Available online at www.sciencedirect.com

SCIENCE DIRECT®

Toxicology and Applied Pharmacology 208 (2005) 99–110

Toxicology and Applied Pharmacology

www.elsevier.com/locate/ytaap

Predicting skin permeability from complex chemical mixtures

Jim E. Riviere*, James D. Brooks

Center for Chemical Toxicology Research and Pharmacokinetics, 4700 Hillsborough Street, North Carolina State University, Raleigh, NC 27606, USA

Received 17 November 2004; accepted 26 February 2005
Available online 25 April 2005

Abstract

Occupational and environmental exposure to topical chemicals is usually in the form of complex chemical mixtures, yet risk assessment is based on experimentally derived data from individual chemical exposures from a single, usually aqueous vehicle, or from computed physiochemical properties. We present an approach using hybrid quantitative structure permeation relationships (QSPeR) models where absorption through porcine skin flow-through diffusion cells is well predicted using a QSPeR model describing the individual penetrants, coupled with a mixture factor (MF) that accounts for physicochemical properties of the vehicle/mixture components. The baseline equation is $\log k_p = c + m\mathrm{MF} + a\Sigma\alpha_2^H + b\Sigma\beta_2^H + s\pi_2^H + rR_2 + vV_x$ where $\Sigma\alpha_2^H$ is the hydrogen-bond donor acidity, $\Sigma\beta_2^H$ is the hydrogen-bond acceptor basicity, π_2^H is the dipolarity/polarizability, R_2 represents the excess molar refractivity, and V_x is the McGowan volume of the penetrants of interest; c, m, a, b, s, r, and v are strength coefficients coupling these descriptors to skin permeability (k_p) of 12 penetrants (atrazine, chlorpyrifos, ethylparathion, fenthion, methylparathion, nonylphenol, ρ-nitrophenol, pentachlorophenol, phenol, propazine, simazine, and triazine) in 24 mixtures. Mixtures consisted of full factorial combinations of vehicles (water, ethanol, propylene glycol) and additives (sodium lauryl sulfate, methyl nicotinate). An additional set of 4 penetrants (DEET, SDS, permethrin, ricinoleic acid) in different mixtures were included to assess applicability of this approach. This resulted in a dataset of 16 compounds administered in 344 treatment combinations. Across all exposures with no MF, R^2 for absorption was 0.62. With the MF, correlations increased up to 0.78. Parameters correlated to the MF include refractive index, polarizability and log (1/Henry's Law Constant) of the mixture components. These factors should not be considered final as the focus of these studies was solely to determine if knowledge of the physical properties of a mixture would improve predicting skin permeability. Inclusion of multiple mixture factors should further improve predictability. The importance of these findings is that there is an approach whereby the effects of a mixture on dermal absorption of a penetrant of interest can be quantitated in a standard QSPeR model if physicochemical properties of the mixture are also incorporated.

Keywords: QSPeR; QSAR; Dermal Absorption; Risk Assessment; Chemical Mixtures

Introduction

A primary exposure route for environmental and occupational chemicals is the skin. Significant progress has been made on defining quantitative structure permeation relationships (QSPeRs) to describe chemical absorption across the skin. The roots of this field extend to the early work of Hansch and Dunn (1972). Existing experimental approaches to quantitate chemical dermal absorption utilize simple in vitro diffusion cell systems possessing an intact stratum corneum barrier. For most chemicals, the outermost layer of the epidermis, the stratum corneum, is considered the rate limiting diffusion barrier to compound penetration into skin. Studies have shown that this barrier is composed primarily of the intercellular lipids that surround dead keratin-filled corneocytes that comprise this layer, with chemical penetration occurring through these intercellular lipids (Elias, 1983). Partition coefficients (ratio of concentration in skin to vehicle) and permeability constants (log k_p) are estimated in such experiments. They generally correlate to the octanol/water partition coefficient ($K_{o/w}$) and are predictive of the absorption of a single chemical across this rate limiting barrier (Bronaugh et al., 1982; Buchwald and Bodor, 2001; Moss et al., 2002; Sartorelli et al., 1998).

* Corresponding author.
E-mail address: Jim_Riviere@ncsu.edu (J.E. Riviere).

0041-008X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.taap.2005.02.016

The first such relationship applied to pharmaceutics and toxicology which received widespread attention was that of Potts and Guy (1992) which incorporated octanol-water partition coefficient (log $K_{o/w}$) and molecular weight (MW) to predict in vitro skin permeation [log k_p = 0.71 log $K_{o/w}$ − 0.0061 MW − 6.3]. This approach has been incorporated into Environmental Protection Agency (EPA) guidelines (EPA, 1999). Numerous approaches have since been formulated. In a number of contributions, Abraham et al. (1995, 1999) and Abraham and Martins (2004) attempted to generalize these solute-solvent interactions for permeability through biological membranes, including skin, in the context of linear-free energy relationships (LFERs) expressed as basic solvation equations. Most QSPeRs applied to dermal absorption are LFERs that link the specific molecular descriptors (molecular size, hydrogen bonding) with the process being modeled (e.g., partitioning, membrane permeation). A number of widely quoted published dermal QSPeRs have recently been critically reviewed by Geinoz et al. (2004).

There is general agreement that such an approach which links skin permeability to a set of basic molecular descriptors is the preferred method to characterize skin absorption across chemicals as mechanistic insight is obtained both when compounds can be predicted and when lack of predictability occurs. The common thread running through existing QSPeR approaches is that individual chemical penetrants are characterized by various physical properties that include solvatochromatic molecular descriptors or experimentally measured partition coefficients for the penetrants of issue.

However, most topical chemical exposures are not to individual chemicals but rather are to penetrants exposed in complex chemical mixtures consisting of different vehicles or chemical additives. Drugs are applied in formulations where additives are present for specific functions related to drug delivery or stability. In contrast, occupational and environmental exposures are often to chemicals more loosely associated with the penetrant of interest (e.g., contaminants, additives, solvents). Risk assessment models are based either on experimental estimates of skin permeability or on the physicochemical properties of the individual penetrant as defined by LFERs established in the system of interest (e.g., skin permeability, log k_p). It is important to realize that these models are only parameterized using molecular properties of the penetrants and not the vehicle or other mixture components in which the penetrants being modeled are dosed. Most such models are defined in aqueous systems. Hostynek and Magee (1997) used indicator variables embedded in LFER equations to allow analysis across exposures consisting of different vehicles or occlusive conditions. These indicator variables did not contain any information concerning the vehicles, but were a statistical regression tool to allow the base LFER model to be applied to penetrants dosed under different experimental conditions.

