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Membrane-Coated Fiber Array Approach for Predicting Skin Permeability of Chemical Mixtures from Different Vehicles

Jim E. Riviere,[1] Ronald E. Baynes, and Xin-Rui Xia

Center for Chemical Toxicology Research and Pharmacokinetics (CCTRP), College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina 27606

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A membrane-coated fiber (MCF) array approach was developed for quantitative assessment of skin absorption from chemical mixtures, which was based on the similarity in the absorption mechanisms of the MCF membrane and the stratum corneum of the skin. A set of probe compounds were used to detect the relative molecular interaction strengths of chemicals with the vehicle and the membranes, which provided a linkage between the skin permeability (log k) and MCF partition coefficients (log K_F). A predictive model was established via multiple linear regression analysis of the data matrix of experimentally measured log k value and log K_{Fm} values; $\log k = a_0 + a_1 \log K_{F1} + a_2 \log K_{F2} + \ldots + a_n \log K_{Fm}$, where m is the number of diverse MCFs. Twenty-five probe compounds and three MCFs (polydimethylsiloxane for lipophilic, polyacrylate for polarizable, and CarboWax for polar interactions) were used to demonstrate the model development processes in the MCF array approach. The skin permeability of the probe compounds was measured with conventional diffusion cell experiments using dermatomed porcine skin. Three predictive models were established for skin permeability prediction from chemical mixtures in water, 50% ethanol, and 1% sodium lauryl sulfate (SLS) with R^2 values of 93, 91, and 83, respectively. The log k and log K_F values were considerably altered by the addition of ethanol or SLS into the dose vehicle; however, their correlations to skin permeability remained strong under various conditions. These results suggested that the experimentally based MCF array approach can be used to predict skin absorption from chemical mixtures in different vehicles or formulations.

Key Words: **chemical mixtures; percutaneous absorption; predictive model; QSAR; solvent effect; vehicle effect.**

Assessment of skin absorption from complex chemical mixtures remains a challenging problem in spite of the increasing knowledge of the barrier function and biological structure of the skin. There are thousands of chemicals, drugs, pharmaceuticals, and cosmetics that result in potential exposure to enormous chemical combinations at various concentration levels and vehicles. It is cost prohibitive to study the skin absorption of all these mixtures using conventional experimental methodologies (Bronaugh and Maibach, 1999; Bronaugh *et al*., 1982; Franz, 1975). A general strategy is to seek the relationships of skin permeability with the physicochemical parameters of the chemicals. Several quantitative structure–permeability relationships (QSPRs) have been derived (Geinoz *et al*., 2004; Moss *et al*., 2002). The first widely accepted model was developed by Potts and Guy (1992), which incorporated lipophilicity (measured by octanol/water partition coefficient, log $K_{o/w}$) and molecular weight to predict skin permeability. The role of lipophilicity in skin absorption has been demonstrated by most of the studies. El Tayar *et al*. (1991) identified that hydrogen bonding plays a significant role while molecular size is not significant in skin absorption. The importance of molecular interactions including hydrogen bonding, dipolarity/polarizability, and van der Waals force is demonstrated in Abraham's solute descriptor approach for skin permeability predictions (Abraham *et al*., 1995, 1999).

Most of the prior developed models are based on compiled literature data, which could have significant variations due to interlaboratory variability using different experimental protocols and skins from different sources and anatomic locations (Moss and Cronin, 2002; Moss *et al*., 2002). Furthermore, the experimental data were measured for individual chemicals, not for chemical mixtures (Flynn, 1990). There are limited experimental data for chemical mixtures in the published literature due to the complexity of chemical mixtures. Riviere and Brooks (2005, 2007) have developed a predictive model for chemical mixtures using experimental data from various dose vehicles. This work demonstrated the promise in prediction of skin absorption from complex chemical mixtures.

