transdermal permeation of oxybenzone.

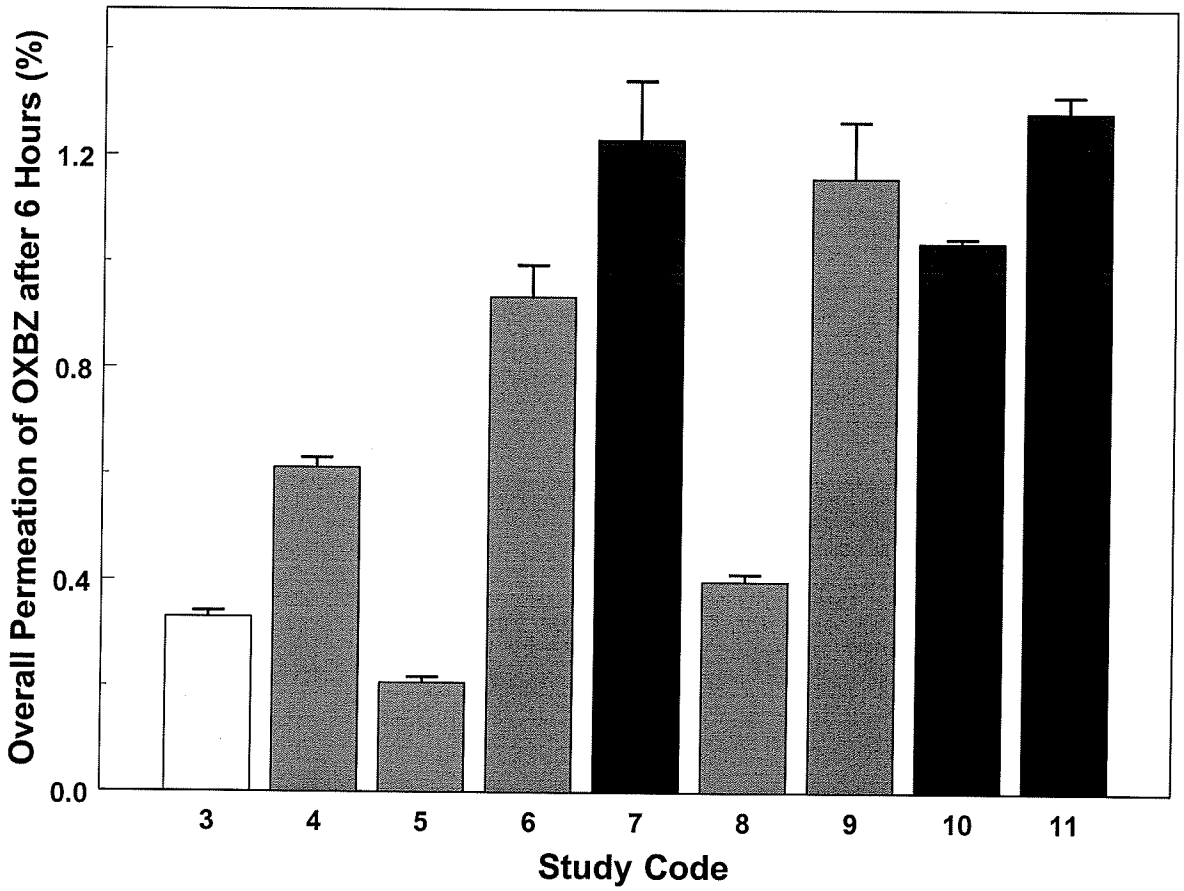


Figure 8.5. Overall permeation percentage of oxybenzone through pigskin after 6 hours

Table 8.5. Overall permeation percentage of oxybenzone through pigskin after 6 hours (%)

No.	Study Code								
	3	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
1	0.29	0.66	0.18	1.09	0.77	0.46	0.96	1.03	1.41
2	0.35	0.55	0.20	0.83	1.16	0.40	0.95	1.05	1.24
3	0.30	0.66	0.24	1.10	1.57	0.39	1.31	1.03	1.30
4	0.36	0.57	0.19	1.00	1.40	0.35	1.03	1.01	1.18
5	0.33	0.63	0.19	0.81	1.15	0.40	1.62	1.07	1.28
6	0.31	0.60	0.24	0.78	1.32	0.39	1.08	1.03	1.28
Mean	0.33	0.61	0.21	0.93	1.23	0.40	1.16	1.04	1.28
SEM	0.01	0.02	0.01	0.06	0.11	0.01	0.11	0.01	0.03

Table 8.6. Comparison of percutaneous permeation of oxybenzone through pigskin (%)

OXBZ	4	5	6	7	8	9	10	11
3	85*	–36	181*	272*	21*	252*	215*	288*
5	190*							
6				32*				
8						190*		

** Significantly different ($p \leq 0.05$)*

Table 8.7. Steady-state flux of oxybenzone through pigskin ($\mu g/cm^2 h$)

No.	Study Code								
	3	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
1	3.56	8.80	1.95	1.77	3.96	4.57	4.87	14.47	14.31
2	4.92	7.43	2.80	2.44	4.23	3.80	4.87	14.90	12.42
3	4.02	9.42	2.61	2.03	2.98	3.90	6.44	13.55	13.87
4	4.70	7.50	2.76	2.08	4.66	3.54	5.46	14.81	13.28
5	4.15	6.90	2.24	1.57	3.62	3.73	4.93	15.09	15.08
6	3.81	7.51	3.17	1.39	3.87	3.52	5.18	13.94	13.88
Mean	4.19	7.93	2.59	1.88	3.89	3.84	5.29	14.46	13.81
SEM	0.21	0.39	0.18	0.16	0.23	0.16	0.25	0.25	0.37

Table 8.8. Comparison of steady-state flux of oxybenzone through pigskin (%)

OXBZ	4	5	6	7	8	9	10	11
3	90*	–38*	–55*	–7	–8	26	245*	230*

** Significantly different ($p \leq 0.05$)*

8.2.3. Discussion

Based on the results of overall permeation percentage and steady-state flux, percutaneous permeation of DEET and oxybenzone across the piglet skin was clearly dependent on the formulation type, application amount and application sequence when insect repellent and sunscreen were applied concurrently.

Between the two test formulations, DEET in OFF® lotion permeated more easily across the piglet skin than that in OFF® spray. DEET in the mixture of OFF® lotion and Coppertone® lotion also permeated more than that in the mixture of OFF® spray and Coppertone® lotion. This may partly be attributed to a weaker affinity of DEET to non-medicinal ingredients in the OFF® lotion (an O/W emulsion). An emulsion is a thermodynamically instable system that consists of two phases. Since DEET is a lipophilic compound, it would be incorporated in the dispersed oil phase. The oil droplets in an O/W emulsion possess larger contact surface with the skin membrane, increasing interaction between DEET and stratum corneum and subsequently allowing more DEET permeation. In addition, emulsifiers in OFF® lotion could also change the structure of stratum corneum in pigskin. Stratum corneum is composed of lipids and keratin. The emulsifiers in the lotion are able to extract lipids, increase the fluidity of lipids, and affect the arrangement of lipids and keratin in stratum corneum, which will result in absorption enhancement of chemicals across the skin. On the other hand, OFF® spray was a homogenous solution. In order to incorporate lipophilic DEET into the preparation at the required concentration (7% DEET), special solvents or co-solvents would have been used to prepare a stable formulation. These solvents could have stronger affinity to DEET molecules, rendering its capability of penetrating across the skin membrane.

Different application sequence resulted in totally different percutaneous profiles of DEET. When OFF® spray was applied on the top of Coppertone® lotion without premixing, transdermal permeation of DEET was negligible. This indicated an effective shielding of sunscreen lotion against DEET diffusion and penetration. On the contrary, percutaneous absorption of DEET was enhanced when Coppertone® lotion was applied on the top of OFF® spray. This was attributed to the role of oxybenzone as a percutaneous absorption enhancer, which also suggested a diffusion capability of oxybenzone from Coppertone® lotion into OFF® spray. Premix of repellent and sunscreen together facilitated profound interactions between DEET and oxybenzone, subsequently resulting in synergistic transdermal penetration of both test substances.

Increasing the proportion of sunscreen in the mixture of repellent and sunscreen enhanced the percutaneous permeation of DEET, which clearly indicated the enhancing effect of oxybenzone to other substances. This property of oxybenzone has been demonstrated in numerous similar studies. In other words, Coppertone® lotion can increase the percutaneous penetration of OFF® products. In our previous study, oxybenzone was able to increase the solubility of DEET in the buffer solution. However, the presence of excipients and additives in commercial Coppertone® lotion could also facilitate the penetration of DEET across the piglet skin. For combined repellent/ sunscreen lotions, the penetration percentages of DEET were higher than individual OFF® lotion, but lower than fresh mixture of OFF® lotion and Coppertone® lotion. The rationale of formulating both repellent and sunscreen ingredients into a single preparation is therefore questionable and unjustifiable because of potential interaction among the active components.

In terms of transdermal permeation of oxybenzone, the overall permeation percentages and steady-state fluxes of oxybenzone in all test groups were lower than their DEET counterparts. However, differences in permeability of oxybenzone from concurrent application of insect repellent and sunscreen were still significantly different from that of Coppertone® control. Specifically, oxybenzone was able to diffuse across OFF® spray to reach the receptor medium, a completely different scenario from when OFF® spray was applied on the top of Coppertone® lotion. This finding suggested an appropriate application sequence for applying repellent and sunscreen; sunscreen should always be applied before insect repellent, in order to minimize overall transdermal penetration of the active ingredients. Permeation percentages of oxybenzone from the two combined repellent/sunscreen products were also higher than Coppertone® control, once again suggesting the disadvantages of the combined formulations. Combined DEET/sunscreen preparations not only decrease the SPF of the active sunscreen components, but also increase the transdermal permeation of both DEET and oxybenzone.

In real-life situation, both insect repellents and sunscreens are commonly applied without well-defined dose regimens at the preference of individual users. The application sequence and proportion are also dictated by personal use habit and availability of products. In addition, environmental conditions such as duration and intensity of sun exposure, temperature and wind, geological location, and number of biting insects/mosquitoes play important roles in the need for using repellents and sunscreens simultaneously. With the arrival of the West Nile virus in North America and its widespread dissemination by mosquitoes, concurrent use of both DEET-based insect repellent and sunscreen products has become inevitable in many regions across the

continent. In the light of enhanced transdermal penetration of DEET and oxybenzone from a concurrent application, more systematical investigations are required to ensure the safety and effectiveness of these over-the-counter consumer care products.

This *in vitro* study did not investigate the clinical implication of concurrent use of insect repellents and sunscreens. Nevertheless, pharmacological and toxicological consequences resulting from transdermal enhancement of DEET and oxybenzone should be further studied. DEET and oxybenzone have been used extensively for decades, but their safety characteristics are only marginally profiled. Guidelines for use of insect repellents and sunscreens are updated continuously on a yearly basis; new information on the safety and effectiveness of these commercially available products is critical and beneficial in assisting healthcare professionals providing criteria for the general public. Dermatologists have widely recommended generous and frequent use of sunscreens for effective sun-blocking efficiency. The use of insect repellents, on the other hand, is always required as whenever necessary. Further studies are therefore warranted to understand the mechanisms of interactions between DEET and oxybenzone, and minimize overall transdermal penetration of these active ingredients *in vivo*.

8.3. Artificial Membranes

Three artificial membranes were used in the thesis to test and compare their feasibility for *in vitro* diffusion studies as membrane models. The use of artificial membranes can not only minimize batch-to-batch variations that are commonly encountered in all biological membranes, but also reduce expense associated with using skin samples from living humans or animals.

8.3.1. LDPE Membrane

LDPE membrane is a plastic membrane that possesses lipophilic characteristics. It has been used as one of the artificial membrane models for *in vitro* diffusion studies by numerous references [55,62,63]. The thickness of the LDPE membrane used in this thesis was 50μm.

8.3.1.1. Penetration of DEET

The overall permeation percentages (OPP) of DEET in No.1 and No.2 were 0.38±0.03% and 2.05±0.13%, respectively. Permeation of DEET from control repellent lotion increased by 439% compared to control repellent spray. The OPP of DEET in No.4 and No.5 were 0% and 0.48±0.01%. No.5 slightly increased the transmembrane permeation of DEET by 26% compared to No.1. The OPP of DEET in No.6 and No.7 were 1.52±0.01% and 3.25±0.16%, respectively. Compared to No.6, DEET permeation in No.7 increased by 114%. While DEET penetration in No.6 was 300% higher than No.1, DEET penetration in No.7 was 59% higher than No.2. Both mixtures of repellent and sunscreen significantly increased DEET permeation when sunscreen was introduced. The

OPP of DEET in No.8 and No.9 were 2.36±0.13% and 1.24±0.07%, respectively. No.8 was 90% higher in DEET permeation than No.9. No.8 and No.9 were 521% and 226% higher than No.1. In No.10, the OPP value of DEET was 1.94±0.15%, which was 410% higher than No.1, but 5% lower than No.2. Figure 8.6 shows the penetration percentages of DEET from all study groups. Table 8.9 and Table 8.10 list the data of DEET penetration across the LDPE membrane.

The steady-state flux (SSF) of DEET (Table 8.11 and Table 8.12) in No.1 and No.2 were 3.55±0.52$\mu g/cm^2h$ and 42.65±2.68$\mu g/cm^2h$. There was no flux of DEET in No.4 in 6 hours. The SSF of DEET in No.5 was 13.24±0.15$\mu g/cm^2h$. The values of SSF in No.5 and No.2 were 273% and 1100% higher than that in No.1. The SSF of DEET in No.6 and No.7 were 5.58±0.16$\mu g/cm^2h$ and 23.69±1.73$\mu g/cm^2h$. No.7 was 325% higher than No.6. At the same time, the flux in No.6 increased by 57% compared to No.1, but the flux in No.7 decreased by 44% compared to No.2. The SSF of DEET in No.8 and No.9 were 6.34±1.01$\mu g/cm^2h$ and 22.77±1.23$\mu g/cm^2h$, respectively. The SSF of DEET in No.8 and No.9 respectively increased by 78% and 542% compared to No.1. The SSF of DEET in No.10 was 36.93±3.30$\mu g/cm^2h$, which was 939% higher than No.1 but 13% lower than No.2.

In summary, same penetration patterns of DEET were found in LDPE membrane, even though the permeation percentages of DEET in this membrane model were much lower than those in the piglet skin. The repellent lotion produced higher transmembrane permeation than the repellent spray. The mixtures of repellent and sunscreen also produced higher transmembrane permeation of DEET than their control counterparts. The combined repellent/sunscreen lotion produced higher DEET penetration as well.