In this manuscript, we present a modeling strategy whereby a term that accounts for mixture effects (additives, vehicles) on the absorption process is added to the LFER equation parameterized only using individual penetrant properties. This results in a hybrid LFER that explicitly incorporates both penetrant and mixture properties. This model was formulated using data obtained from a complete factorial experimental study of 12 diverse compounds (MW: 94–350 g/mol; log $K_{o/w}$: −0.1 to 5.8) administered in 24 vehicle/mixture additive combinations (water, ethanol, propylene glycol, sodium lauryl sulfate, methyl nicotinate) resulting in a dataset of 288 treatment combinations with 4–5 replicates per treatment. This model was then further assessed using an additional 4 compounds plus new triazine mixtures administered in a total of 56 treatment combinations. All studies were conducted under identical experimental conditions using in vitro porcine skin flow-through diffusion cells (PSFT). This model system was selected because porcine skin is widely accepted as being an animal model for human dermal absorption; and in vitro systems are considered predictive of in vivo absorption when the stratum corneum is the rate limiting step in absorption (Bronaugh and Stewart, 1985; Bronaugh et al., 1982; Monteiro-Riviere, 1991).

We present these data as a novel approach to dermal QSPeR analysis as it incorporates physical chemical properties of the mixture components independent of the physical properties of the penetrants that are accounted for by the molecular descriptors in the LFER equation.

Materials and methods

Chemicals. Atrazine-ring-UL-^{14}C (specific activity = 15.1 mCi/mmol, purity = 98.1%), MethylParathion-ring-UL-^{14}C (specific activity = 13.8 mCi/mmol, purity = 99.5%), 4-Nitrophenol-UL-^{14}C (specific activity = 6.4 mCi/mmol, purity = 99.6%), Parathion-ring-UL-^{14}C (specific activity = 9.2 mCi/mmol, purity = 97.1%), Pentachlorophenol-ring-UL-^{14}C (PCP) (specific activity = 11.9 mCi/mmol, purity = 98.0%), Permethrin-benzyl-ring-UL-^{14}C (specific activity = 10.9 mCi/mmol, purity = 96.1%), Phenol-UL-^{14}C (specific activity = 9.0 mCi/mmol, purity = 98.5%) and Simazine-ring-UL-^{14}C (specific activity = 15.5 mCi/mmol, purity = 99.0%) were obtained from Sigma Chemical Co., St. Louis, MO, USA. Chlorpyrifos[pyridine-2,6-^{14}C] (specific activity = 32 mCi/mmol, purity =99.0%), Fenthion-ring-UL-^{14}C (specific activity = 55 mCi/mmol, purity = 98.5%), Propazine-ring-UL-^{14}C (specific activity = 15 mCi/mmol, purity = 96.6%), Ricinoleic acid [12-^{3}H] (specific activity = 20,000 mCi/mmol, purity = 99%), and 1,3,5,-Triethyl hexahydro-S-triazine [methylene-^{14}C] (specific activity = 10 mCi/mmol, purity = 98.6%) were obtained from American Radiolabeled Chemicals, Inc., St. Louis, MO, USA. *p*-Nonylphenol-ring-^{14}C (specific activity = 76.6 mCi/mmol) was obtained from BioDynamics Radiochemicals, Billingham, UK. Sodium 2-dodecylbenzene sulfonate-ring-UL-^{14}C (SDS) (specific activity = 50.77 mCi/mmol, purity = 99.1%) was obtained from Wizard Laboratories, Sacramento, CA. N,N Diethyl-m-toluamide (DEET) (purity = 98%) was

obtained from Chem Service Company, West Chester, PA. (DEET was not radiolabeled, but was analyzed via HPLC). Absolute ethyl alcohol (200 proof) was obtained from Aaper Alcohol and Chemical Co. Shelbyville, KY, USA. Propylene glycol (purity = 99%), Sodium lauryl sulfate (purity = 99%), and Methyl nicotinic acid (purity = 99%) were obtained from Sigma Chemical Co., St. Louis, MO, USA. Water was distilled in our in-house still.

Porcine skin flow-through diffusion cells and mixture exposures. The flow-through diffusion cell (Bronaugh and Stewart, 1985; Chang and Riviere, 1991) was used to perfuse porcine skin obtained from the dorsal area of weanling female Yorkshire pigs according to protocols approved by the North Carolina State University Institutional Animal Care and Use Committee. Skin was dermatomed to a thickness of 500 μm with a Padgett Dermatome (Padgett Instruments, Inc., Kansas City, MO, USA). Each circular skin disk was punched to provide a dosing surface area of 0.64 cm^2 and then placed into a two-compartment Teflon flow-through diffusion cell (Crowne Glass, Somerville, NJ). Skin was perfused using a Krebs–Ringer bicarbonate buffer spiked with dextrose and bovine serum albumin, and topically dosed with 20 μL of one of 12 marker penetrant compounds listed in Table 1 (10 μg/cm^2) formulated in one of 24 specified mixtures listed in Table 2. This resulted in a total of 288 treatments with n = 4–5 replicates/treatment designed as a randomized complete factorial experiment. To extend and validate this analysis, a second series of four compounds (DEET, permethrin, ricinoleic acid, SDS) in multiple mixtures (combinations of chemicals in water, ethanol or aqueous ethanol) and triazine in new mixtures from previously published data from our laboratory (Baynes and Riviere, 2004; Baynes et al., 2002a, 2003; Riviere and Monteiro-Riviere, 2002; Riviere et al., 2003) yielding 56 treatment combinations were also included in a second analysis of 16 compounds in 344 treatment combinations. The temperature of the perfusate and flow-through cell was maintained at 37 °C using a Brinkmann constant temperature circulator (Brinkmann, Inc., Wesbury, NY, USA) and the pH was maintained between 7.3 and 7.5. Perfusate flow-rate was 4 mL/h, and perfusate samples were collected every 15 min for 2 h and every h thereafter until the end of the 8-h dosing period. A representative 1 mL sample of each perfusate sample was analyzed for radioactivity using Bioscint scintillation cocktail (National Diagnostics, Atlanta, GA, USA) and a Packard 2500TR Tricarb Scintillation Counter (Downers Grove, IL, USA). The permeability constant [k_p] (cm/h) of chemical through porcine skin was determined by dividing the steady state flux, calculated using regression as the slope of cumulative μg/cm^2 versus time regression, by applied surface concentration.

Table 1
Molecular descriptor values of penetrants used to parameterize LFER model

Marker	$\Sigma\alpha_2^H$	$\Sigma\beta_2^H$	π_2^H	R_2	V_x
Substituted phenols					
Nonylphenol	0.5295	0.3799	0.7434	0.7911	2.0432
Pentachlorophenol	0.7467	0.0412	0.8710	1.2624	1.3871
Phenol	0.5295	0.3135	0.8713	0.8095	0.7751
ρ-Nitrophenol	0.8677	0.2250	1.6711	1.0712	0.9493
Organophosphates					
Chlorpyrifos	0.0030	0.9687	2.1268	1.4788	2.1503
Ethylparathion	0.0030	0.8730	2.1084	1.2678	1.9984
Fenthion	0.0030	0.9183	1.7252	1.3858	1.9877
MethylParathion	0.0030	0.8702	2.1108	1.2702	1.7166
Triazine herbicides					
Atrazine	0.1801	0.8802	1.2398	1.5097	1.6196
Propazine	0.1801	0.8974	1.1993	1.4944	1.7605
Simazine	0.1801	0.8630	1.2803	1.5250	1.4787
Triazine	0.0030	1.8501	1.0621	0.5015	1.5675

Values obtained from ABSOLV Solute Property Prediction Software (Sirius Analytical Instruments, Ltd., East Sussex, UK).