We have developed a membrane-coated fiber (MCF) array approach for predicting skin permeability from an aqueous vehicle (Xia *et al*., 2007). The MCF array approach is an experimentally based methodology that utilizes a high-throughput MCF technique to measure the physicochemical parameters required for model development; no literature data or molecular structure information is required. In the MCF technique, a polymer membrane coated onto a fiber is used as the absorption membrane to determine the partition coefficients of chemicals

[1] To whom correspondence should be addressed at CCTRP/CVM, North Carolina State University, 4700 Hillsborough St., Raleigh, NC 27606-8401. Fax: (919)513-6358. E-mail: jim_riviere@ncsu.edu.

from any liquid vehicle (Xia *et al*., 2003). The MCF technique integrates the membrane absorption and quantitative analysis into one step and fully utilizes the separation power of the automatic chromatographic instruments (gas chromatography [GC] or high-performance liquid chromatography). It completely eliminates the emulsion problem and the other error sources associated with sample treatment and handling in liquid–liquid systems, such as in measuring log $K_{o/w}$ values (OECD, 1981). These features allow the MCF technique to have greater sensitivity, accuracy, and high throughput in the quantitative assessment of the mixture effects.

The utilization of the MCF array approach for skin permeability prediction is based on the similarity in the absorption mechanisms of the MCF membrane and the stratum corneum of the skin. Several types of molecular interactions were identified to be the primary factors in skin absorption: lipophilic, hydrogen bonding, and polarizable π*-electron interactions (Moss *et al*., 2002). These molecular interactions could be simulated with an array of MCFs having diverse physicochemical properties. A set of probe compounds is used to detect the relative molecular interaction strengths of chemicals with the vehicle and the stratum corneum or the MCF membranes, which provide the linkage between the skin permeability (log k) and MCF partition coefficients (log K_F). A predictive model is established via multiple linear regression analysis of the data matrix of experimental measured log k value and log K_{Fm} values;

$$\log k = a_0 + a_1 \log K_{F1} + a_2 \log K_{F2} + \cdots + a_m \log K_{Fm}, \quad (1)$$

where m is the number of diverse MCFs. The values of [a_0, a_1, a_2, ... a_m] are characteristic properties of the absorption system (the skin, the MCFs, and the vehicle), therefore, denoted as system coefficients in the following context. The system coefficients of a given absorption system can be obtained via multiple linear regression analysis of the data matrix [log k: log K_{F1} log K_{F2} ... log K_{Fm}]$_n$, where ''n'' is the number of the probe compounds. Once a robust set of system coefficients are obtained, skin permeability for compounds of interest can be estimated via the predictive model (Equation 1) by only measuring log K_{Fm} values of the compounds with the multiple MCFs; no conventional diffusion experiment using human or animal skin is required. This not only reduces the animal usage, but also reduces the cost in studying complex chemical mixtures, particularly for large scale assessments (Cassee *et al*., 1998; USEPA, 2000).

In more complex scenarios, the major components or vehicle of the chemical mixtures may vary and influence the skin absorption from chemical mixtures such as pharmaceutical and cosmetic formulations or various industrial formulations in occupational exposures. The complex formulation and vehicle effects on skin absorption were seldom considered in most of the existing predictive models (Moss *et al*., 2002; Riviere and Brooks, 2005, 2007). In this study, it is hypothesized that the system coefficients will be altered when the major components of the vehicle are changed in composition or proportion. Therefore, the changes in the system coefficients can be used to account quantitatively for the formulation and vehicle effects. The purpose of the present study is to verify this hypothesis and to explore the feasibility of the MCF array approach for predicting skin permeability from chemical mixtures in different vehicles.

MATERIALS AND METHODS

Chemicals and materials. Acetone (GC grade), ethanol (200 proof), and sodium lauryl sulfate (SLS) (99% in purity) were purchased from Sigma-Aldrich (St Louis, MO). Deionized water was prepared from a Picotech Water System (Research Triangle Park, NC). A set of probe compounds having purity better than 98% were purchased from Sigma-Aldrich. 100-μm polydimethylsiloxane (PDMS), 85-μm polyacrylate (PA), and 50-μm carbowax/template resin (Wax) fiber assembles were purchased from Supelco (Bellfonte, PA).