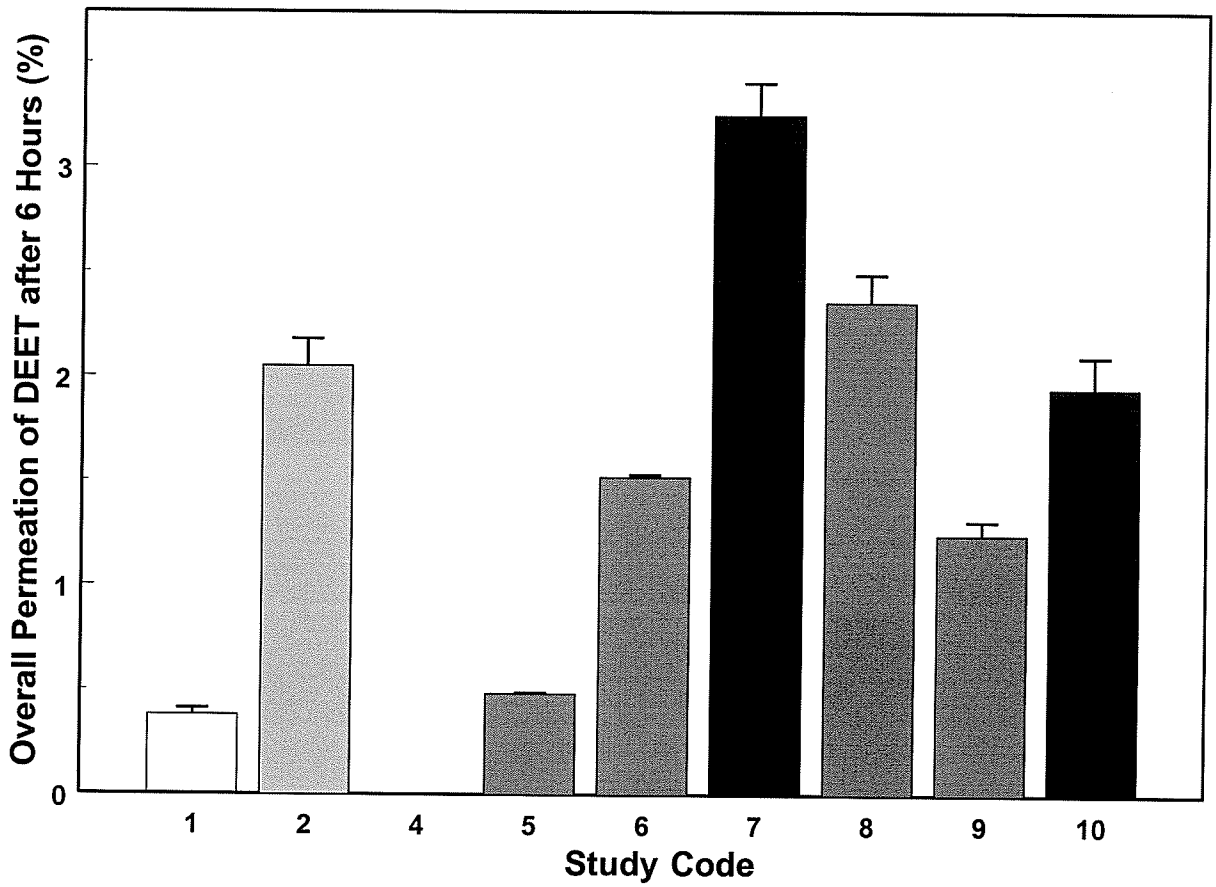


Figure 8.6. Overall permeation percentage of DEET through LDPE membrane after 6 hours

Table 8.9. Overall permeation percentage of DEET through LDPE membrane after 6 hours (%)

No.	Study Code								
	1	**2**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1	0.50	2.51	0.00	0.49	1.49	3.84	2.83	1.21	2.55
2	0.34	1.62	0.00	0.50	1.57	2.89	2.23	1.03	1.54
3	0.32	2.01	0.00	0.46	1.50	2.90	1.92	1.48	1.67
4	0.38	2.05	0.00	0.49	1.51	3.22	2.35	1.24	1.92
5	0.35	1.83	0.00	0.47	1.49	3.05	2.19	1.11	2.23
6	0.43	2.27	0.00	0.48	1.55	3.58	2.61	1.37	1.74
Mean	0.38	2.05	0.00	0.48	1.52	3.25	2.36	1.24	1.94
SEM	0.03	0.13	0.00	0.01	0.01	0.16	0.13	0.07	0.15

Table 8.10. Comparison of transmembrane permeation of DEET through LDPE membrane (%)

DEET	2	5	6	7	8	9	10
1	439*	26	300*		521*	226*	410*
2				59*			–5*
6				114*			
9					90*		

** Significantly different ($p \leq 0.05$)*

Table 8.11. Steady-state flux of DEET through LDPE membrane ($\mu g/cm^2 h$)

No.	Study Code								
	1	**2**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1	5.10	51.95	0.00	13.47	4.87	29.89	9.86	22.18	50.11
2	(1.30)	33.35	0.00	13.68	5.92	18.10	5.48	18.95	28.54
3	3.68	42.68	0.00	12.74	5.71	21.49	2.83	27.13	31.95
4	3.42	42.77	0.00	13.36	5.51	23.04	6.49	22.72	35.61
5	3.58	37.96	0.00	12.83	5.57	22.38	5.12	20.46	42.93
6	4.18	47.19	0.00	13.34	5.89	27.26	8.24	25.18	32.47
Mean	3.55	42.65	0.00	13.24	5.58	23.69	6.34	22.77	36.93
SEM	0.52	2.68	0.00	0.15	0.16	1.73	1.01	1.23	3.30

Table 8.12. Comparison of steady-state flux of DEET through LDPE membrane (%)

DEET	2	5	6	7	8	9	10
1	1100*	273*	57		78*	542*	939*
2				–44*			–13
6				325*			

* *Significantly different ($p \leq 0.05$)*

8.3.1.2. Penetration of Oxybenzone

The OPP of oxybenzone of No.3 was 1.04±0.03%. The OPP of oxybenzone of No.4 and No.5 were 1.38±0.06% and 2.61±0.03%. No.5 was 89% higher than No.4, while No.4 and No.5 were 33% and 151% higher than No.3. The OPP of oxybenzone of No.6 and No.7 were 6.34±0.08% and 1.92±0.04%, respectively. No.6 was 232% higher than No.7. Additionally No.6 and No.7 increased by 510% and 84% compared to the control No.3. The OPP of oxybenzone in No.8 and No.9 were 3.91±0.18% and 11.10±0.29%, respectively. The No.9 increased 184% compared to No.8. No.9 and No.8 were 970% and 276% higher than No.3, respectively. The OPP of oxybenzone in No.10 was 2.36±0.03%, which was higher by 127% than No.3. Figure 8.7 and Tables 8.13 and 8.14 show the original data of oxybenzone permeation.

The SSF of oxybenzone in No.3 was 14.23±0.37$\mu g/cm^2h$. The SSF of oxybenzone in No.4 and No.5 were 18.19±0.84$\mu g/cm^2h$ and 34.87±0.44$\mu g/cm^2h$. No.5 and No.4 increased by 145% and 27% compared to No.3 (Tables 8.15 and 8.16). The SSF of oxybenzone in No.6 and No.7 were 42.35±0.50$\mu g/cm^2h$ and 11.75±0.30$\mu g/cm^2h$. No.6 and No.7 respectively increased by 198% and decreased by 17% compared to No.3. The SSF of oxybenzone in No.8 and No.9 were 35.67±1.60$\mu g/cm^2h$ and 51.95±1.49$\mu g/cm^2h$. No.8 and No.9 respectively increased by 150% and 265% while compared with No.3. The SSF in No.10 was 33.48±0.35$\mu g/cm^2h$, which were 135% higher than No.3.

In summary, the penetration percentages of oxybenzone across the LDPE membrane were higher than what was observed in piglet skin. The physical mixture of repellent spray and sunscreen lotion at 1:2 produced the highest permeation of oxybenzone across the LDPE membrane.

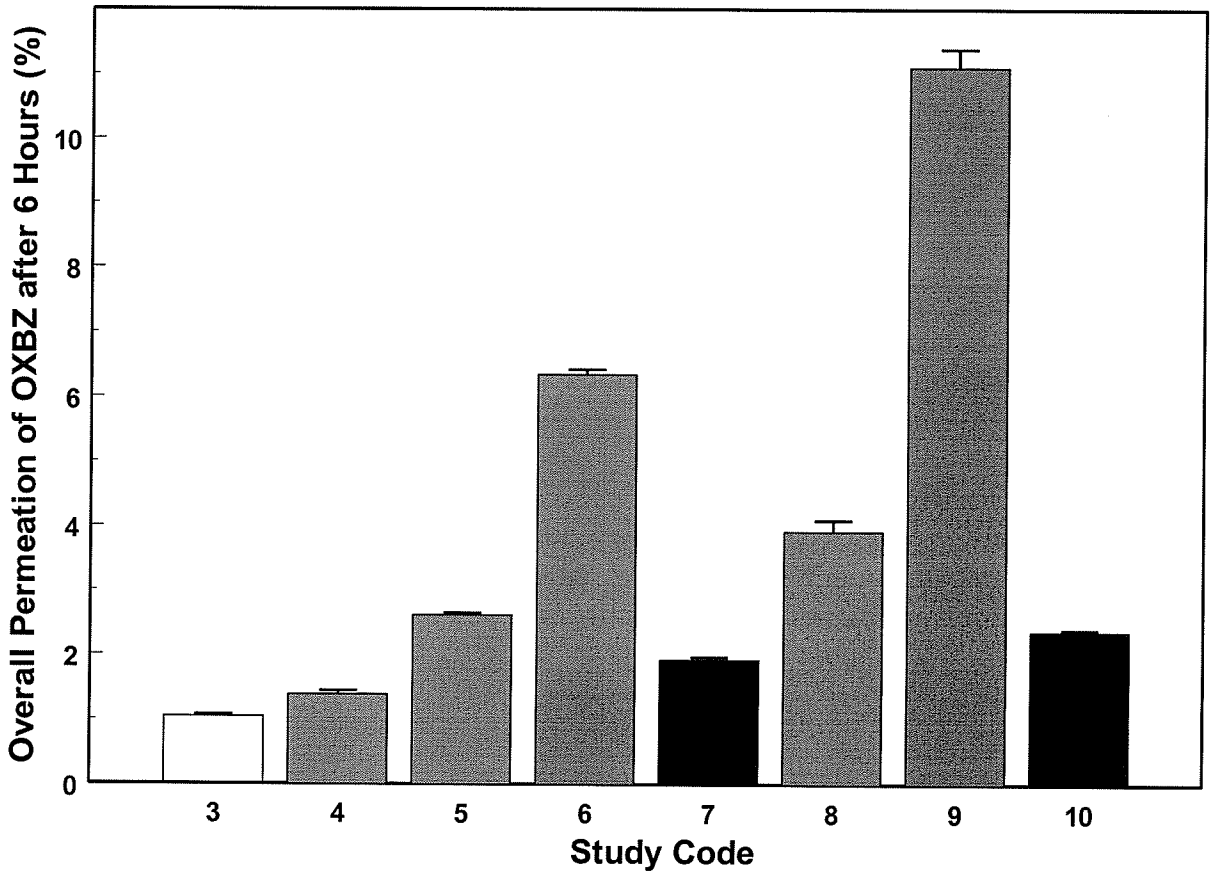


Figure 8.7. Overall permeation percentage of oxybenzone through LDPE membrane after 6 hours

Table 8.13. Overall permeation percentage of oxybenzone through LDPE membrane after 6 hours (%)

No.	Study Code							
	3	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1	1.01	1.52	2.74	6.05	1.78	3.45	10.10	2.26
2	0.92	1.07	2.59	6.49	2.04	4.48	11.01	2.42
3	1.15	1.41	2.53	6.55	2.01	3.45	12.10	2.41
4	1.03	1.38	2.61	6.34	1.87	4.06	11.20	2.38
5	1.01	1.44	2.69	6.42	1.98	4.26	11.70	2.31
6	1.09	1.44	2.57	6.19	1.82	3.75	10.60	2.40
Mean	1.04	1.38	2.61	6.34	1.92	3.91	11.10	2.36
SEM	0.03	0.06	0.03	0.08	0.04	0.18	0.29	0.03

Table 8.14. Comparison of transmembrane permeation of oxybenzone through LDPE membrane (%)

OXBZ	**4**	**5**	**6**	**7**	**8**	**9**	**10**
3	33	151*	510*	84*	276*	967*	127*
4		89*					
7			232*				
8						184*	

**Significantly different ($p \leq 0.05$)*

Table 8.15. Steady-state flux of oxybenzone through LDPE membrane (μg/cm²h)

No.	Study Code							
	3	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1	14.02	19.62	36.59	40.79	11.25	31.53	47.06	32.24
2	12.80	14.05	34.44	42.19	12.39	40.31	50.59	34.35
3	15.51	18.82	33.72	44.52	12.49	30.93	57.11	34.30
4	14.09	18.83	34.75	42.50	11.44	37.81	52.31	33.48
5	14.07	18.81	35.68	42.40	12.25	38.81	54.91	32.73
6	14.88	19.03	34.06	41.71	10.68	34.63	49.74	33.81
Mean	14.23	18.19	34.87	42.35	11.75	35.67	51.95	33.48
SEM	0.37	0.84	0.44	0.50	0.30	1.60	1.49	0.35

Table 8.16. Comparison of steady-state flux of oxybenzone through LDPE membrane (%)

OXBZ	**4**	**5**	**6**	**7**	**8**	**9**	**10**
3	27	145*	198*	–17	150*	265*	135*

** Significantly different ($p \leq 0.05$)*

8.3.2. LFC1 Membrane

LFC1 is a membrane with neutral surface charge and hydrophilic characteristics. Although this type of artificial membrane had been extensively used in the municipal and industrial surface and wastewater applications, its composite structure was regarded as a substitute for biological membranes. The thickness of the membrane used in the experiments was 150μm. The material that faced the donor compartment was polyester, while polyamide was the material that was in direct contact with the receptor medium.

8.3.2.1. Penetration of DEET

The overall permeation percentages (OPP) of DEET in No.1 and No.2 were 1.20±0.15% and 1.26±0.11%. There was no significant difference in DEET permeation between No.1 and No.2. The OPP of DEET in No.4 and No.5 were 0% and 1.10±0.28% respectively. Compared to No.1, permeation of DEET in No.5 decreased by 8% (a statistically significant difference). The OPP of DEET of No.6 and No.7 were 0.49±0.08% and 2.95±0.50%. No. 7 increased by 500% compared to No.6, while No.6 was 59% lower than No.1 and No.7 was 134% higher than No.2. The OPP of DEET in No.8 and No.9 were 0.62±0.09% and 1.09±0.15%. No.9 was 76% higher than No.8. Additionally No.8 and No.9 respectively decreased by 48% and 9% compared to No.1. The OPP of DEET in No.10 was 0.80±0.03%, which was 36% lower than No.2. Figure 8.8 shows the overall penetration percentages of DEET across LCF1 membrane in 6 hours for all test groups. Table 8.17 and Table 8.18 list the original DEET penetration data and the comparison of DEET increases among the test groups respectively.

The steady-state flux (SSF) of DEET in No.1 and No.2 were 21.03±2.48$\mu g/cm^2h$ and 31.68±2.89$\mu g/cm^2h$. There was no flux of DEET in No.4 in 6 hours. The SSF of DEET in No.5 was 25.63±6.69$\mu g/cm^2h$. The values in No.2 and No.5 increased by 50% and 22% compared to No.1. The SSF of DEET in No.6 and No.7 were 4.08±0.89$\mu g/cm^2h$ and 35.36±5.42$\mu g/cm^2h$. No.7 was 767% higher than No.6. At the same time, No.6 decreased by 81% compared to No.1, and No.7 increased by 12% compared to No.2. The SSF of DEET in No.8 and No.9 were 2.04±0.69$\mu g/cm^2h$ and 13.94±2.53$\mu g/cm^2h$. No.8 and No.9 respectively decreased by 90% and 34% compared to No.1. The SSF of DEET in No.10 was 14.58±0.59$\mu g/cm^2h$, which was 54% lower than No.2. Table 8.19 and Table 8.20 list the original steady-state flux values of DEET and the comparison of DEET fluxes among the test groups respectively.