Table 2
Composition of the 24 mixtures investigated

EtOH	PG
EtOH + MNA	PG + MNA
EtOH + SLS	PG + SLS
EtOH + MNA + SLS	PG + MNA + SLS
EtOH + Water	PG + Water
EtOH + Water + MNA	PG + Water + MNA
EtOH + Water + SLS	PG + Water + SLS
EtOH + Water + MNA + SLS	PG + Water + MNA + SLS
EtOH + PG + Water	Water
EtOH + PG + Water + MNA	Water + MNA
EtOH + PG + Water + SLS	Water + SLS
EtOH + PG + Water + MNA + SLS	Water + MNA + SLS

EtOH—Ethanol; PG—Propylene glycol; MNA—Methyl nicotinate; SLS—Sodium lauryl sulfate.

The model and its parameters. We elected to use Abraham's LFER model as our base equation since it is representative of the dermal QSPeR approaches presently available (Abraham and Martins, 2004). Preliminary analyses applying 16 LFER equations reviewed by Geinoz et al. (2004) confirmed a superior fit of our data set to the Abraham equation compared to most other models reviewed. It must be stressed that the purpose of this paper is not to identify the *optimal* LFER for predicting dermal permeation, nor to validate that this model is predictive of dermal absorption. Rather, we selected this model since it is broadly accepted by the scientific community as being descriptive of the key molecular/physiochemical parameters relevant to solute absorption across skin. Our focus was to apply such a widely accepted model to the mixture problem. This base model can be written as:

$$\log k_p = c + a\Sigma\alpha_2^H + b\Sigma\beta_2^H + s\pi_2^H + rR_2 + vV_x \quad (1)$$

where k_p is the permeability constant for the PSFT experiments, $\Sigma\alpha_2^H$ is the hydrogen-bond donor acidity, $\Sigma\beta_2^H$ is the hydrogen-bond acceptor basicity, π_2^H is the dipolarity/

polarizability, R_2 represents the excess molar refractivity, and V_x is the McGowan volume. All of these parameters are for the 12 penetrants being studied. The parameters c, a, b, s, r, and v are strength coefficients coupling the molecular descriptors to skin permeability in our experimental system. These are the regression coefficients obtained when the LFER equation describing the system (e.g., k_p in skin) is solved across multiple chemicals where the molecular descriptors for each chemical are known. Table 1 is a list of the molecular descriptor values used in this analysis for the 12 defining original compounds. Descriptor values for the 5 validation chemicals were obtained from ABSOLV (Sirius Analytical Instruments, Ltd., East Sussex, UK) and ADME Boxes (Pharma Algorithms, Toronto, Canada).

In order to incorporate mixture effects, we add another term to this Eq. (1) called the mixture factor (MF) yielding:

$$\log k_p = c + m\mathrm{MF} + a\Sigma\alpha_2^H + b\Sigma\beta_2^H + s\pi_2^H + rR_2 + vV_x \quad (2)$$

The nature of the MF is determined by examining the residual plot (actual-predicted log k_p) generated from Eq. (1) based on molecular descriptors of the permeants as a function of the physical chemical properties of the mixture/solvents in which they were dosed. Twenty non-duplicate physical chemical properties of the mixture components (see Table 2) were analyzed; including parameters of molecular size and volume, hydrogen bonding properties, pK_a, ovality, Henry's Law Constant, polarizability, refractive indices, melting point, boiling point, and vapor pressure. We then computed a composite physical chemical MF (MF_1, MF_2, etc.) by weighting the component's physical chemical parameter (e.g., refraction index, etc) by its contribution to its MF based on the summation of the weight percentage of each of the bulk components in the mixtures for a particular parameter. Minor mixture components based on weight percentages, in this case the actual penetrant, did not materially contribute to the value of the MF for a specific treatment and could be excluded. The following is an example of this calculation for PCP in the EtOH + PG + MNA + SLS mixture.

Component	μg	Percentage	log (1/HC)	Contribution
EtOH	5920	28.6	5.008	1.430
Water	5000	24.1	5.579	1.345
PG	7771	37.5	7.666	2.873
MNA	25.4	0.1	6.235	0.007
SLS	2000	9.6	8.124	0.783
PCP	15.2	0.1	3.393	0.002
			$\sum$ log (1/HC) = 6.443	

Statistical analysis. Multiple regression analysis was carried out using SAS 8.1 for Windows (SAS Institute, Cary, NC). Data was initially fit to Eq. (1) using the molecular descriptor values in Table 1 for the 12 penetrants across all 288 treatment combinations (12 penetrants × 24 mixtures) ignoring the dosing mixture into which the chemicals were administered. Four to five replicates per treatment combination were studied. Due to the wide range of possible responses across these diverse treatments, no attempt was made to remove individual treatment outliers. All collected data in the complete factorial block design was used in these analyses. The "predicted vs. observed" k_p residuals were then analyzed to determine if a covariate (termed mixture factor-MF) could be identified which explained any trend evidenced in these data related to the effect of the vehicle/mixture. In other words, a covariate was included in the model if it were correlated to the residual pattern as evidenced by R^2. Twenty physical chemical properties of the mixture components were analyzed based on strength of the correlation against the residuals of Eq. (1). To confirm selection of these specific parameters, principal component analyses of these descriptor's effects on k_p also yielded three groups of descriptors which accounted for different patterns of variability seen in the 12 compound × 24 mixture balanced data set. Refraction index, polarizability, and log (1/Henry's Law Constant) were representative of these primary properties. These parameters were then incorporated as the MF in Eq. (2). To generate final equations covering a wider set of compounds and mixtures, the additional 5 so-called validation chemicals in 56 treatment combinations (note triazine was present in both sets but were exposed in different mixtures) were also analyzed against the three MFs selected from the original balanced design, yielding a final dataset of 16 compounds administered in 344 different treatment combinations. Correlation matrices for these regressions were also determined.

Results

Fig. 1 is a plot of observed versus predicted log k_p and its associated residual plot from PSFT experiments for the original 288 treatment combinations. The residual plot for this regression illustrates no specific pattern is evident, except that data were grouped within the penetrants (vertical columns of spread). The LFER equation (mean ± SEM for strength coefficients) describing these data was:

$$\log k_p = -0.412(\pm 0.417) - \left(1.403(\pm 0.303)\Sigma\alpha_2^H\right) + \left(0.129(\pm 0.166)\Sigma\beta_2^H\right) + \left(0.297(\pm 0.085)\pi_2^H\right) - \left(0.498(\pm 0.135)R_2\right) - \left(1.885(\pm 0.110)V_x\right) \quad (3)$$

As expected, this model did not accurately predict log k_p for these penetrants since they were dosed in multiple vehicles, in