Individual stock solutions with a concentration of 10 mg/ml in acetone were prepared for each of the probe compounds. A standard mixture in acetone containing the probe compounds with a concentration of 100 μg/ml for each component was prepared from the individual stock solutions. A series of standard solutions in acetone were prepared from the standard mixture to be used as external calibration standards for GC/MS analysis. Some of the probe compounds were volatile and some of them were toxic. All of the solution preparation processes were conducted in a fume hood with gloves and goggles.

Flow-through diffusion cell experiments. The skin permeability of the probe compounds was measured by using a flow-through diffusion cell system (Bronaugh and Stewart, 1985). Porcine skin was obtained from the dorsal area of weanling female Yorkshire pigs. The skin was dermatomed to a thickness of 350 μm with a Padgett Dermatome (Kansas City, MO). Each circular skin section was punched out and placed into a two-compartment Teflon flow-through diffusion cell. The skin membranes were perfused using Krebs–Ringer bicarbonate buffer spiked with dextrose and bovine serum albumin of 4.5 (wt/vol) %. The temperature of the perfusate and flow-through cells was maintained at 37°C using a Brinkman circulator (Westbury, NY). The pH of the receptor solution was maintained between 7.3 and 7.5. The flow rate of the receptor solution was 4.0 ml/h. After careful release of any air-bubbles underneath the skin in the receptor compartments, blank samples were collected before dose. The chemical mixture in water, 1 (wt/vol) % SLS and 50 (vol/vol) % ethanol (500 μl) were dosed on the skin in the donor compartment, and sampled every 30 min throughout the 8-h diffusion experiments. The concentrations of different probe compounds in dose vehicles were in the range of 6–160 μg/ml to ensure that the dosing concentrations were below the solubility of the compounds at 20°C. The pH of the dosing solutions was in the range of 5.5–6.5. The concentrations of the probe compounds in the collected samples were analyzed by a headspace/solid-phase microextraction (SPME) and GC/MS method.

Headspace/SPME GC/MS analyses. A Combi PAL automatic sampler was used to perform the headspace/SPME analysis. The sample vials containing 1 ml of the receptor solutions sampled at different time points was transferred into an incubator and shaken at 500 rpm for 10 min to equilibrate the sample temperature to 60°C. A preconditioned PDMS/divinylbenzene (DVB) fiber was inserted into the headspace of the sample vial. The chemicals in the gas phase equilibrating with those in the sample phase were absorbed into the stationary phase of the fiber. The headspace absorption was held static at 60°C for 20 min; then the fiber was removed from the sample vial and injected into the GC/MS for quantitative analysis. The quantitative analysis was calibrated with a set of external calibration solutions. The external calibration solutions were prepared

by stepwise dilution of the dosing solutions with the receptor solution, which ensured the matrixes of the calibration solutions were similar to those of the collected samples in headspace/SPME analysis.

Determination of the partition coefficients. The partition coefficients of the probe compounds were determined with three MCFs (100-μm PDMS, 85-μm PA, and 50-μm Wax). The 100-μm PDMS fibers were conditioned at 250°C for 30 min and 85-μm PA fiber at 300°C for 2 h as recommended by the manufacture. The 50-μm Wax fibers were preconditioned at 220°C for 30 min. A Combi PAL automatic sampler (CTC Analytics, Switzerland) was used to perform the partitioning experiments. The detailed procedures for measuring partition coefficients using the MCF technique were described elsewhere (Xia *et al*., 2003). Here briefly, a glass vial containing 8.0 ml of the working solution was transferred into an incubator and shaken at 500 rpm for 5 min to equilibrate the sample temperature to 37°C. A preconditioned MCF was immersed into the working solution to start the absorption experiment under constant stirring at 400 rpm and 37°C. For a given period of time, the fiber was removed from the vial and transferred into the injector of a gas chromatograph for quantitative analysis. The concentrations of the probe compounds in the aqueous working solution were optimized for quantitative analysis in the range of 0.01–2 μg/ml. The partition coefficients of the probe compounds were also measured using the three MCFs (PDMS, PA, and Wax) in aqueous solutions containing 0, 10, 25, 40, and 50 (vol/vol) % of ethanol or 0, 0.1, 0.5, 1, 2, and 5 (wt/vol) % of SLS.