In summary, the highest overall permeation of DEET was found from the physical mixture of repellent lotion and sunscreen lotion at 1:1 ratio. However, there was no significant difference between the control repellent spray and the control repellent lotion in terms of overall DEET permeation. In addition, the extent of permeation percentage of DEET across LFC1 membrane was much lower than what was found with the biological piglet membrane.

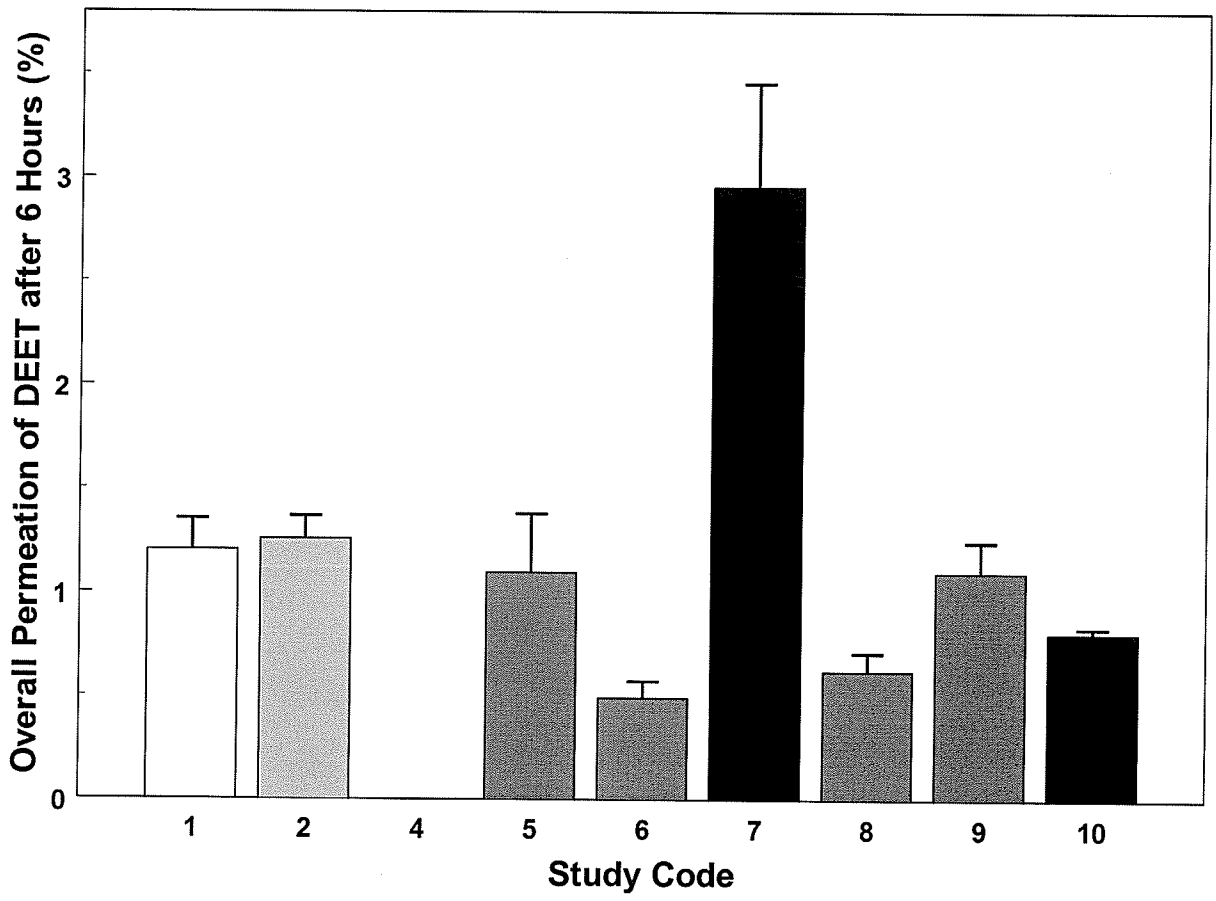


Figure 8.8. Overall permeation percentage of DEET through LFC1 membrane after 6 hours

Table 8.17. Overall permeation percentage of DEET through LFC1 membrane after 6 hours (%)

No.	Study Code								
	1	**2**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1	0.87	1.45	0.00	1.63	0.48	4.72	0.44	1.01	0.81
2	1.23	1.58	0.00	0.39	0.34	3.61	0.67	0.74	0.76
3	1.51	1.39	0.00	0.41	0.28	2.91	0.78	0.96	0.78
4	1.80	1.24	0.00	1.06	0.39	1.30	0.95	1.23	0.92
5	0.88	0.97	0.00	2.14	0.69	3.28	0.47	0.87	0.73
6	0.95	0.97	0.00	0.94	0.77	1.91	0.43	1.75	0.81
Mean	1.20	1.26	0.00	1.10	0.49	2.95	0.62	1.09	0.80
SEM	0.15	0.11	0.00	0.28	0.08	0.50	0.09	0.15	0.03

Table 8.18. Comparison of transmembrane permeation of DEET through LFC1 membrane (%)

DEET	2	5	6	7	8	9	10
1	5	-8^{*}	-59^{*}		-48^{*}	-9^{*}	-33^{*}
2				134			-36^{*}
6				500^{*}			
8						76	

** Significantly different ($p \leq 0.05$)*

Table 8.19. Steady-state flux of DEET through LFC1 membrane (μg/cm²h)

No.	Study Code								
	1	**2**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1	15.63	36.73	0.00	37.00	4.26	48.51	1.29	12.94	13.73
2	21.97	40.29	0.00	8.64	2.16	47.38	1.71	8.59	13.10
3	25.15	34.24	0.00	9.96	2.15	35.36	1.77	12.35	14.74
4	30.66	32.24	0.00	25.62	2.55	15.52	5.43	15.49	16.96
5	15.46	24.39	0.00	51.31	6.16	41.47	1.30	8.87	15.39
6	17.32	22.17	0.00	21.22	7.19	23.90	0.74	25.41	13.55
Mean	21.03	31.68	0.00	25.63	4.08	35.36	2.04	13.94	14.58
SEM	2.48	2.89	0.00	6.69	0.89	5.42	0.69	2.53	0.59

Table 8.20. Comparison of steady-state flux of DEET through LFC1 membrane (%)

DEET	2	5	6	7	8	9	10
1	50	22	−81*		−90*	−34	−31
2				12*			−54*
6				767*			

** Significantly different ($p \leq 0.05$)*

8.3.2.2. Penetration of Oxybenzone

The OPP of oxybenzone of No.3 was 0.04±0.00% (Figure 8.9, Table 8.21). The OPP of oxybenzone of No.4 and No.5 were both 0.12±0.00%, which were 200% higher than the control No.3 (Table 8.22). The OPP of oxybenzone of No.6 and No.7 were 0.77±0.07% and 0.55±0.06%. No.6 was 40% higher than No.7. In addition, No.6 and No.7 increased by 1825% and 1275% respectively compared to No.3. The OPP of oxybenzone in No.8 and No.9 were 0.42±0.06% and 2.46±0.16% respectively. No.9 increased by 486% compared to No.8. And No.8 and No.9 were 950% and 6050% higher than No.3. The OPP of oxybenzone in No.10 was 0.47±0.03%, which was 1075% higher than No.3.

The SSF of oxybenzone in No.3 was 0.19±0.05$\mu g/cm^2h$ (Tables 8.23 and 8.24). The SSF of oxybenzone in No.4 and No.5 were 0.20±0.01$\mu g/cm^2h$ and 0.15±0.01$\mu g/cm^2h$. No.5 decreased by 21% compared to No.3 but No.4 increased by 5% compared to No.3. The SSF of oxybenzone in No.6 and No.7 were 5.98±0.56$\mu g/cm^2h$ and 3.12±0.51$\mu g/cm^2h$. At the same time, No.6 and No.7 increased by 3047% and 1542% compared to No.3. The SSF of oxybenzone in No.8 and No.9 were 2.65±0.63$\mu g/cm^2h$ and 10.45±0.88$\mu g/cm^2h$. No.8 and No.9 respectively increased by 1295% and 5400% compared to No.3. No.9 was also 294% higher than No.8. The SSF of oxybenzone in No.10 was 4.34±0.33$\mu g/cm^2h$, which was 2184% higher than No.3.

In summary, the highest permeation of oxybenzone across the LFC1 membrane was found in the mixture of repellent spray and sunscreen lotion at the ratio of 2:1.

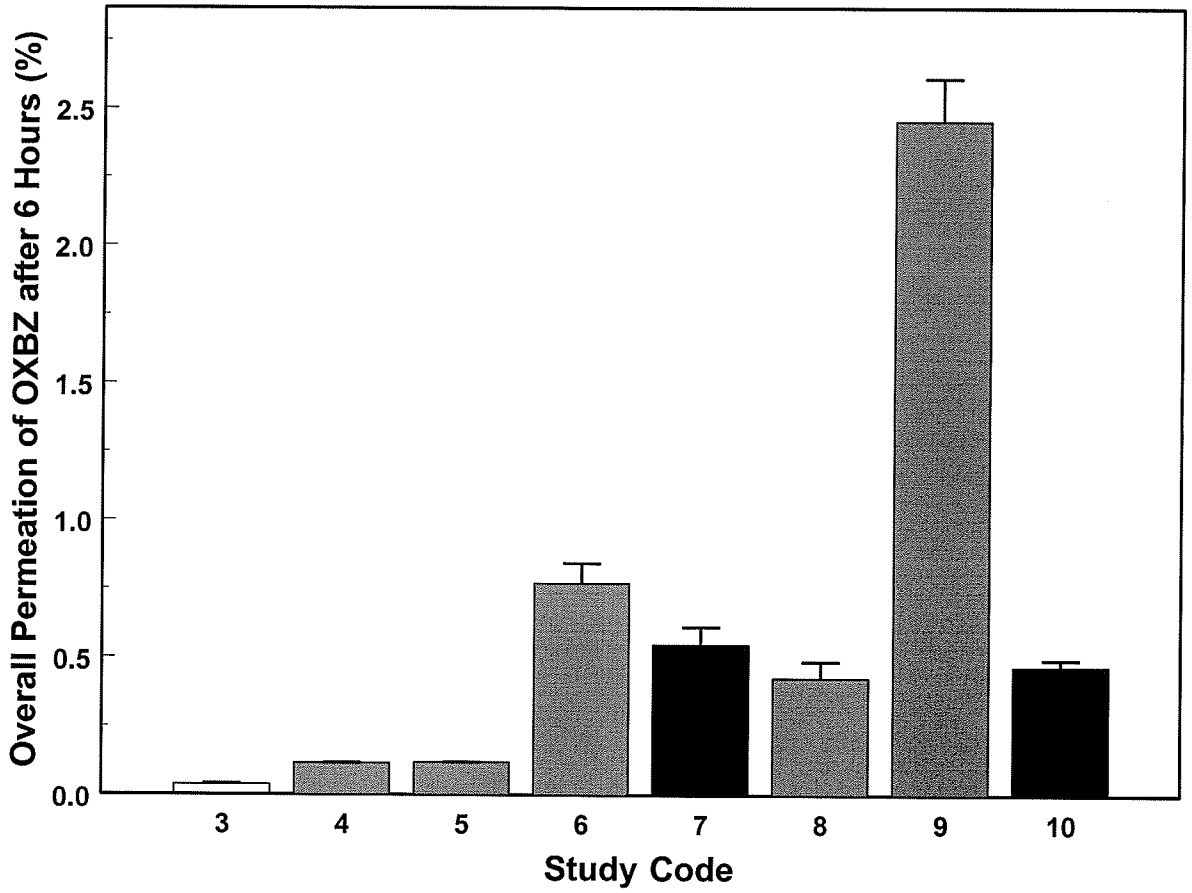


Figure 8.9. Overall permeation percentage of oxybenzone through LFC1 membrane after 6 hours

Table 8.21. Overall permeation percentage of oxybenzone through LFC1 membrane after 6 hours (%)

No.	Study Code							
	3	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1	0.03	0.11	0.12	0.78	0.72	0.28	1.96	0.55
2	0.04	0.12	0.12	0.46	0.66	0.33	2.44	0.46
3	0.03	0.12	0.12	0.74	0.56	0.37	2.27	0.43
4	0.05	0.11	0.12	0.77	0.29	0.69	3.04	0.51
5	0.03	0.12	0.12	0.99	0.62	0.48	2.30	0.37
6	0.05	0.12	0.12	0.88	0.44	0.39	2.74	0.47
Mean	0.04	0.12	0.12	0.77	0.55	0.42	2.46	0.47
SEM	0.00	0.00	0.00	0.07	0.06	0.06	0.16	0.03

Table 8.22. Comparison of transmembrane permeation of oxybenzone through LFC1 membrane (%)

OXBZ	4	5	6	7	8	9	10
3	200*	200*	1825*	1275*	950*	6050*	1075*
7			40				
8						486*	

** Significantly different ($p \leq 0.05$)*

Table 8.23. Steady-state flux of oxybenzone through LFC1 membrane (μg/cm²h)

No.	Study Code							
	3	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1	0.12	0.16	0.12	5.35	4.01	1.16	7.75	5.22
2	0.21	0.22	0.18	3.56	4.53	1.63	10.35	4.06
3	0.07	0.21	0.18	6.15	3.37	2.08	9.71	3.94
4	0.44	0.18	0.17	6.49	1.06	5.47	13.81	5.19
5	0.12	0.20	0.12	6.76	3.39	3.12	9.16	3.10
6	0.15	0.22	0.11	7.53	2.34	2.45	11.93	4.52
Mean	0.19	0.2	0.15	5.98	3.12	2.65	10.45	4.34
SEM	0.05	0.01	0.01	0.56	0.51	0.63	0.88	0.33

Table 8.24. Comparison of steady-state flux of oxybenzone through LFC1 membrane (%)

OXBZ	**4**	**5**	**6**	**7**	**8**	**9**	**10**
3	5	–21	3047*	1542*	1295*	5400*	2184*

** Significantly different ($p \leq 0.05$)*

8.3.3. Mill-F Membrane

The hydrophilic Mill-F membrane is composed of biologically inert materials cellulose acetate and cellulose nitrate. The pore size and the thickness of the membrane used in the experiments were 0.025μm and 130μm, respectively.

8.3.3.1. Penetration of DEET

The overall permeation percentages (OPP) of DEET in No.1 and No.2 were 5.43±0.28% and 14.85±0.32%, respectively. The OPP of DEET in No.4 and No.5 were 0% and 8.96±0.25%, respectively. No.5 produced an increase of 71% DEET permeation compared to No.1, but no statistically significant difference was found between the two groups. The OPP of DEET of No.6 and No.7 were 11.70±1.19% and 9.69±0.07%. No.6 increased by 21% compared to No.7 (no significant difference), while No.6 was 123% higher than No.1 (significantly different) and No.7 was 35% lower than No.2 (significantly different). In No.8 and No.9, the OPP of DEET after 6 hours were 17.20±0.49% and 8.17±0.52% respectively. No.8 was 111% higher than No.9. No.8 and No.9 were respectively 228% and 56% higher than No.1. The OPP of DEET in No.10 was 19.40±0.66%, which was 270% and 31% higher than No.1 and No.2 respectively. Figure 8.10 shows the overall penetration percentages of DEET across Mill-F membrane in 6 hours for all test groups. Table 8.25 and Table 8.26 list the original DEET penetration data and the comparison of DEET increases among the test groups respectively.