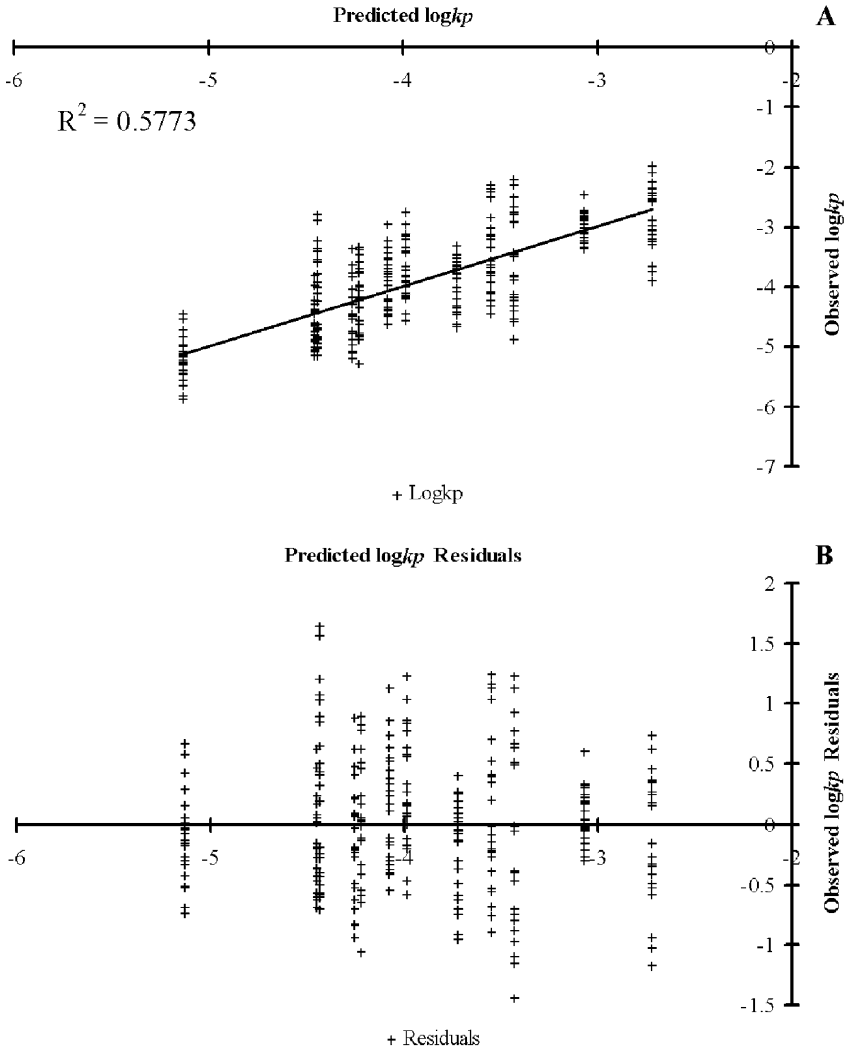

Fig. 1. Plot of predicted versus observed log k_p (A) and associated residual plot (B) with no MF for the 12 compounds in Table 1 dosed in all combinations of mixtures listed in Table 2. The predicted line (—) is based on Eq. (3).

contrast to single vehicle studies where QSPeR studies are normally defined. The residuals of this model showed no further correlation to any penetrant property.

In contrast, when vehicle/mixture component properties (mixture components listed in Table 2) were analyzed against these residuals (x axis of residual plot is now mixture property of interest), trends in residuals became evident which suggests that a mixture property could explain variance in the LFER model defined from penetrant properties alone. Of the 20 properties analyzed for mixture components, three were clearly superior based upon R^2 predicting the residuals as well as the increase in the R^2 of the LFER multiple regression analysis compared to the model without a MF based on this property (Eq. (3) above). Separate covariance and principal component analysis also indicated that these three factors were relatively independent in reducing residual variance. The final values for these parameters were obtained from the SPARC online calculator (http://ibmlc2.chem.uga.edu/sparc/).

The predicted versus observed plots for a MF equal to refractive index (Fig. 2 also illustrates residual plot), polarizability (Fig. 3) or log (1/Henry's Law Constant) (Fig. 4) demonstrated a significant improvement in predicting k_p. Note that these residual plots are before inclusion of the specific MF to show correlation of the Eq. (3) residuals against the specific physical chemical property studied. All other molecular descriptors in these analyses were fixed to the values of the penetrants listed in Table 1. The improvement in k_p predictability can best be appreciated when correlations are compared across equations in Table 3. In addition, these three parameters were relatively independent as evidenced by low covariances between each pair of parameters, suggesting that they explain different components of the residual variances reflecting different physical chemical properties of the mixtures/solvents. This is also evident by comparing the different patterns in the residual plots for each of the variables. The resulting LFERs now including a mixture factor are:

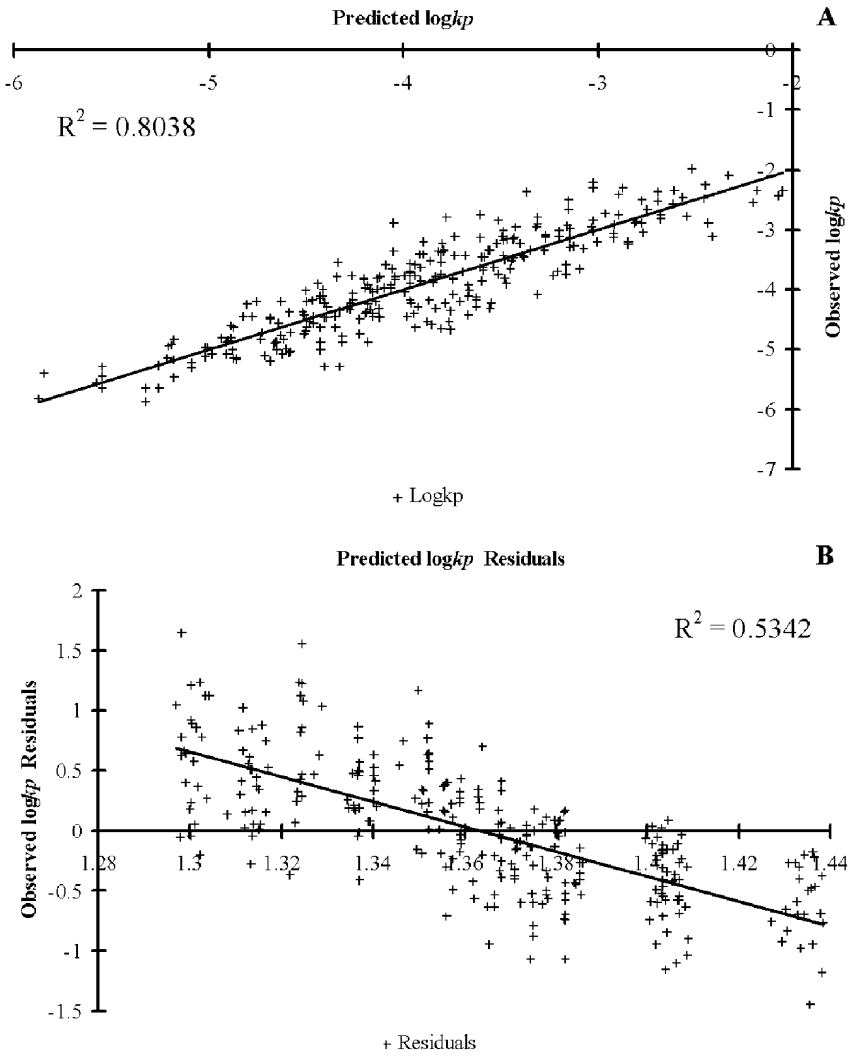

Fig. 2. Plot of predicted versus observed log k_p (A) and associated residual plot (B) for the 12 compounds in Table 1 dosed in all combinations of mixtures listed in Table 2 where MF_1 is Refractive Index. The predicted line (−) is based on Eq. (4).