Quantitative analysis. The quantitative analysis of the chemicals was performed with a Varian GC/MS 4000 equipped with ion trap mass selective detector. A Combi PAL automatic sampler was used for liquid injection, fiber absorption, and headspace experiments. The injection port was maintained at 280°C when using PDMS and PA fibers, 250°C and 270°C when using Wax and PDMS/DVB fibers, respectively. These temperatures were selected for optimal thermal desorption and useful life of the fibers. The analytical conditions were improved to reduce analytical time and increase analytical sensitivity. Separation was performed on a 30 m× 0.25 mm (i.d.)× 0.25 μm (df) HP-5MS capillary column (Agilent, Palo Alto, CA). The column oven was programmed as follows: the initial temperature was 40°C and held for 1 min, ramped at 20°C/min to 60°C and 3°C/min to 97°C, held at 97°C for 3.5 min, then ramped at 20°C/min to 200°C and 40°C/min to 250°C, and finally held at 250°C for 5 min. An electronic pressure control was used to maintain a carrier gas flow of 1 ml/min helium.

Data analysis. The permeability coefficient of a chemical through the skin membrane (k) is calculated via the following equation:

$$k = \frac{J_{ss}}{AC_d} \tag{2}$$

where J_{ss} is the steady-state flux (μg/h), A is the dose area (0.64 cm^2), and C_d is the concentration of the chemical in the donor solution (μg/ml). The resulting unit for the permeability coefficient (k) is cm/h. The steady-state flux was the slope of the accumulation absorption amounts at different time points (Addicks *et al*., 1987).

The partition coefficient of a calibration compound in a given absorption system (log K_F) was calculated from the equilibrium absorption amount ($n°$) by the definition of the partition coefficient (Xia *et al*., 2003):

$$K_F = \frac{C_{pe}}{C_{me}} = \frac{n^o V_m}{V_p\left(V_m C_o - n^o\right)} \tag{3}$$

where C_o is the initial concentration of the compound in the working solution; V_m is the volume of the working solution; V_p is the volume of the MCF membrane (V_p = 0.612 μl for 100-μm PDMS, 0.520 μl for 85-μm PA, and 0.330 μl for 50-μm Wax); C_{pe} is the equilibrium concentration in the membrane ($C_{pe} = n°/V_p$); and C_{me} is the equilibrium concentration in the working solution ($C_{me} = C_o - n°/V_m$).

The compounds having a complete set of log k and log K_F values were used for statistical analysis. The multiple linear regression analysis was performed by using SAS Analyst from SAS Institute, Inc. (Cary, NC).

RESULTS

Skin Permeability of the Calibration Compounds

The skin permeability was measured by traditional diffusion experiments using dermatomed porcine skin. Figure 1 shows the absorption profiles (absorption amounts in the receptor solutions versus time) of four compounds in 50% ethanol vehicle; these four compounds were selected to be representatives across the entire chromatogram of the 32 probe compounds for illustrating the data handing and analysis. The error bars for the absorption profiles were the standard error of measurement from four diffusion cells (n = 4).

The steady-state flux (J_{ss}) of a compound across the skin was obtained from the slope of the cumulative amounts versus time profile (Fig. 2). The skin was occluded over the 8-h experiment, but the skin integrity was maintained known from the steady-state flux of the compounds. The J_{ss} values of chlorobenzene, iodobenzene, naphthalene, and biphenyl were 0.487, 0.427, 0.175, and 0.061, respectively. The skin permeability (log k) of the probe compounds was obtained via Equation 2 from the donor concentration (C_d) and the steady-state flux (J_{ss}).