The steady-state flux (SSF) of DEET through Mill-F in No.1 and No.2 were 116.34±4.62$\mu g/cm^2h$ and 305.48±6.35$\mu g/cm^2h$, respectively. There was no flux of DEET

in No.4 in 6 hours. The SSF of DEET in No.5 was 175.97±9.80$\mu g/cm^2h$. The fluxes in No.5 and No.2 were 51% and 160% higher than that of No.1, respectively. The SSF of DEET in No.6 and No.7 were 91.35±2.52$\mu g/cm^2h$ and 95.07±0.79$\mu g/cm^2h$, respectively. No.6 and No.7 decreased by 22% and 69% respectively compared to No.1 and No.2. The SSF of DEET in No.8 and No.9 were 115.05±3.45$\mu g/cm^2h$ and 114.05±8.95$\mu g/cm^2h$, respectively. No statistically significant differences were found in the values of SSF among the control No.1, test groups No.8 and No.9. The SSF of DEET in No.10 was 393.54±17.60$\mu g/cm^2h$, which was 29% higher than No.2. Tables 8.27 and 8.28 list the steady-state flux values of DEET and comparisons of various test groups, respectively.

In summary, the highest overall permeation of DEET across the Mill-F membrane in 6 hours was found from the mixture of repellent spray and sunscreen lotion at the mixing ratio of 1:2.

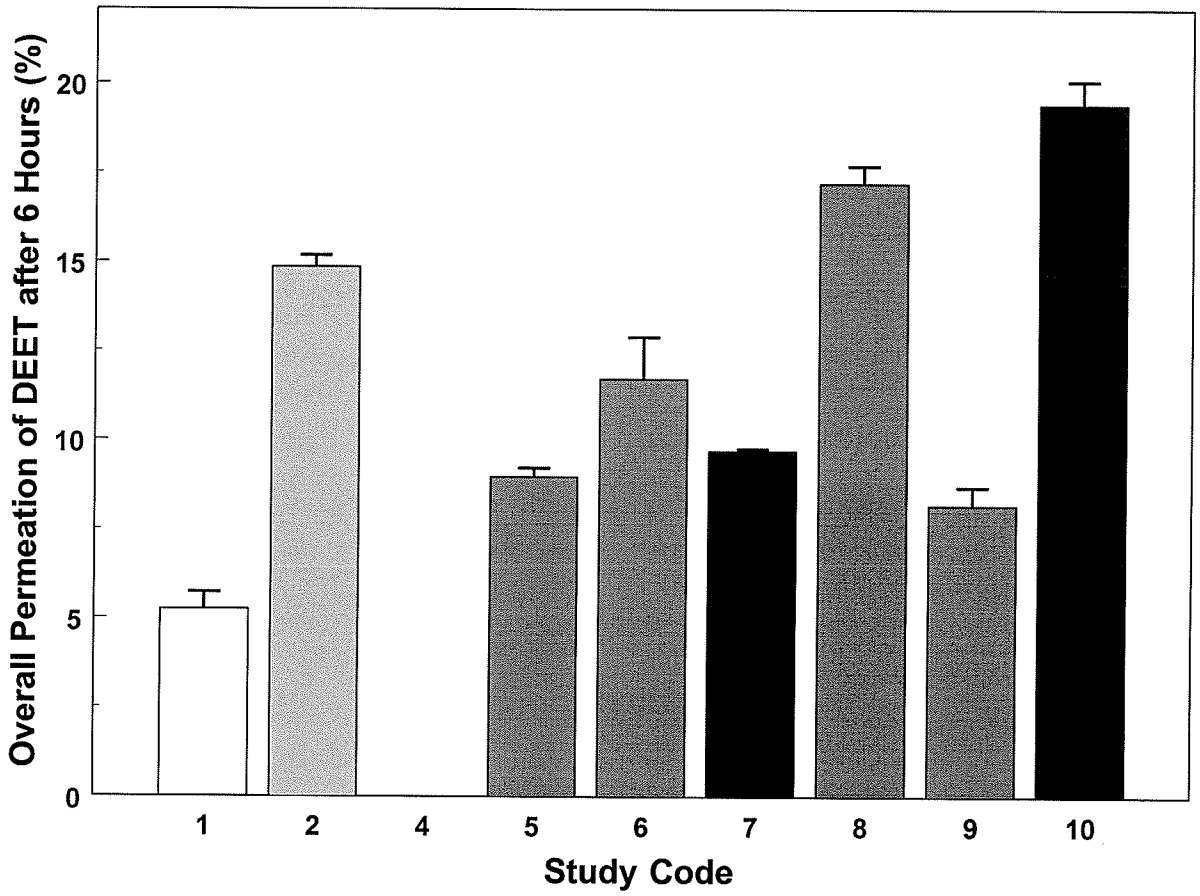


Figure 8.10. Overall permeation percentage of DEET through Mill-F membrane after 6 hours

Table 8.25. Overall permeation percentage of DEET through Mill-F membrane after 6 hours (%)

No.	Study Code								
	1	**2**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1	5.63	13.89	0.00	8.64	8.60	9.57	15.44	9.10	16.56
2	6.70	16.03	0.00	7.55	7.41	9.47	17.28	9.57	19.32
3	5.10	14.60	0.00	10.63	13.95	9.92	18.87	6.16	21.30
4	5.09	14.86	0.00	8.24	13.79	9.81	17.20	8.28	19.16
5	5.36	15.45	0.00	8.94	13.47	9.65	18.00	8.69	19.30
6	4.73	14.39	0.00	9.78	13.00	9.72	16.30	7.24	20.50
Mean	5.25	14.85	0.00	8.96	11.70	9.69	17.20	8.17	19.40
SEM	0.48	0.32	0.00	0.25	1.19	0.07	0.49	0.52	0.66

Table 8.26. Comparison of transmembrane permeation of DEET through Mill-F membrane (%)

DEET	5	6	7	8	9	10
1	71	123*		228*	56*	270*
2			–35*			31*
7		21				
9				111*		

** Significantly different ($p \leq 0.05$)*

Table 8.27. Steady-state flux of DEET through Mill-F membrane (μg/cm^{2}h)

No.	Study Code								
	1	2	4	5	6	7	8	9	10
1	130.27	289.18	0.00	154.25	93.99	92.41	103.81	128.33	316.44
2	98.24	329.94	0.00	154.62	81.01	96.87	113.46	140.16	396.33
3	117.25	293.60	0.00	215.55	94.84	97.15	127.70	79.42	443.41
4	118.11	304.99	0.00	164.58	96.70	94.24	114.99	116.12	389.19
5	124.62	317.51	0.00	174.82	95.08	93.60	120.98	122.00	393.40
6	109.53	297.66	0.00	192.00	86.75	96.12	109.35	98.254	422.46
Mean	116.34	305.48	0.00	175.97	91.35	95.07	115.05	114.05	393.54
SEM	4.62	6.35	0.00	9.80	2.52	0.79	3.45	8.95	17.60

Table 8.28. Comparison of steady-state flux of DEET through Mill-F membrane (%)

DEET	2	5	6	7	8	9	10
1	160*	51*	–22		–1	–2	238*
2				–69*			29*

** Significantly different ($p \leq 0.05$)*

8.3.3.2. Penetration of Oxybenzone

The OPP of oxybenzone in No.3 was 0.83±0.01%. The OPP of oxybenzone of No.4 and No.5 were 0.84±0.03% and 1.54±0.12%, respectively. No.5 was 86% and 83% higher than both No.4 and No.3. The OPP of oxybenzone in No.6 and No.7 was 3.26±0.16% and 1.42±0.04%. No.6 was 129% higher than No.7, while both No.6 and No.7 increased by 290% and 71% compared to No.3, respectively. In No.8 and No.9, the OPP of oxybenzone were 1.84±0.04% and 4.91±0.06%, respectively. No.9 increased by 168% compared to No.8. Additionally No.8 and No.9 increased by 120% and 490% compared to No.3, respectively. The OPP of oxybenzone in No.10 was 2.08±0.14%, which was 150% higher than No.3 (Figure 8.11, Tables 8.29 and 8.30).

The steady-state flux (SSF) of oxybenzone in No.3 was 14.78±0.10$\mu g/cm^2h$. The SSF of oxybenzone in No.4 and No.5 were 13.37±0.62$\mu g/cm^2h$ and 29.32±2.26$\mu g/cm^2h$, respectively. No.5 increased by 98% compared to No.3. The SSF of oxybenzone in No.6 and No.7 were 29.86±1.32$\mu g/cm^2h$ and 11.47±0.44$\mu g/cm^2h$, respectively. No.6 increased by 102% compared to No.3, but No.7 was not different from No.3. The SSF of oxybenzone in No.8 and No.9 were 22.36±0.61$\mu g/cm^2h$ and 29.52±0.38$\mu g/cm^2h$, respectively. No.8 and No.9 respectively increased by 51% and 100% compared to No.3. The SSF of oxybenzone in No.10 was 31.61±1.65$\mu g/cm^2h$, which was 114% higher than the control No.3 (Tables 8.31 and 8.32).

In summary, the mixture of repellent spray and sunscreen lotion at a ratio of 2:1 produced the highest transmembrane permeation for oxybenzone.

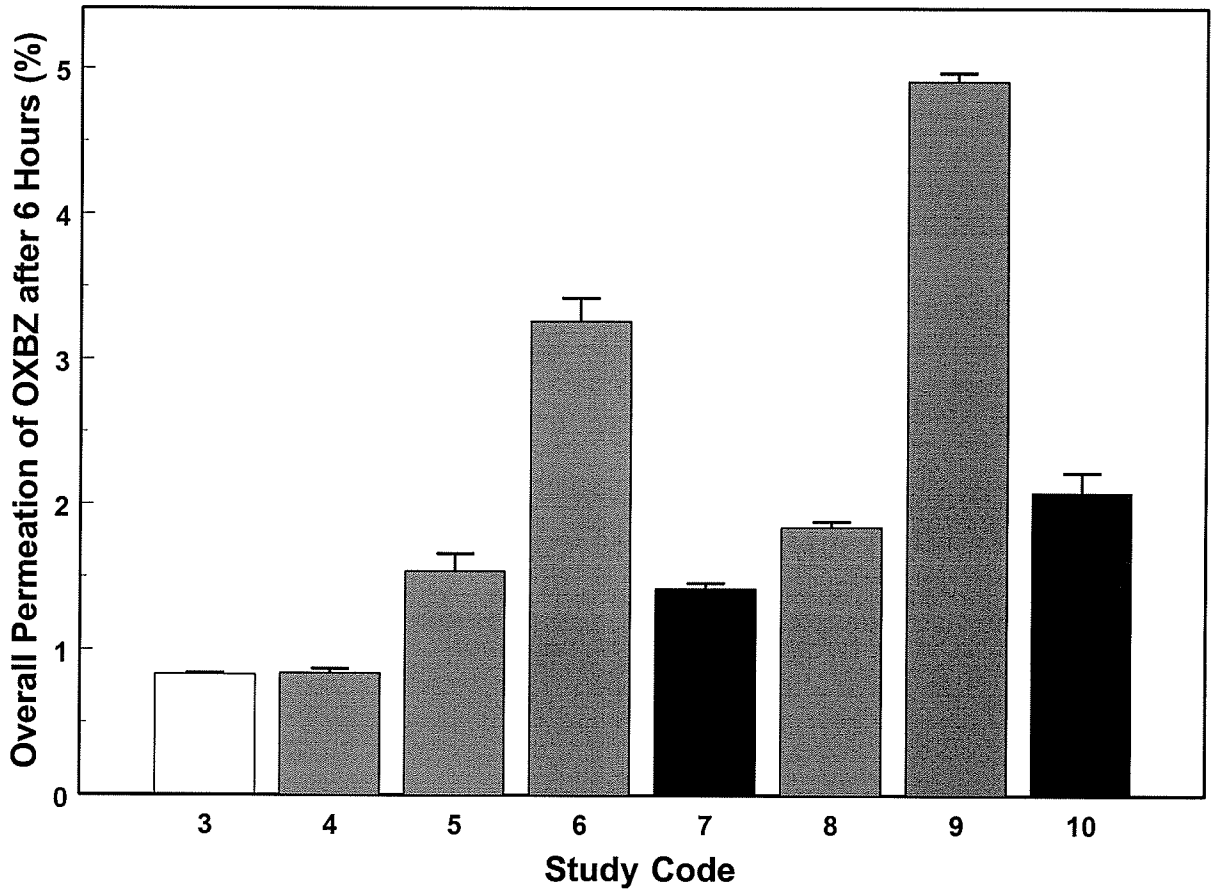


Figure 8.11. Overall permeation percentage of oxybenzone through Mill-F membrane after 6 hours

Table 8.29. Overall permeation percentage of oxybenzone through Mill-F membrane after 6 hours (%)

No.	Study Code							
	3	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1	0.81	0.83	1.23	2.87	1.25	1.76	5.15	1.60
2	0.82	0.93	1.38	3.20	1.46	1.80	4.78	1.95
3	0.85	0.72	1.98	2.78	1.57	2.03	4.78	2.18
4	0.82	0.88	1.53	3.45	1.40	1.76	4.89	2.15
5	0.84	0.75	1.37	3.75	1.47	1.76	4.85	1.94
6	0.81	0.90	1.76	3.50	1.36	1.90	5.02	2.66
Mean	0.83	0.84	1.54	3.26	1.42	1.84	4.91	2.08
SEM	0.01	0.03	0.12	0.16	0.04	0.04	0.06	0.14

Table 8.30. Comparison of transmembrane permeation of oxybenzone through Mill-F membrane (%)

OXBZ	4	5	6	7	8	9	10
3		86*	290*	71*	120*	490*	150*
4		83*					
7			129*				
8						168*	

** Significantly different ($p \leq 0.05$)*

Table 8.31. Steady-state flux of oxybenzone through Mill-F membrane ($\mu g/cm^2 h$)

No.	Study Code							
	3	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1	14.49	14.05	22.92	26.25	9.75	21.51	31.04	25.97
2	14.73	16.13	26.45	29.36	11.97	21.60	28.89	32.66
3	15.09	12.28	38.01	26.07	12.91	25.12	28.44	35.78
4	14.75	15.32	28.99	31.75	11.20	21.42	29.41	35.42
5	15.03	13.03	25.99	34.06	11.94	21.48	29.19	32.32
6	14.57	15.43	33.54	31.64	11.06	23.01	30.19	27.53
Mean	14.78	13.37	29.32	29.86	11.47	22.36	29.52	31.61
SEM	0.10	0.62	2.26	1.32	0.44	0.61	0.38	1.65

Table 8.32. Comparison of steady-state flux of oxybenzone through Mill-F membrane (%)

OXBZ	4	5	6	7	8	9	10
3	–10	98*	102*	–22	51*	100*	114*

** Significantly different ($p \leq 0.05$)*

8.3.4. Discussion

One of the main objectives for testing the artificial membranes was to evaluate their appropriateness in any *in vitro* diffusion study, with the hope of minimizing the wide batch-to-batch variations with biological specimens and reducing study costs and resource demands associated with the collection of skin samples from humans or animals. However, the selection and use of artificial membranes should always be investigated carefully and individually, because data correlation is critical in drug development and delivery. Significant discrepancy between biological and artificial membranes could lead to incorrect experimental results and prediction, which would subsequently potentiate erroneous medical consequences in humans.