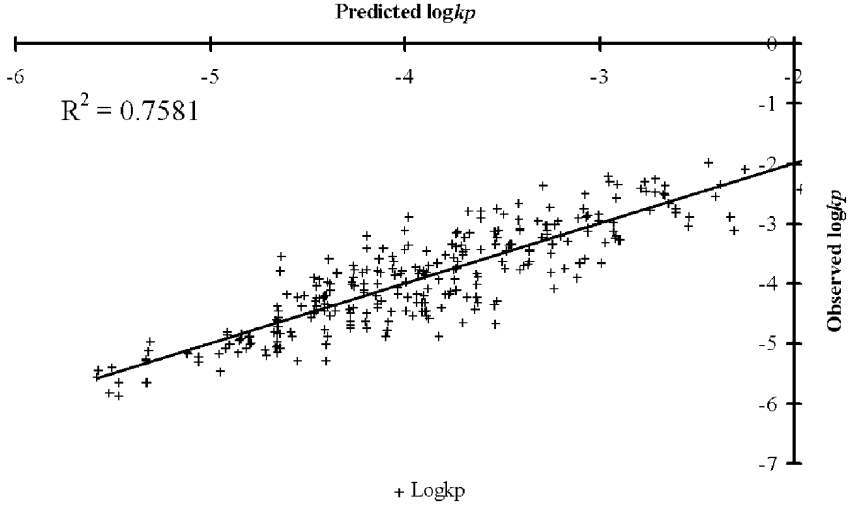

Fig. 3. Plot of predicted versus observed log k_p for the 12 compounds in Table 1 dosed in all combinations of mixtures listed in Table 2 where MF_2 is Polarizability. The predicted line (−) is based on Eq. (5).

Refractive Index

$$\log k_p = 13.921(\pm 0.845) - (10.394(\pm 0.577)\mathrm{MF}_1) - \left(1.527(\pm 0.207)\Sigma\alpha_2^H\right) + \left(0.045(\pm 0.113)\Sigma\beta_2^H\right) + \left(0.327(\pm 0.058)\pi_2^H\right) - (0.561(\pm 0.092)R_2) - (1.904(\pm 0.075)V_x) \quad (4)$$

Polarizability

$$\log k_p = 0.801(\pm 0.327) - (0.318(\pm 0.022)\mathrm{MF}_2) - \left(1.529(\pm 0.229)\Sigma\alpha_2^H\right) + \left(0.043(\pm 0.126)\Sigma\beta_2^H\right) + \left(0.327(\pm 0.064)\pi_2^H\right) - (0.563(\pm 0.102)R_2) - (1.902(\pm 0.083)V_x) \quad (5)$$

log(1/Henry's Law Constant)

$$\log k_p = 2.372(\pm 0.359) - (0.479(\pm 0.032)\mathrm{MF}_3) - \left(1.282(\pm 0.225)\Sigma\alpha_2^H\right) + \left(0.195(\pm 0.123)\Sigma\beta_2^H\right) + \left(0.280(\pm 0.063)\pi_2^H\right) - (0.453(\pm 0.099)R_2) - (1.863(\pm 0.082)V_x) \quad (6)$$

In order to assess the robustness of this approach, the additional five compounds in 56 treatment combinations were analyzed using the same approach as presented for the original 12 compounds. Mixtures studied were very different from the original analysis. Triazine was included in both sets of data however different mixtures were involved. These studies are not as balanced as the complete factorial design of the original group. The three mixture factors identified earlier were used in this validation analysis to determine if k_p from these new compounds could be predicted. The LFERs for the complete dataset of 16 compounds in 344 treatment combinations are:

No MF

$$\log k_p = -2.200(\pm 0.296) - \left(0.174(\pm 0.213)\Sigma\alpha_2^H\right) + \left(0.574(\pm 0.136)\Sigma\beta_2^H\right) + \left(0.382(\pm 0.082)\pi_2^H\right) - (0.326(\pm 0.127)R_2) - (1.391(\pm 0.063)V_x) \quad (7)$$

Refractive index (Fig. 5A)

$$\log k_p = 10.751(\pm 0.830) - (9.242(\pm 0.571)\mathrm{MF}_1) - \left(0.525(\pm 0.162)\Sigma\alpha_2^H\right) + \left(0.329(\pm 0.103)\Sigma\beta_2^H\right) + \left(0.407(\pm 0.062)\pi_2^H\right) - (0.411(\pm 0.096)R_2) - (1.385(\pm 0.048)V_x) \quad (8)$$

Polarizability (Fig. 5B)

$$\log k_p = -1.804(\pm 0.268) - (0.149(\pm 0.016)\mathrm{MF}_2) - \left(0.155(\pm 0.191)\Sigma\alpha_2^H\right) + \left(0.546(\pm 0.122)\Sigma\beta_2^H\right) + \left(0.421(\pm 0.074)\pi_2^H\right) - (0.433(\pm 0.115)R_2) - (1.255(\pm 0.059)V_x) \quad (9)$$

log(1/Henry's Law Constant) (Fig. 5C)

$$\log k_p = 0.105(\pm 0.296) - (0.419(\pm 0.031)\mathrm{MF}_3) + \left(0.050(\pm 0.174)\Sigma\alpha_2^H\right) + \left(0.693(\pm 0.110)\Sigma\beta_2^H\right) + \left(0.388(\pm 0.067)\pi_2^H\right) - (0.323(\pm 0.103)R_2) - (1.328(\pm 0.052)V_x) \quad (10)$$

The correlations for this dataset [all 16 compounds in 344 treatment combinations] are listed in Table 4. These data suggest that the same factors identified in the original 12 compound analysis also improved prediction of k_p for these additional compounds in different vehicle/mixture exposure scenarios.

The regression correlation matrices for Eqs. (7) Eqs. (8) Eqs. (9) Eqs. (10) are presented in Table 5. Across all equations, including the no-MF control, the strongest parameter correlations are with the two hydrogen bond parameters. As expected, incorporation of the mixture factors had minimal effect on the penetrant parameter correlations (e.g., those in Eq. (1)). However, the intercept correlations are now different due to the ability of the mixture factors to explain this source of variation not related to penetrant descriptors.

Conclusions

Our results suggest that incorporating properties of a solvent or mixture improves prediction of a chemical's

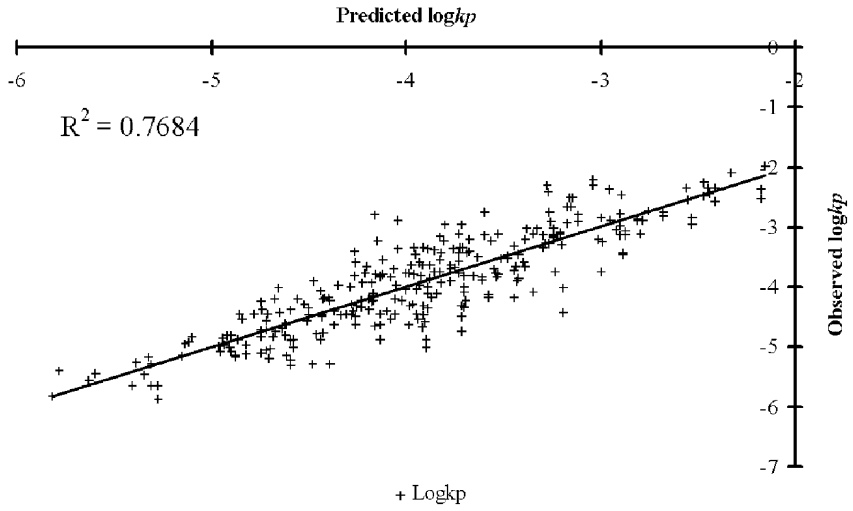

Fig. 4. Plot of predicted versus observed log k_p for the 12 compounds in Table 1 dosed in all combinations of mixtures listed in Table 2 where MF_3 is log(1/Henry's Law Constant). The predicted line (—) is based on Eq. (6).

permeability across skin. The preponderance of QSPeR skin absorption studies reported in the literature to date have focused on selecting the appropriate molecular descriptors that correlate to dermal permeability of individual chemical permeants using in vitro excised skin. These studies, epitomized in the Abraham model used in the present work (Abraham and Martins, 2004), and comprehensively reviewed in Geinoz et al. (2004), employ aqueous donor and receptor solutions. However, as is widely acknowledged by the risk assessment community, most occupational and environmental exposures to chemicals are to complex mixtures, and not individual compounds in defined aqueous vehicles. Experimental skin absorption studies have previously shown that mixture/vehicle effects significantly modify an individual chemical's dermal absorption and may even overshadow the magnitude of permeability differences between individual compounds (Baynes et al., 2002b; Brooks and Riviere, 1996; Idson, 1983; Qiao et al., 1996; Riviere and Monteiro-Riviere, 2002; Riviere et al., 2001, 2003; Rosado et al., 2003). The mechanism of the ethanol and SLS effects on penetrant absorption seen in the present studies are described elsewhere (van der Merwe and Riviere, 2004a, 2004b). These experimental absorption mixture studies suggest that for QSPeR models to be useful for realistic risk assessment estimates in the field, vehicle and mixture component effects should also be considered. The data presented in this work are a promising start to this type of analysis.

Table 3
Improvement of log k_p predictability (R^2) using physical chemical properties of the mixture components listed in Table 2

	MF	Predicted vs. Observed log k_p	Residuals
No MF	–	0.58	0
Refractive Index	MF_1	0.80	0.53
Polarizability	MF_2	0.76	0.43
log (1/Henry's Law constant)	MF_3	0.77	0.45

It should be stressed that only vehicle or mixture components that make up a large fraction of a mixture will contribute to the value of a computed MF. This is clearly seen in the example of a MF computation in Material and methods. Because the marker compounds are in such low concentration compared to the rest of the mixture components, the R^2 values do not change if the contribution of the markers were not included. This is consistent with minor (low weight percentage) versus major/bulk (high weight percentage) component effects on the physical chemistry properties of a solution. However, if the mixture factor were to directly chemically interact with a penetrant, its presence would affect k_p although it would not be predicted from LFER parameters. This limitation is discussed below.

In comparing this work with other QSPeR approaches, a few points should be made. First, our strength coefficients differ from those of Abraham and Martins (2004) because the experimental systems were different, in terms of our use of 24 different donor solutions, a protein-based receptor solution, and the use of excised pig rather than human skin. The strength coefficients in a LFER equation link the values of the molecular descriptors for a penetrant to a specific experimental system. It is interesting to note that the relatively large variability in estimates of the hydrogen bonding descriptors were also the descriptors that seemed relatively more variable in many of the solvatochromatic QSPeRs analyzed in the Geinoz et al. (2004) review. When the five validation compounds and new mixtures were added to our original analyses, the hydrogen bonding parameters remained the most variable, but now $\Sigma\beta_2^H$ was more variable than $\Sigma\alpha_2^H$. This linkage is supported by the relatively high correlations for these two parameters (0.85–0.86) in the correlation matrices in Table 5. It was not the purpose of our study to select the optimal LFER equation to