Three artificial membranes with variable physical and chemical properties were tested in this thesis for their transmembrane profiles of DEET and oxybenzone. The overall permeation percentages of DEET and oxybenzone across these artificial membrane models were compared with those from the piglet skin. Table 8.33 and Table 8.34 list the comparison results of DEET and oxybenzone for all study groups respectively.

In general, transmembrane permeation of DEET was reduced in LDPE and LCF1 membranes, but increased in Mill-F membrane. For oxybenzone, transmembrane permeation was reduced only in LCF1 membrane, but increased in both LDPE and Mill-F membranes. These permeation profiles may be dictated by physicochemical properties of the test compounds or formulations as well as the characteristics of the membranes such as the thickness, the pore size and the affinity to lipophilicity or hydrophilicity.

Table 8.33. Differences in overall permeation percentage of DEET from three artificial membranes compared to pigskin (%)

No.	Study Code								
	1	**2**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
LDPE	–32	–49	0	–79	–84	–79	–32	–42	–69
LCF1	114	–69	0	–53	–95	–81	–82	–49	–87
Mill-F	838	267	0	286	21	–37	394	285	213

Table 8.34. Differences in overall permeation percentage of oxybenzone from three artificial membranes compared to pigskin (%)

No.	Study Code							
	3	**4**	**5**	**6**	**7**	**8**	**9**	**10**
LDPE	215	126	1148	582	56	877	857	127
LCF1	–88	–80	–43	–17	–55	5	112	–55
Mill-F	151	38	633	251	15	360	323	100

The solubility of DEET and oxybenzone in the test preparations might have played an important role in their transmembrane characterization. DEET is more soluble than oxybenzone [142], which could indicate its higher affinity to formulation additives and lower permeation percentages across the artificial membranes. On the other hand, oxybenzone is more lipophilic than DEET, so its transmembrane permeation across lipophilic LDPE membrane was increased compared to piglet skin. Previous studies in

this laboratory indicated that permeation of DEET and oxybenzone across another lipophilic artificial membrane poly(dimethylsiloxane) (PDMS) in polar solvents such as ethanol and propylene glycol was higher than that in nonpolar solvents such as poly(ethylene glycol) 400 [142]. The results may support the selection and use of artificial membranes with lipophilic properties for *in vitro* diffusion test of lipophilic compounds like oxybenzone and DEET, which is also relevant to biological membranes because most skin components from living animals are lipophilic in nature.

The permeability of DEET and oxybenzone across an artificial membrane might also be dependent on the properties of the membrane. Interactions between the test compounds and membrane material could produce variable permeation results, which could differ significantly from what would be expected from the biological membranes. LDPE membrane possessed lipophilic characteristics in nature. This property had a tendency to exclude hydrogen bonding between diffusant and polyethylene molecules, rendering its ability to permeate across the membrane. The partition coefficients ($\log P_{octanol/water}$) of DEET and oxybenzone are 2.0 and 3.8 respectively, which suggests that oxybenzone possess a higher affinity to lipophilic components than DEET. This property might reflect in the observation of LDPE membrane where permeation of oxybenzone was increased compared to the pigskin while permeation of DEET was reduced compared to the pigskin. For hydrophilic membranes LCF1 and Mill-F, the results may indicate that this type of membranes is more appropriate for hydrophilic compounds if closer correlation is the study objective. Specifically, Mill-F membrane did not reflect the actual permeation characterization of DEET and oxybenzone, because there were significant differences between this membrane and the piglet skin.

The thickness and pore size of a membrane model also dictate the extent and rate of permeation of test materials. Even though all three artificial membranes tested were thinner than the piglet skin (LDPE 50μm, LCF1 150μm, Mill-F 130μm and piglet skin 380μm), their pore size might be variable from the piglet skin, thus producing different permeation percentages of DEET and oxybenzone. In addition, biological membranes tend to expand the pore size when in contact with receptor medium for an extended period of time (skin hydration). Artificial membranes, on the other hand, do not normally deform due to special manufacturing process. All these factors should be taken into consideration when interpreting permeation data from experiments with artificial membrane models. As discussed previously, stratum corneum is the main rate-controlling barrier to transdermal drug absorption. LFC1 membrane was tested for the iontophoretic transdermal delivery of timolol maleate [62] and was found simulating the function of stratum corneum because of its unique three-layer structure. It was found from this study that LCF1 produced the smallest difference compared to the piglet skin, even though overall permeation percentages in this membrane model were suppressed. This property could be used to investigate the permeation profiles of drug delivery across stratum corneum.

Same as biological membrane, the physical properties of the formulation played an important role in permeability of DEET and oxybenzone across the artificial membranes. Specifically, the viscosity of a test sample could influence significantly the outcome of transmembrane permeation. Contrary to the piglet skin from which the highest permeation of DEET and oxybenzone was normally produced by the mixture of repellent and sunscreen lotions, test groups with the presence of repellent spray showed

higher permeation of DEET and oxybenzone than their counterparts of repellent lotion in hydrophilic membranes LCF1 and Mill-F. This may be explained by the fact that repellent spray as a liquid formulation interacts readily with the membrane surface, promoting diffusion process of the active ingredients across the membranes. Lotions on the other hand retarded the diffusion rate across the formulation, producing smaller permeability of DEET and oxybenzone.

In summary, the percutaneous enhancement of DEET and oxybenzone from a concurrent application of commercially available insect repellent and sunscreen products was further observed in three artificial membrane models. There were variations among the membranes in terms of rate and extent of transmembrane permeation, due mainly to physical and chemical characteristics of the membranes and the test molecules. For lipophilic compounds such as DEET and oxybenzone, lipophilic membrane LDPE and hydrophilic membrane LCF1 produced relatively correlated data that were comparable to what observed in piglet skin. The results obtained from Mill-F membrane somehow deviated significantly from those obtained from the biological membrane. For *in vitro* evaluation purposes, artificial membrane models are still considered satisfactory candidates for diffusion experiments, because they are relatively inexpensive, readily commercially available and batch-to-batch reproducible. Results from the artificial membranes should however be compared with those from biological membranes in order to realistically predict transdermal penetration characteristics of percutaneous drug delivery systems. This is of particular importance at later stages of drug research and development.

8.4. Formulations

Numerous studies have proven that different formulation types could influence the transdermal characteristics of the active ingredients from topical application [148-151]. Previous experiments with commercially available insect repellent and sunscreen products also showed similar characterization. This percutaneous enhancement resulted from not only the interactions between DEET and oxybenzone, but also various factors from formulations such as viscosity, proportion of oil/water phases, and lipophilicity/hydrophilicity of the additives. In order to further investigate the influence of the excipients on the penetration of DEET and oxybenzone, and to find appropriate formulation parameters to minimize percutaneous absorption of the two compounds, two emulsion-type formulations with different ratio of oil/water phase were prepared and tested. Both preparations were oil-in-water emulsion-based lotions. Formulation A was composed of 22% oil phase and 78% water phase, while Formulation B was composed of approximately 40% oil phase and 60% water phase. Similar diffusion protocols were used to evaluate the percutaneous penetration of DEET and oxybenzone from these preparations across both biological and artificial membranes. Table 8.35 lists the six prepared products that were tested in this preliminary study.

Table 8.35. Concentrations of DEET/oxybenzone in prepared formulations

Code	**FA1**	**FA2**	**FA3**	**FB1**	**FB2**	**FB3**
Ingredient	OXBZ 5%	DEET 7%	D+O 5/7%	OXBZ 5%	DEET 7%	D+O 5/7%

8.4.1 Pigskin

The OPP of DEET in FA2 and FA3 were 8.72±0.23% and 13.31±0.93% respectively. The OPP of DEET in FB2 and FB3 were 3.80±0.11% and 7.42±0.58% respectively. Compared to the commercially available repellent lotion control (No.2), permeation of DEET from these preparations all increased (Figure 8.12, Tables 8.36 and 8.37). The combined formulations further increased the percutaneous permeation of DEET compared to its single-component counterpart. Table 8.38 and Table 8.39 list the steady-state flux of DEET from these formulations and the comparisons among test groups and control studies respectively.

The OPP of oxybenzone in FA1 and FA3 were 0.43±0.03% and 1.15±0.02% respectively. The OPP of oxybenzone in FB1 and FB3 were 0.19±0.02% and 0.23±0.00% respectively. Compared to the commercially available sunscreen lotion control (No.3), permeation of oxybenzone from A preparations increased, but permeation of oxybenzone from B preparations decreased (Figure 8.13, Tables 8.40 and 8.41). Again, the combined formulations further increased the percutaneous permeation of oxybenzone compared to its single-component counterpart. Table 8.42 and Table 8.43 list the steady-state flux of oxybenzone from these formulations and the comparisons among test groups and control studies respectively.

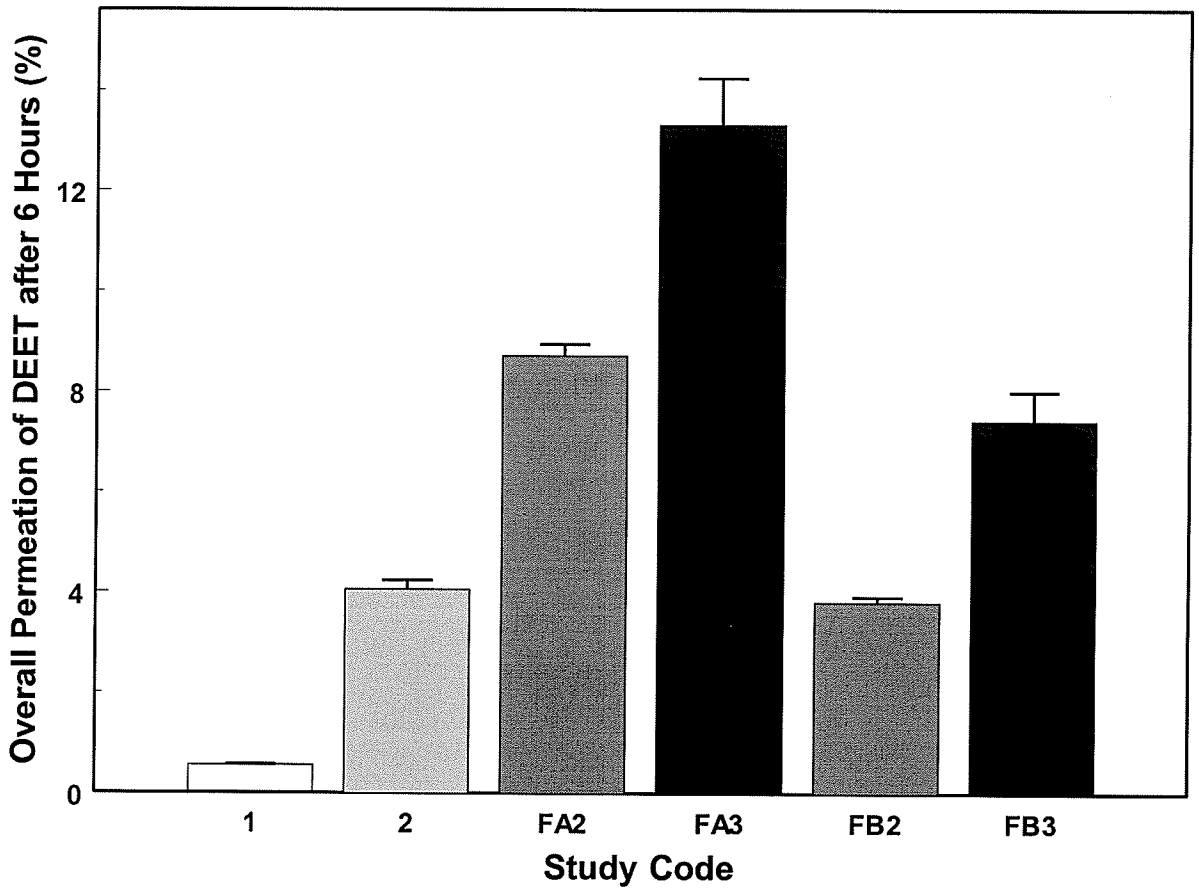


Figure 8.12. Overall permeation percentage of DEET in prepared formulations through pigskin after 6 hours

Table 8.36. Overall permeation percentage of DEET from prepared formulations through pigskin after 6 hours (%)

No.	Study Code					
	No.1	**No.2**	**FA2**	**FA3**	**FB2**	**FB3**
1	0.57	3.90	7.83	11.20	3.16	5.84
2	0.56	3.34	8.76	12.04	3.24	7.34
3	0.60	3.91	8.51	13.28	4.24	7.43
4	0.57	4.57	9.38	12.03	4.55	9.08
Mean	0.56	4.04	8.72	13.31	3.80	7.42
SEM	0.01	0.18	0.23	0.93	0.11	0.58

Table 8.37. Comparison of percutaneous permeation of DEET from prepared formulations through pigskin (%)

DEET	FA2	FA3	FB2	FB3
No.2	115*	229*	–6	84*
FA2		53*	–56*	
FA3				–44*
FB2				95*

** Significantly different ($p \leq 0.05$)*

Table 8.38. Steady-state flux of DEET from prepared formulations through pigskin (μg/cm²h)

No.	Study Code					
	No.1	**No.2**	**FA2**	**FA3**	**FB2**	**FB3**
1	13.09	101.25	74.48	129.04	36.78	72.35
2	12.51	83.70	101.20	128.29	33.15	84.28
3	13.78	101.45	90.74	156.19	49.50	82.95
4	12.62	120.09	105.60	150.27	54.47	100.80
Mean	12.52	104.83	93.00	140.95	43.47	85.11
SEM	0.35	5.29	6.92	7.20	5.07	5.89

Table 8.39. Comparison of steady-state flux of DEET from prepared formulations through pigskin (%)