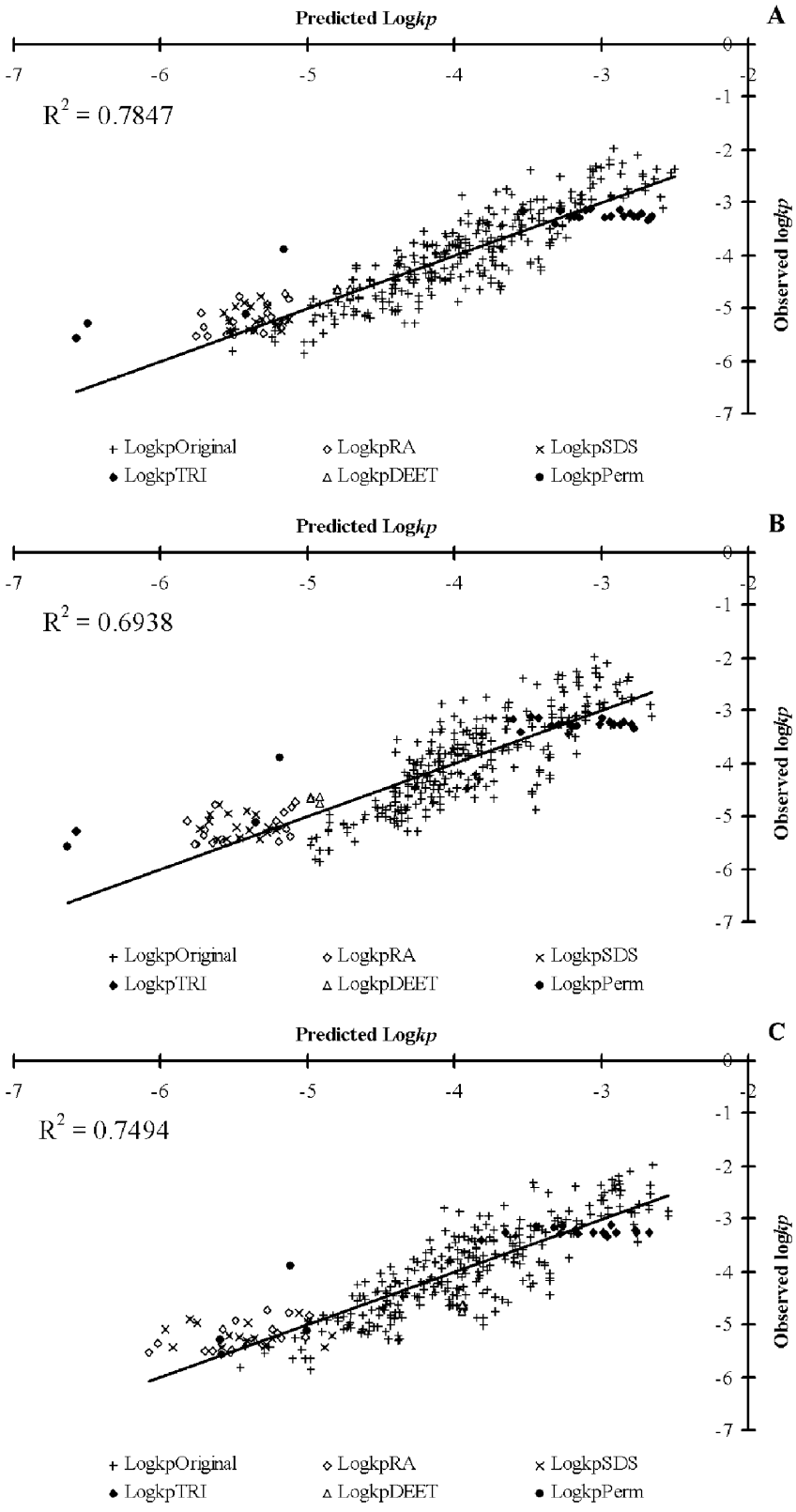

Fig. 5. Plot of predicted versus observed log k_p for full data set of 16 compounds across all mixtures where: (A) MF_1 is Refractive Index, predicted line (—) based on Eq. (8); (B) MF_2 is Polarizability, predicted line (—) is based on Eq. (9); (C) MF_3 is log (1/Henry's Constant), predicted line based on Eq. (10).

predict chemical absorption (e.g., minimum number of independent parameters needed to describe the data). We selected this equation as being representative of those previously reported in the literature for predicting chemical absorption in a defined solvent, so that we could assess whether incorporation of a MF improved predictability

Table 4
Improvement of log k_p predictability (R^2) using physical chemical properties of the mixture components listed in Table 2 and of the five validation compounds

	MF	Predicted vs. Observed log k_p	Residuals
No MF	–	0.62	0
Refractive index	MF_1	0.78	0.41
Polarizability	MF_2	0.69	0.17
log (1/Henry's Law Constant)	MF_3	0.75	0.34

across different solvent systems. One advantage of the present study, which is not shared by many studies based on an analysis of historic literature, is that the present studies were done under the constraints of a single experimental design thereby removing any inter-laboratory variation that exists in many modeling studies.

Concerning attempts to account for vehicle effects, Hostynek and Magee (1997) used an indicator variable to develop LFER models for chemical penetrants across different solvent system. However, they did not make an attempt to predict these solvent effects as we have done in the present work. The need for such an approach can be seen by the data patterns when MF was not incorporated into the equations. In this scenario, log k_p values clustered around penetrants (Fig. 1) as only data related to penetrant descriptors are included in the base LFER model (Eqs. (1), (3), and (7)). When a MF was included, these clusters dissipated as now vehicle/mixture specific properties explained some of the variance. All three MFs were minimally correlated to the penetrant descriptors as appreciated in Table 5. It is also suggested, based on examining the pattern of data scattering when different MFs were used, that different mixture interactions were being predicted.

Table 5
Correlation matrices of parameters

No MF

	Intercept	$\Sigma\alpha_2^H$	$\Sigma\beta_2^H$	π_2^H	R_2	V_x
Intercept	1.00					
$\Sigma\alpha_2^H$	−0.87	1.00				
$\Sigma\beta_2^H$	−0.83	0.85	1.00			
π_2^H	−0.30	0.31	0.14	1.00		
R_2	−0.71	0.58	0.66	−0.25	1.00	
V_x	−0.10	−0.15	−0.26	−0.22	−0.12	1.00

MF = refractive index

	Intercept	Refractive index	$\Sigma\alpha_2^H$	$\Sigma\beta_2^H$	π_2^H	R_2	V_x
Intercept	1.00						
Refractive index	−0.96	1.00					
$\Sigma\alpha_2^H$	−0.36	0.13	1.00				
$\Sigma\beta_2^H$	−0.36	0.15	0.85	1.00			
π_2^H	−0.05	−0.03	0.31	0.13	1.00		
R_2	−0.24	0.06	0.58	0.66	−0.25	1.00	
V_x	−0.02	−0.01	−0.15	−0.26	−0.22	−0.12	1.00

MF = polarizability

	Intercept	Polarizability	$\Sigma\alpha_2^H$	$\Sigma\beta_2^H$	π_2^H	R_2	V_x
Intercept	1.00						
Polarizability	−0.16	1.00					
$\Sigma\alpha_2^H$	−0.86	−0.01	1.00				
$\Sigma\beta_2^H$	−0.82	0.03	0.85	1.00			
π_2^H	−0.28	−0.06	0.31	0.13	1.00		
R_2	−0.72	0.10	0.57	0.66	−0.26	1.00	
V_x	−0.05	−0.25	−0.14	−0.26	−0.20	−0.14	1.00

MF = log(1/Henry's Law Constant)

	Intercept	log(1/HC)	$\Sigma\alpha_2^H$	$\Sigma\beta_2^H$	π_2^H	R_2	V_x
Intercept	1.00						
Log(1/HC)	−0.59	1.00					
$\Sigma\alpha_2^H$	−0.65	−0.10	1.00				
$\Sigma\beta_2^H$	−0.62	−0.08	0.85	1.00			
π_2^H	−0.24	−0.01	0.31	0.14	1.00		
R_2	−0.58	0.00	0.57	0.66	−0.25	1.00	
V_x	−0.03	−0.09	−0.14	−0.25	−0.22	−0.12	1.00

This is not surprising since principal components grouped the three MFs as independent predictors. When the new validation compounds and mixtures were added, polarizability was not as predictive. These observations suggest that a combination of independent properties would better predict mixture effects, that is a composite factor derived as a function of MF_1, MF_2, and MF_3. Unfortunately, the present data set was not comprehensive enough, nor properly balanced, to allow for such a complex analysis of both penetrant and mixture properties to be undertaken at this time.

The primary finding of the present study is that considering physical chemical properties of the solvent/mixture in which a topical chemical is dosed on skin, significantly improves the prediction of permeability in a QSPeR framework based on LFER relationships. This can be accomplished through the use of a covariate in the equation, the MF, which is related to properties of the mixture/solvent rather than to the individual penetrants.

Based on the analysis of a number of such mixture properties, three—the refractive index, polarizability and log (1/Henry's Law Constant) seem to explain upwards of 50% of the variance not predicted from the penetrant-based LFER model. These three parameters are related to different physical chemical properties of the mixtures, that is size, hydrophobicity, and volatility, respectively. There were insufficient mixture combinations to make a credible effort at combining these factors to improve predictability. Additionally, only solvatochromatic interactions are predicted using this approach, since this experimental framework restricts detection of mixture effects to those that would be predictable from the molecular properties quantitated in a LFER equation. Factors that modified diffusivity of a penetrant would also not be predicted from these solvatochromatic descriptors. Direct chemical reactions (e.g., covalent binding) and biological effects in skin (e.g., altered stratum corneum lipids) would not be explained by such properties.