DEET	FA2	FA3	FB2	FB3
No.2	–11	34*	–59*	–19*
FA2		52*	–53*	
FA3				–40*
FB2				96*

** Significantly different ($p \leq 0.05$)*

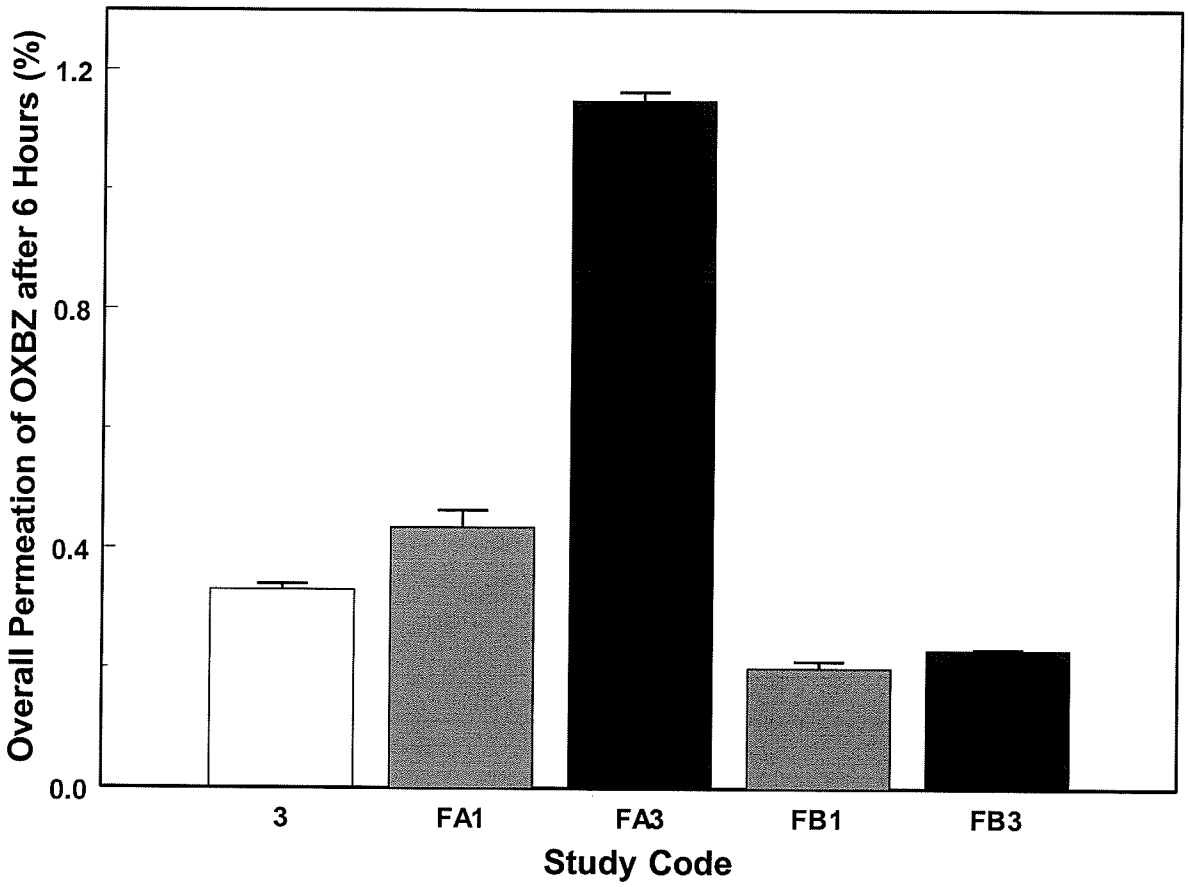


Figure 8.13. Overall permeation percentage of oxybenzone in prepared formulations through pigskin after 6 hours

Table 8.40. Overall permeation percentage of oxybenzone from prepared formulations through pigskin after 6 hours (%)

No.	Study Code				
	No.3	**FA1**	**FA3**	**FB1**	**FB3**
1	0.29	0.50	1.14	0.19	0.23
2	0.35	0.39	1.10	0.23	0.23
3	0.30	0.40	1.17	0.18	0.24
4	0.36	0.36	1.15	0.15	0.24
Mean	0.33	0.43	1.15	0.19	0.23
SEM	0.01	0.03	0.02	0.02	0.00

Table 8.41. Comparison of percutaneous permeation of oxybenzone from prepared formulations through pigskin (%)

DEET	FA1	FA3	FB1	FB3
No.3	30*	248*	–42*	–30*
FA1		167*	–55*	
FA3				–80*
FB1				21

** Significantly different ($p \leq 0.05$)*

Table 8.42. Steady-state flux of oxybenzone from prepared formulations through pigskin (μg/cm²h)

No.	Study Code				
	No.3	**FA1**	**FA3**	**FB1**	**FB3**
1	3.00	4.61	11.15	0.96	2.13
2	3.56	3.16	10.55	1.83	1.99
3	4.92	3.33	11.92	1.39	2.18
4	4.02	3.00	11.72	1.06	1.97
Mean	4.19	3.53	11.33	1.31	2.06
SEM	0.21	0.37	0.31	0.2	0.05

Table 8.43. Comparison of steady-state flux of oxybenzone from prepared formulations through pigskin (%)

OXBZ	FA1	FA3	FB1	FB3
No.3	–16	170*	–69*	–51*
FA1		220*	–63*	
FA3				–82*
FB1				57

* *Significantly different ($p \leq 0.05$)*

8.4.2. LDPE Membrane

The OPP of DEET through LDPE in FA2 and FA3 were 2.49±0.01% and 2.74±0.01%, respectively. The OPP of DEET through LDPE in FB2 and FB3 were 1.17±0.02% and 1.47±0.01%, respectively. Compared to the commercially available repellent lotion control (No.2), permeation of DEET from A preparations all increased (Figure 8.14, Tables 8.44 and 8.45), but the permeation of DEET from B preparations decreased. Moreover, the combined formulations significantly increased the percutaneous permeation of DEET compared to its single-component counterpart. Table 8.46 and Table 8.47 list the steady-state flux of DEET from these formulations and the comparisons among test groups and control studies respectively.

The OPP of oxybenzone through LDPE in FA1 and FA3 were 2.77±0.02% and 4.18±0.02% respectively. The OPP of oxybenzone through LDPE in FB1 and FB3 were 1.86±0.02% and 2.51±0.02% respectively. Compared to the commercially available sunscreen lotion control (No.3), permeation of oxybenzone from A preparations increased, but permeation of oxybenzone from B preparations decreased (Figure 8.15, Tables 8.48 and 8.49). Again, the combined formulations further increased the transmembrane permeation of oxybenzone compared to its single-component counterpart. Table 8.50 and Table 8.51 list the steady-state flux of oxybenzone from these formulations and the comparisons among test groups and control studies respectively.

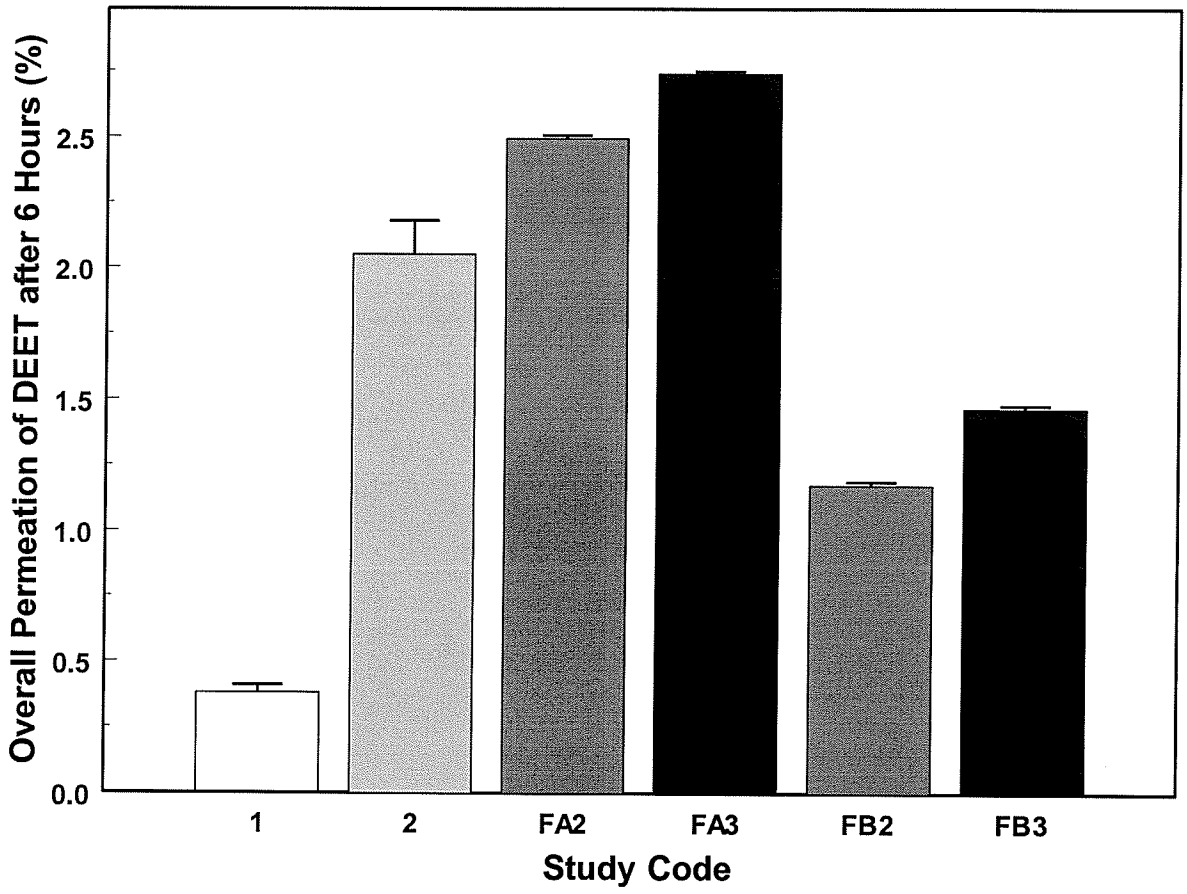


Figure 8.14. Overall permeation percentage of DEET in prepared formulations through LDPE membrane after 6 hours

Table 8.44. Overall permeation percentage of DEET from prepared formulations through LDPE membrane after 6 hours (%)

No.	Study Code					
	No.1	**No.2**	**FA2**	**FA3**	**FB2**	**FB3**
1	0.50	2.51	2.49	2.75	1.17	1.46
2	0.34	1.62	2.47	2.72	1.20	1.49
3	0.32	2.01	2.52	2.75	1.15	1.44
Mean	0.38	2.05	2.49	2.74	1.17	1.47
SEM	0.03	0.13	0.01	0.01	0.02	0.01

Table 8.45. Comparison of transmembrane permeation of DEET from prepared formulations through LDPE membrane (%)

DEET	FA2	FA3	FB2	FB3
No.2	21	33*	–42*	–28*
FA2		10	–53*	
FA3				–46*
FB2				26

** Significantly different ($p \leq 0.05$)*

Table 8.46. Steady-state flux of DEET from prepared formulations through LDPE membrane (μg/cm²h)

No.	Study Code					
	No.1	**No.2**	**FA2**	**FA3**	**FB2**	**FB3**
1	3.41	52.00	42.83	50.19	32.06	40.25
2	3.58	33.34	42.86	49.82	33.12	41.21
3	4.18	42.68	43.00	49.96	31.66	39.83
Mean	3.99	42.65	42.90	49.99	32.28	40.43
SEM	0.31	2.68	0.05	0.11	0.44	0.41

Table 8.47. Comparison of steady-state flux of DEET from prepared formulations through LDPE membrane (%)

DEET	FA2	FA3	FB2	FB3
No.2	1	17	–24	–5
FA2		17	–25*	
FA3				–19*
FB2				25

** Significantly different ($p \leq 0.05$)*

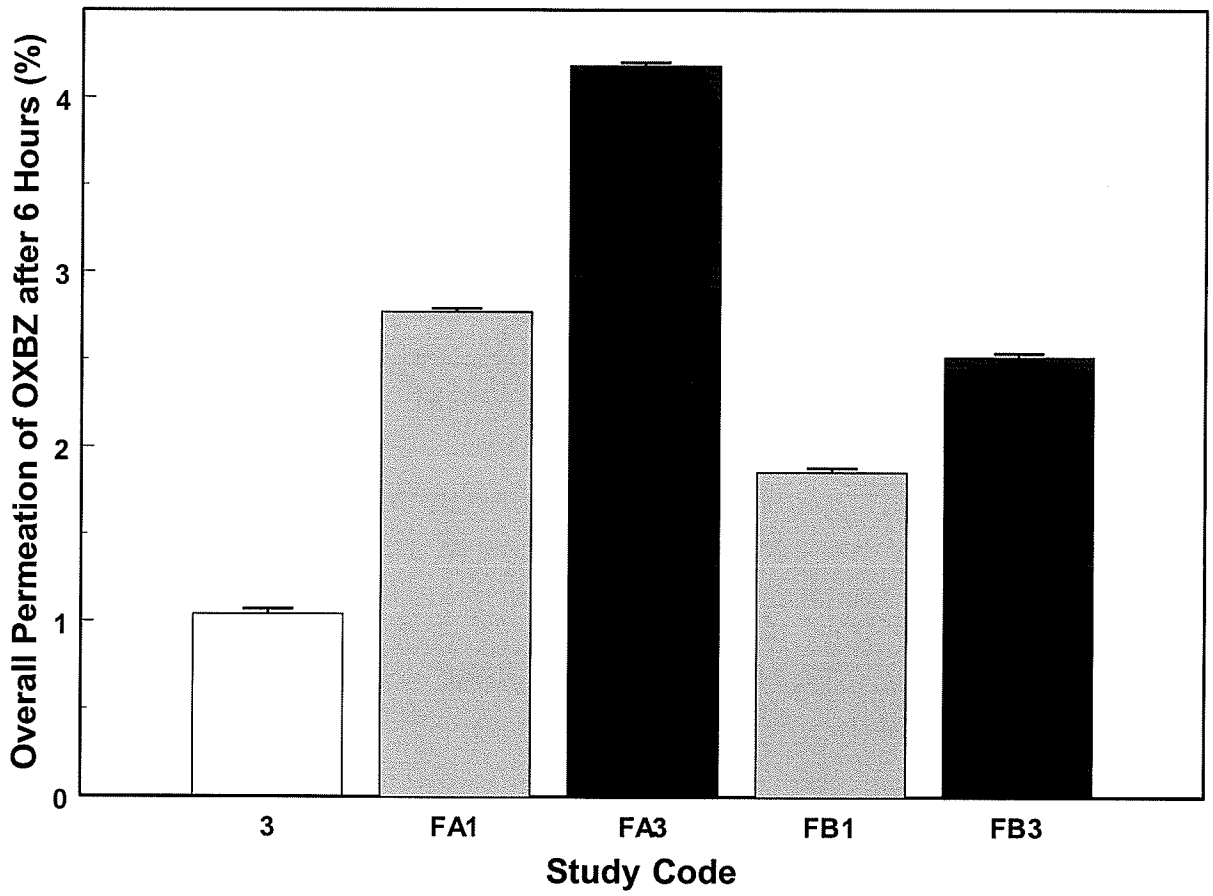


Figure 8.15. Overall permeation percentage of oxybenzone in prepared formulations through LDPE membrane after 6 hours

Table 8.48. Overall permeation percentage of oxybenzone from prepared formulations through LDPE membrane after 6 hours (%)

No.	Study Code				
	No.3	**FA1**	**FA3**	**FB1**	**FB3**
1	1.03	2.76	4.18	1.84	2.51
2	1.01	2.74	4.14	1.91	2.55
3	1.09	2.80	4.21	1.83	2.48
Mean	1.04	2.77	4.18	1.86	2.51
SEM	0.03	0.02	0.02	0.02	0.02

Table 8.49. Comparison of transmembrane permeation of oxybenzone from prepared formulations through LDPE membrane (%)

OXBZ	FA1	FA3	FB1	FB3
No.3	170*	300*	79*	140*
FA1		51*	–33*	
FA3				–40*
FB1				35*

** Significantly different ($p \leq 0.05$)*

Table 8.50. Steady-state flux of oxybenzone from prepared formulations through LDPE membrane (μg/cm²h)

No.	Study Code				
	No.3	**FA1**	**FA3**	**FB1**	**FB3**
1	14.09	33.82	49.64	23.75	33.87
2	14.07	33.60	49.33	24.09	33.97
3	14.88	33.82	49.60	24.01	34.19
Mean	14.30	33.75	49.52	23.95	34.01
SEM	0.37	0.07	0.10	0.11	0.09

Table 8.51. Comparison of steady-state flux of oxybenzone from prepared formulations through LDPE membrane (%)

OXBZ	FA1	FA3	FB1	FB3
No.3	136*	246*	67*	138*
FA1		47*	–29*	
FA3				–31*
FB1				42*

** Significantly different ($p \leq 0.05$)*

8.4.3. LFC1 Membrane

The OPP of DEET through LFC1 in FA2 and FA3 were 2.91±0.02% and 3.46±0.32%, respectively. The OPP of DEET through LFC1 in FB2 and FB3 were 1.69±0.25% and 1.90±0.28%, respectively. Compared to the commercially available repellent lotion control (No.2), permeation of DEET from these preparations all increased (Figure 8.16, Tables 8.52 and 8.53). The combined formulations significantly increased the percutaneous permeation of DEET compared to its single-component counterpart. Table 8.54 and Table 8.55 list the steady-state flux of DEET from these formulations and the comparisons among test groups and control studies respectively.