It is also unrealistic to expect three physical–chemical properties, selected from the original solvents, to explain interactions for very different mixtures. When the validation mixtures were added, the impact of polarizability decreased. One potential approach to address this concern would be to use MFs specifically related to solvent classes. Creation of a composite MF should also address this concern. To further define such interactions, more compounds in a broader variety of solvents should be studied in the framework.

We do not present this work as a final solution to the mixture problem defined in the context of a QSPeR LFER model relative to the specific structure of the MF. Rather, we present data that clearly demonstrate that if mixture properties are incorporated into a LFER permeability model that is based on molecular descriptors of the penetrant, significantly improved prediction of k_p results. This would increase estimation of the internal dose in many risk assessment scenarios. This finding has numerous implications to occupational and environmental risk assessment for topical chemical exposure where the predominant exposure scenario is to complex mixtures.

Acknowledgments

The authors thank the significant conceptual input of Drs. Ronald Baynes, Patrick Williams and Xin-Rui Xia into the modeling approach utilized in this study, to Dr. Deon van der Merwe for his contributions to selecting molecular descriptors, and to Dr. Charles Smith for statistical advise. This work was supported by NIH Grant R01-OH-07555.

References

Abraham, M.H., Martins, F., 2004. Human skin permeation and partition: general linear free-energy relationship analyses. J. Pharm. Sci. 93, 1508–1523.

Abraham, M.H., Chada, H.S., Mitchell, R.C., 1995. The factors that influence skin penetration of solutes. J. Pharm. Pharmacol. 47, 8–16.

Abraham, M.H., Chada, H.S., Martins, F., Mitchell, R.C., Bradbury, M.W., Gratton, J.A., 1999. Hydrogen bonding part 46: a review of the correlation and prediction of transport properties by an LFER method: physicochemical properties, brain penetration and skin permeability. Pestic. Sci. 55, 78–88.

Baynes, R.E., Riviere, J.E., 2004. Mixture additives inhibit the dermal permeation of the fatty acid, ricinoleic acid. Toxicol. Lett. 147, 15–26.

Baynes, R.E., Brooks, J.D., Barlow, B.M., Riviere, J.E., 2002a. Physiochemical determinants of linear alkylbenzene sulfonate (LAS) disposition in skin exposed to aqueous cutting fluid mixtures. Toxicol. Indust. Health 18, 237–248.

Baynes, R.E., Brooks, J.D., Mumtaz, M., Riviere, J.E., 2002b. Effect of chemical interactions in pentachlorophenol mixtures on skin and membrane transport. Toxicol. Sci. 69, 295–305.

Baynes, R.E., Barlow, B.M., Riviere, J.E., 2003. Dermal disposition of triazine in cutting fluid mixtures. J. Toxicol. Cutaneous Ocular Toxicol. 22, 215–229.

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

Bronaugh, R.L., Stewart, R.F., Congdonm, E.R., Giles, A.L., 1982. Methods for in vitro percutaneous absorption studies. I. Comparison with in vivo results. Toxicol. Appl. Pharmacol. 62, 481–488.

Brooks, J.D., Riviere, J.E., 1996. Quantitative percutaneous absorption and cutaneous distribution of binary mixtures of phenol and ρ-nitrophenol in isolated perfused porcine skin. Fundam. Appl. Toxicol. 32, 233–243.

Buchwald, P., Bodor, N., 2001. A simple, predictive, structure-based skin permeability model. J. Pharm. Pharmacol. 53, 1087–1098.

Chang, S.K., Riviere, J.E., 1991. Percutaneous absorption of parathion in vitro in porcine skin. Fundam. Appl. Toxicol. 17, 494–504.

Elias, P.M., 1983. Epidermal lipids, barrier function and desquamation. J. Invest. Dermatol. 80, 44–49.

EPA-Environmental Protection Agency, 1999. Risk Assessment Guidance for Superfund. Human Health Evaluation Manual (Part E, Supplemental Guidance for Dermal Risk Assessment) Interim Guidance, vol. I. Office of Emergency and Remedial Response, Washington, DC.

Geinoz, S., Guy, R.H., Testa, B., Carrupt, P.A., 2004. Quantitative structure–permeation relationships (QSPeRs) to predict skin permeation: a critical evaluation. Pharm. Res. 21, 83–92.

Hansch, C., Dunn, W.J., 1972. Linear relationships between lipophilic character and biological activity of drugs. J. Pharm. Sci. 61, 1–19.

Hostynek, J.J., Magee, P.S., 1997. Modeling in vivo human skin absorption. Quant. Struct.-Act. Relat. 16, 473–479.

Idson, B., 1983. Vehicle effects in percutaneous absorption. Drug Metab. Rev. 14, 207–222.

Monteiro-Riviere, N.A., 1991. Comparative anatomy, physiology, and biochemistry of mammalian skin. In: Hobson, D.W. (Ed.), Dermal and Ocular Toxicology: Fundamentals and Methods. CRC Press, Boca Raton, pp. 3–71.

Moss, G.P., Dearden, J.C., Patel, H., Cronin, M.T.D., 2002. Quantitative structure–permeability relationships (QSPRs) for percutaneous absorption. Toxicol. In Vitro 16, 299–317.

Potts, R.O., Guy, R.H., 1992. Predicting skin permeability. Pharm. Res. 9, 663–669.

Qiao, G.L., Brooks, J.D., Baynes, R.E., Monteiro-Riviere, N.A., Williams, P.L., Riviere, J.E., 1996. The use of mechanistically defined chemical mixtures (MDCM) to assess component effects on the percutaneous absorption and cutaneous disposition of topically-exposed chemicals: I. Studies with parathion mixtures in isolated perfused porcine skin. Toxicol. Appl. Pharmacol. 141, 473–486.

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

Riviere, J.E., Qiao, G.L., Baynes, R.E., Brooks, J.D., Mumtaz, M., 2001. Mixture component effects on the in vitro dermal absorption of pentachlorophenol. Arch. Toxicol. 75, 329–334.

Riviere, J.E., Baynes, R.E., Brooks, J.D., Yeatts, J.L., Monteiro-Riviere, N.A., 2003. Percutaneous absorption of topical diethyl-m-toluamide(-DEET): effects of exposure variables and coadministered toxicants. J. Toxicol. Environ. Health A 66, 131–151.

Rosado, C., Cross, S.E., Pugh, W.J., Roberts, M.S., Hadgraft, J., 2003. Effect of vehicle pretreatment on the flux, retention, and diffusion of topically applied penetrants in vitro. Pharm. Res. 20, 1502–1507.

Sartorelli, P., Aprea, C., Cenni, A., Novelli, M.T., Orsi, D., Palmi, S., Matteucci, G., 1998. Prediction of percutaneous absorption from physiochemical data: a model based on data of in vitro experiments. Ann. Occup. Hyg. 42, 267–276.

van der Merwe, D., Riviere, J.E., 2004a. Effect of vehicles and sodium lauryl sulfate on xenobiotic permeability and stratum corneum partitioning in porcine skin. Toxicology 206, 325–335.

van der Merwe, D., Riviere, J.E., 2004b. Comparative studies on the effect of water, ethanol, and water/ethanol mixtures on chemical partitioning into porcine stratum corneum and silastic membrane. Toxicol. In Vitro 19, 69–77.