The transmembrane permeation of oxybenzone from formulation FA1 was negligible, but the OPP of oxybenzone from FA3 was 0.92±0.02%. The OPP of oxybenzone through LFC1 in FB1 and FB3 were 0.07±0.00% and 0.47±0.06%, respectively. Compared to the commercially available sunscreen lotion control (No.3), permeation of oxybenzone increased in FB1, FA3 and FB3 (Figure 8.17, Tables 8.56 and 8.57). The combined formulations further increased the transmembrane permeation of oxybenzone compared to its single-component counterpart. Table 8.58 and Table 8.59 list the steady-state flux of oxybenzone from these formulations and the comparisons among test groups and control studies respectively.

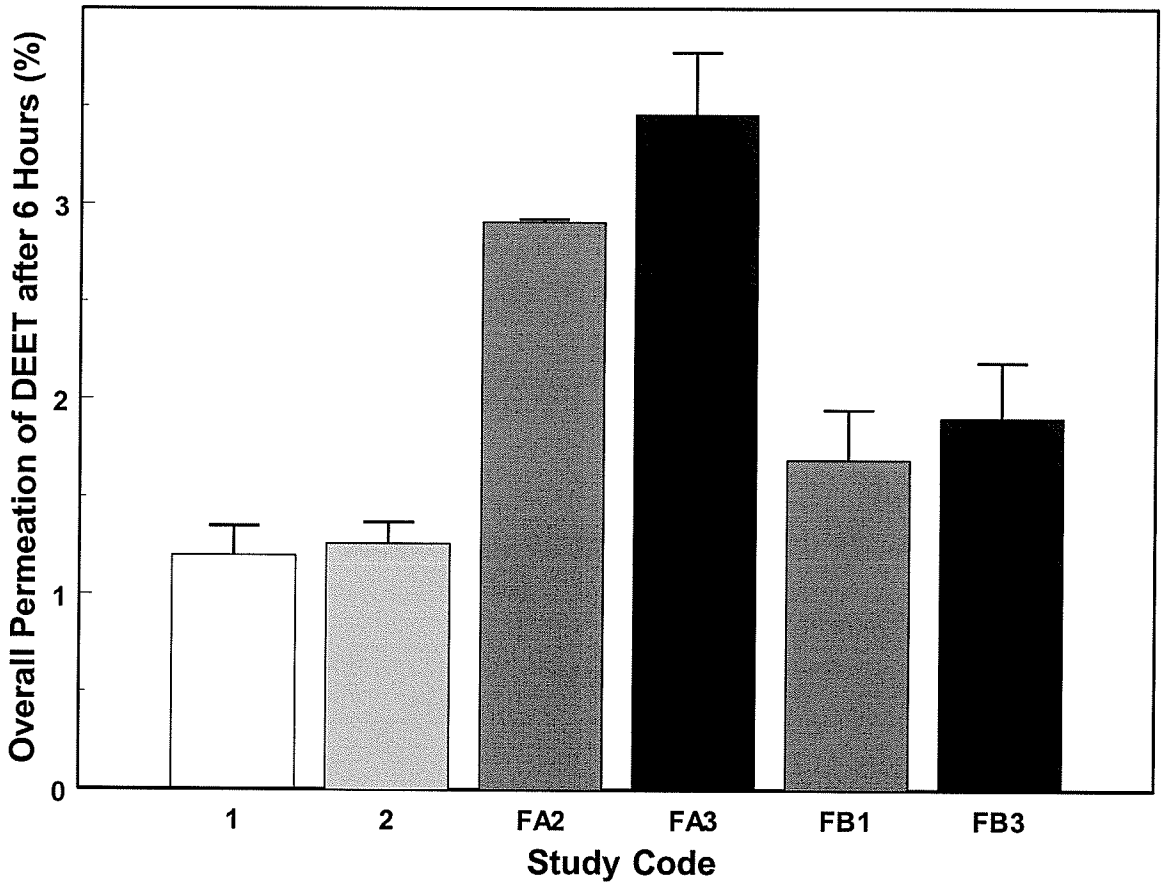


Figure 8.16. Overall permeation percentage of DEET in prepared formulations through LFC1 membrane after 6 hours

Table 8.52. Overall permeation percentage of DEET from prepared formulations through LFC1 membrane after 6 hours (%)

No.	Study Code					
	No.1	**No.2**	**FA2**	**FA3**	**FB2**	**FB3**
1	0.87	1.45	2.88	3.75	2.2	2.47
2	1.23	1.58	2.91	3.80	1.43	1.61
3	1.51	1.39	2.93	2.82	1.44	1.63
Mean	1.20	1.26	2.91	3.46	1.69	1.90
SEM	0.16	0.11	0.02	0.32	0.25	0.28

Table 8.53. Comparison of transmembrane permeation of DEET from prepared formulations through LFC1 membrane (%)

DEET	**FA2**	**FA3**	**FB2**	**FB3**
No.2	130*	175*	34	51
FA2		19	–42*	
FA3				–45*
FB2				12

** Significantly different ($p \leq 0.05$)*

Table 8.54. Steady-state flux of DEET from prepared formulations through LFC1 membrane (μg/cm^{2}h)

No.	Study Code					
	No.1	**No.2**	**FA2**	**FA3**	**FB2**	**FB3**
1	15.63	34.24	47.27	85.82	38.49	43.35
2	21.97	32.24	47.62	86.67	39.32	44.38
3	25.15	24.39	47.60	86.95	39.69	45.07
Mean	21.03	31.69	47.50	86.48	39.17	44.27
SEM	2.48	2.89	0.11	0.34	0.36	0.50

Table 8.55. Comparison of steady-state flux of DEET from prepared formulations through LFC1 membrane (%)

DEET	FA2	FA3	FB2	FB3
No.2	50*	173*	24	40
FA2		82	–17*	
FA3				–49*
FB2				13

** Significantly different ($p \leq 0.05$)*

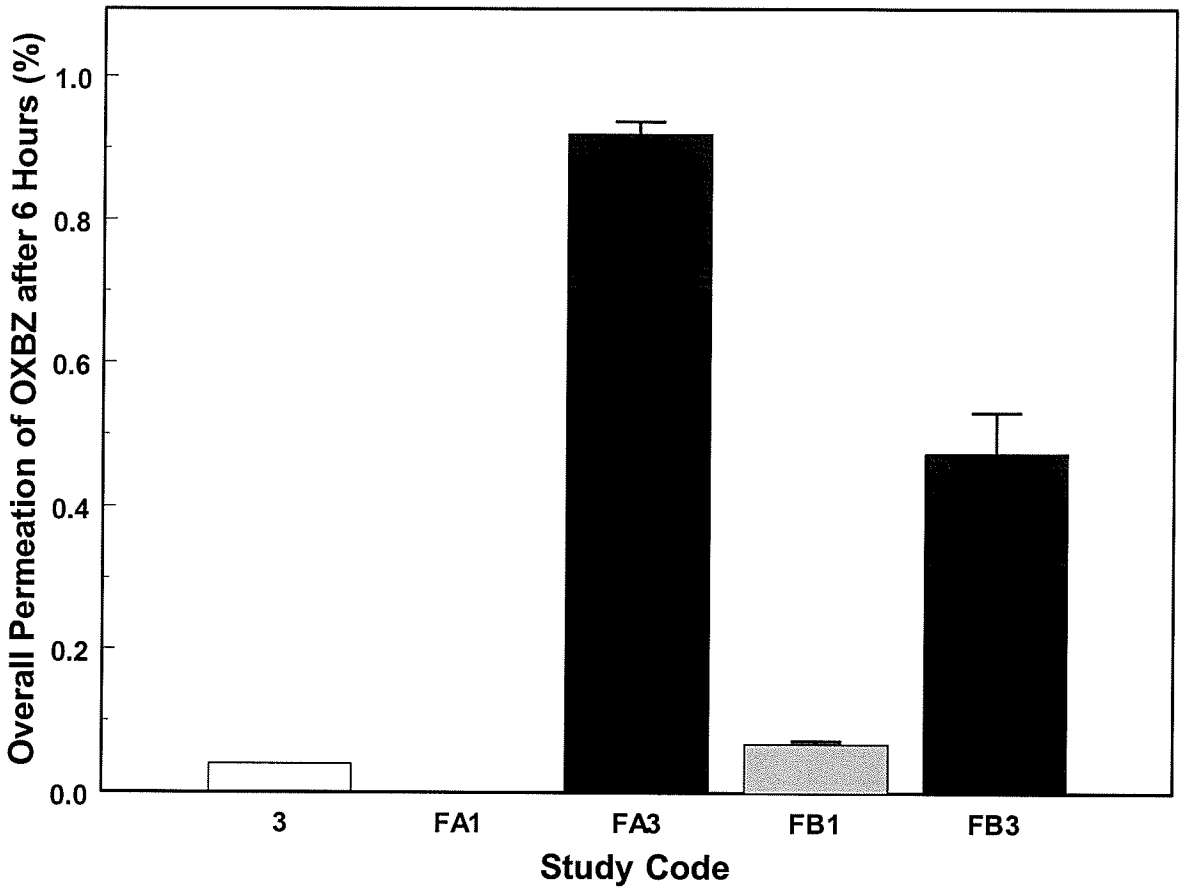


Figure 8.17. Overall permeation percentage of oxybenzone in prepared formulations through LFC1 membrane after 6 hours

Table 8.56. Overall permeation percentage of oxybenzone from prepared formulations through LFC1 membrane after 6 hours (%)

No.	Study Code				
	No.3	**FA1**	**FA3**	**FB1**	**FB3**
1	0.03	0.00	0.89	0.06	0.59
2	0.04	0.00	0.92	0.07	0.40
3	0.03	0.00	0.95	0.08	0.43
Mean	0.04	0.00	0.92	0.07	0.47
SEM	0.00	0.00	0.02	0.00	0.06

Table 8.57. Comparison of transmembrane permeation of oxybenzone from prepared formulations through LFC1 membrane (%)

OXBZ	FA1	FA3	FB1	FB3
No.3		2200*	75	1075*
FA3				–49*
FB1				571*

** Significantly different ($p \leq 0.05$)*

Table 8.58. Steady-state flux of oxybenzone from prepared formulations through LFC1 membrane (μg/cm²h)

No.	Study Code				
	No.3	**FA1**	**FA3**	**FB1**	**FB3**
1	0.12	0.00	14.59	0.38	5.39
2	0.21	0.00	14.86	0.45	5.70
3	0.15	0.00	15.21	0.52	5.85
Mean	0.19	0.00	14.89	0.45	5.65
SEM	0.05	0.00	0.18	0.04	0.14

Table 8.59. Comparison of steady-state flux of oxybenzone from prepared formulations through LFC1 membrane (%)

OXBZ	FA1	FA3	FB1	FB3
No.3		7737*	137*	2874*
FA3				−63*
FB1				1156*

** Significantly different ($p \leq 0.05$)*

8.4.4. Mill-F Membrane

The OPP of DEET through Mill-F in FA2 and FA3 were 27.41±1.31% and 44.52±0.91%, respectively. The OPP of DEET through Mill-F in FB2 and FB3 were 19.60±1.10% and 29.38±1.06%, respectively. Compared to the commercially available repellent lotion control (No.2), permeation of DEET from these preparations all increased (Figure 8.18, Tables 8.60 and 8.61). Moreover, the combined formulations further increased the transmembrane permeation of DEET compared to its single-component counterpart. Table 8.62 and Table 8.63 list the steady-state flux of DEET from these formulations and the comparisons among test groups and control studies respectively.

The OPP of oxybenzone through Mill-F in FA1 and FA3 were 2.28±0.05% and 2.55±0.44%, respectively. The OPP of oxybenzone through Mill-F in FB1 and FB3 were 1.14±0.02% and 1.34±0.02%, respectively. Compared to the commercially available sunscreen lotion control (No.3), permeation of oxybenzone obviously all increased (Figure 8.19, Tables 8.64 and 8.65). Also, the combined formulations further increased the transmembrane permeation of oxybenzone compared to its single-component counterpart. Table 8.66 and Table 8.67 list the steady-state flux of oxybenzone from these formulations and the comparisons among test groups and control studies respectively.

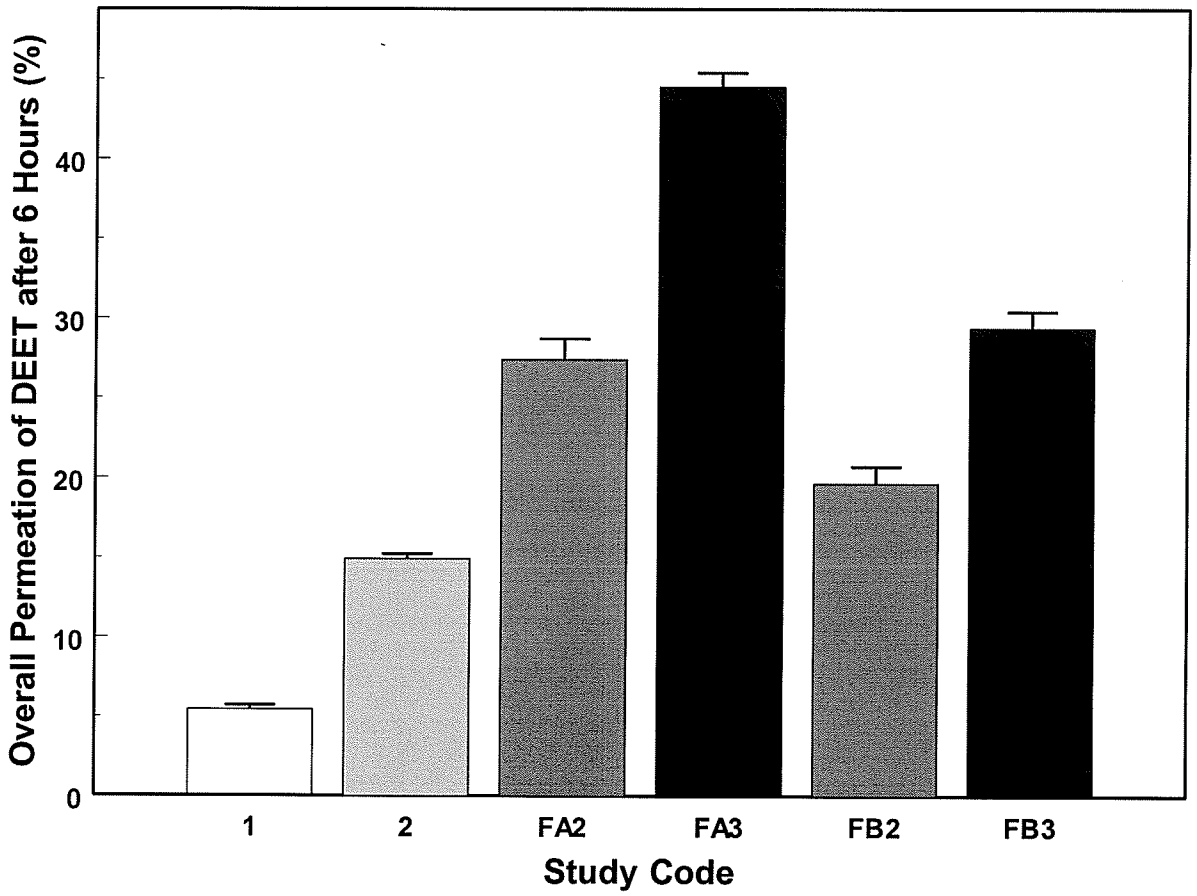


Figure 8.18. Overall permeation percentage of DEET in prepared formulations through Mill-F membrane after 6 hours

Table 8.60. Overall permeation percentage of DEET from prepared formulations through Mill-F membrane after 6 hours (%)

No.	Study Code					
	No.1	**No.2**	**FA2**	**FA3**	**FB2**	**FB3**
1	5.63	14.60	26.46	44.68	18.89	29.45
2	5.36	14.86	29.99	42.86	21.75	31.18
3	4.73	14.39	25.78	46.01	18.14	27.52
Mean	5.43	14.85	27.41	44.52	19.60	29.38
SEM	0.48	0.32	1.31	0.91	1.10	1.06

Table 8.61. Comparison of transmembrane permeation of DEET from prepared formulations through Mill-F membrane (%)

DEET	FA2	FA3	FB2	FB3
No.2	84*	199*	32*	98*
FA2		62*	–28*	
FA3				–34*
FB2				50*

** Significantly different ($p \leq 0.05$)*

Table 8.62. Steady-state flux of DEET from prepared formulations through Mill-F membrane (μg/cm²h)

No.	Study Code					
	No.1	**No.2**	**FA2**	**FA3**	**FB2**	**FB3**
1	130.27	289.18	659.98	714.67	298.60	508.91
2	98.24	329.94	755.62	678.16	371.20	550.66
3	117.25	293.60	642.39	717.95	289.60	485.68
Mean	116.34	305.48	686.00	703.59	319.80	515.09
SED	4.62	6.35	35.18	12.75	25.83	19.01

Table 8.63. Comparison of steady-state flux of DEET from prepared formulations through Mill-F membrane (%)

DEET	FA2	FA3	FB2	FB3
No.2	124*	130*	5	69*
FA2		3	–53*	
FA3				–27*
FB2				61*

** Significantly different ($p \leq 0.05$)*

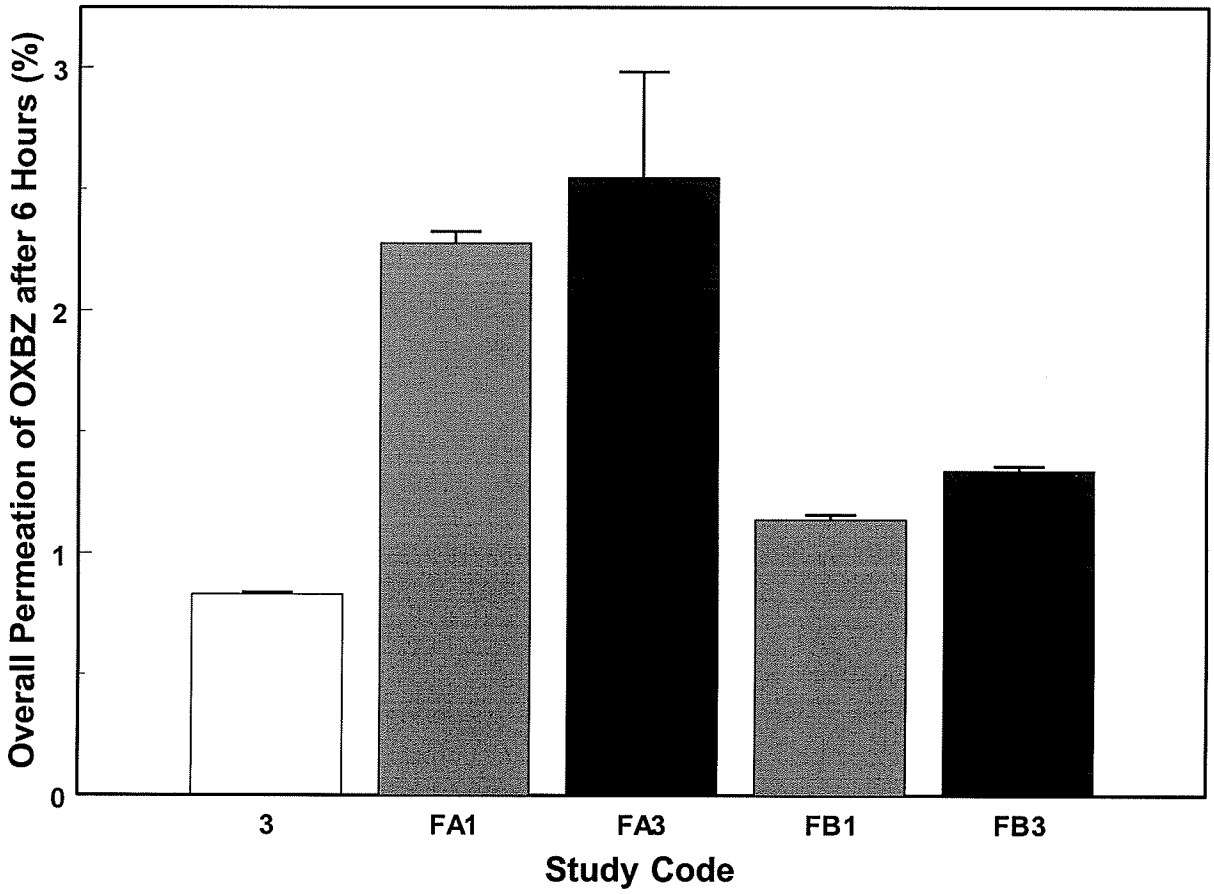


Figure 8.19. Overall permeation percentage of oxybenzone in prepared formulations through Mill-F membrane after 6 hours

Table 8.64. Overall permeation percentage of oxybenzone from prepared formulations through Mill-F membrane after 6 hours (%)

No.	Study Code				
	No.3	**FA1**	**FA3**	**FB1**	**FB3**
1	0.81	2.27	2.13	1.14	1.34
2	0.82	2.37	2.10	1.10	1.31
3	0.85	2.20	3.42	1.17	1.37
Mean	0.83	2.28	2.55	1.14	1.34
SEM	0.01	0.05	0.44	0.02	0.02

Table 8.65. Comparison of transmembrane permeation of oxybenzone from prepared formulations through Mill-F membrane (%)

OXBZ	FA1	FA3	FB1	FB3
No.3	175*	207*	37	61
FA1		11	–50*	
FA3				–47*
FB1				18

* *Significantly different ($p \leq 0.05$)*

Table 8.66. Steady-state flux of oxybenzone from prepared formulations through Mill-F membrane (μg/cm²h)

No.	Study Code				
	No.3	**FA1**	**FA3**	**FB1**	**FB3**
1	14.49	35.85	31.11	18.42	21.77
2	14.73	35.99	30.99	17.93	21.38
3	15.09	34.16	31.68	18.55	21.89
Mean	14.78	35.33	31.26	18.30	21.68
SEM	0.10	0.59	0.21	0.19	0.15

Table 8.67. Comparison of steady-state flux of oxybenzone from prepared formulations through Mill-F membrane (%)

OXBZ	FA1	FA3	FB1	FB3
No.3	139*	110*	24*	47*
FA1		−12*	−48*	
FA3				−31*
FB1				19*

** Significantly different ($p \leq 0.05$)*

8.4.5 Discussion

Previous experiments have indicated that concurrent application of insect repellent and sunscreen formulations will increase the percutaneous permeation of DEET and oxybenzone. This penetration characterization is also dependent on the formulation type, application sequence and application amount. Before the clinical pharmacology and toxicology of concurrent use of repellents and sunscreens can be further assessed *in vivo*, it appears logical and justifiable to design and develop alternative repellent and sunscreen formulations that would minimize or optimize the percutaneous profiles of penetration and absorption of the active ingredients. In this preliminary experiment, two formulation parameters, i.e., the proportion of water and oil phases, the formulation viscosity, were adjusted with the hope of reducing overall percutaneous permeation of DEET and oxybenzone.

The variation in the proportion of water phase and oil phase in an o/w emulsion can influence the solubility and distribution patterns of DEET and oxybenzone in the preparation, and consequently resulting in different outcomes in percutaneous penetration and absorption. Formulation A was composed of 77% water phase and 23% oil phase, while formulation B was composed of 60% water phase and 40% oil phase. Since both DEET and oxybenzone are lipophilic compounds, larger oil phase would promote their dissolution and distribution in the formulation, minimizing overall transdermal permeation of the active ingredients. The percutaneous penetration of DEET and oxybenzone from formulation A was generally higher than that from formulation B. In addition, oxybenzone has a higher lipophilic affinity than DEET. Increasing the proportion of oil phase in the formulation would affect oxybenzone more than DEET,

reducing its capability of leaving the vehicle and interacting with the membrane. The consequence of this difference was the observed smaller percutaneous permeation of oxybenzone than DEET. This phenomenon could also be explained by the concept of emulsion and its thermodynamic characteristics. An oil-in-water emulsion is a thermodynamically instable system in which oil droplets are evenly dispersed in the continuous water phase. Increasing the proportion of the oil phase in the emulsion would result in larger and more numerous oil droplets. This will prevent oil droplets from interacting with the skin membrane and reducing transfer of DEET and oxybenzone from lipophilic oil phase to the membrane surface. On the other hand, fewer oil particles in an emulsion with a high continuous water phase would have more interaction opportunities with the skin surface. Small particles also possess larger particle surface areas, which could subsequently enhance permeation of DEET and oxybenzone across the membrane.

The viscosity of a lotion formulation might also affect the percutaneous penetration of DEET and oxybenzone through the membranes. In formulation B, the xanthan gum was added to increase the consistency of the formulation. It has been proven that rheological properties of a formulation could dictate the extent and rate of drug penetration across the skin [151]. Increasing the consistency of the emulsion would retard the diffusivity of a compound from transferring from one point to another point along the concentration gradient, which would be reflected in reduced flux values and transdermal permeation percentages. However, this particular formulation property should be applied based on the requirement of individual preparation. Sunscreens, for example, could be designed in a favorable way to increase formulation viscosity to retain long contact period of time for optimal UV protection. This character is especially important for

products intended for outdoor water sports application. Insect repellents, on the other hand, may not be ideal formulations for this physical parameter, because short retention time and easy wash-off of the active repellent ingredients are critical in minimizing systemic transdermal absorption of DEET.

It was not possible from this preliminary formulation study to evaluate the overall effects of formulation excipients on transdermal permeation of DEET and oxybenzone, since no commercially available products tested in this thesis disclosed any non-medicinal ingredients or additives by the manufacturers. The prepared formulations contained only two active ingredients, but their protection efficacy (protection time for insect repellent and SPF for sunscreen) was not evaluated separately. However, results from this study proved that the combined use of DEET and oxybenzone could enhance transdermal penetration of both chemical compounds, and that modifying formulation parameters could reduce overall percutaneous absorption of the components. Further systematical investigation is being proposed for next stage of experiments.

Chapter 9. Conclusions

Insect repellents and sunscreens have played a very important role in promoting healthy life styles and preventing vector-borne diseases and skin cancers for the general public. With the continuous improvement in living standards and the extensive public awareness of protection against the West Nile virus and skin cancer, the application of both insect repellent and sunscreen preparations will become more and more acceptable by the general public around the world. Concurrent use of insect repellents and sunscreens has been widely practiced in some regions across North America for many years. This practice is going to gradually become an inevitable summer routine procedure in many countries. Information on transdermal penetration and systemic absorption of topically-applied active repellent and sunscreen substances will not only benefit the healthcare professionals in understanding the pharmacology and toxicology of these chemical compounds in humans, but also provide safer and more effective application guidelines and products to the society as a whole.

In vitro diffusion studies from this thesis indicated a synergistic permeation of the repellent DEET and the sunscreen oxybenzone when both insect repellent and sunscreen preparations were used simultaneously. Of the five commercially available products tested, insect repellent lotion produced higher transmembrane permeation of DEET than repellent spray. Mixing repellent and sunscreen products at different proportions also significantly increased the permeation of DEET and oxybenzone across the membrane models. The concept of combining both repellent and sunscreen ingredients into a single formulation was not supported from the experimental results, because the two combined commercial repellent/sunscreen products generally produced enhanced permeation of DEET and oxybenzone compared to their individual counterparts. Oxybenzone acted as a

transdermal absorption enhancer to DEET, although its transmembrane permeation was also influenced by the presence of DEET. It was found from the experiments that in an ideal real-life situation, sunscreens should always be applied first before insect repellents is used on the top of sunscreens, and that minimal physical mixing should be executed for the concurrent application in order to reduce overall transdermal penetration and absorption of the active ingredients.

It was possible to alter the transmembrane permeability of DEET and oxybenzone by modifying formulation characteristics and selecting appropriate type and amount of excipients and additives used in the preparations. Commercial insect repellent and sunscreen products are generally composed of multiple active ingredients and non-medicinal compounds for optimal protection efficacy and elegant product stability. Preliminary formulation evaluation from this thesis supported the objectives of reducing overall percutaneous permeation of DEET and oxybenzone from formulation modifications. Further studies should be carried out to achieve this ultimate research goal.

The use of artificial membranes could substitute biological membrane models in early screening stages when various physical and chemical parameters of the diffusants are assessed and compared. However, the diffusion results from using artificial membranes should always be judged individually and realistically to obtain accurate and reproducible conclusions. It appeared from this thesis that lipophilic membranes such as LDPE membrane were appropriate for testing lipophilic compounds like DEET and oxybenzone. Correlative comparisons between artificial and biological membranes should be carried out to accurately predict transdermal penetration and absorption of drug candidates across the skin *in vivo*.

In conclusion, percutaneous permeation characteristics of DEET and oxybenzone from concurrent application of commercially available insect repellent and sunscreen products are considered undesirable due to synergistic interaction between the two active ingredients. Since both preparations are extensively used by the general public as over-the-counter consumer-care products, further systematical investigations, both *in vitro* and *in vivo*, are therefore warranted to ensure the application safety and efficacy of the two topical formulations.

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