Figure 3 shows the skin permeability of the full set of 25 probe compounds from water, 1% SLS, and 50% ethanol. The compounds were depicted in the order of permeability from water. Addition of SLS or ethanol into the dose vehicle considerably decreased the permeability of the probe compounds. The SLS effect on the skin permeability was less significant for compounds on the left side of benzonitrile. The effect of 50% ethanol on the permeability was considerably

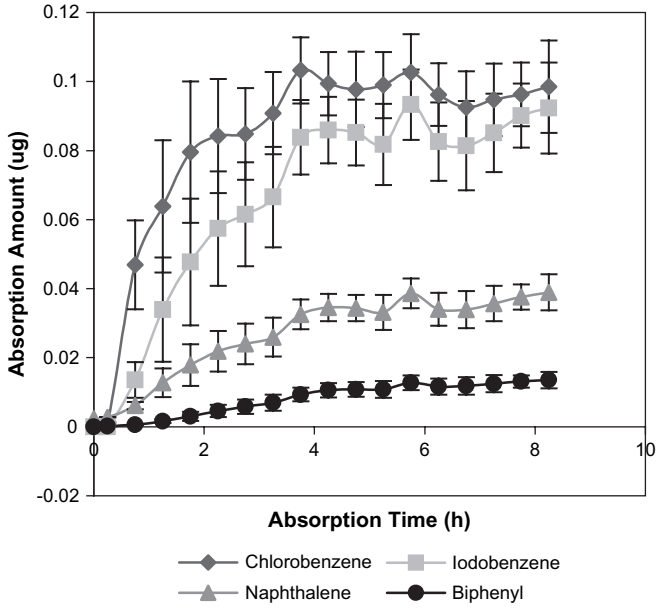

FIG. 1. Absorption profiles of chemicals through porcine skin. The four chemicals were selected to be chromatographically representative of the 32 chemicals studied. The dose vehicle was 50 (vol/vol) % ethanol–water solution.

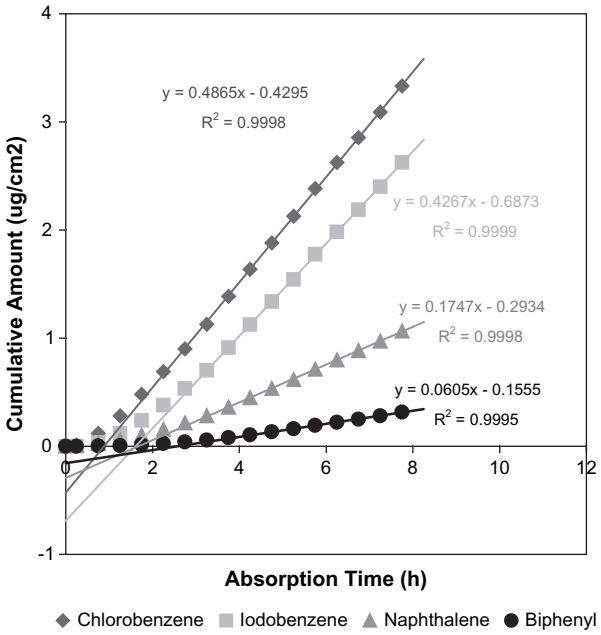

FIG. 2. The cumulative amount versus absorption time.

stronger than that of 1% SLS, while similar trends of modulations were observed for ethanol and SLS.

Partition Coefficients of the Probe Compounds

The absorption profiles of the four compounds by the Wax MCF in water solution are shown in Figure 4. The absorption equilibrium was achieved within 20 min for chlorobenzene and iodobenzene, within 60 min for naphthalene and biphenyl. The absorption equilibrium of the compounds was achieved earlier in ethanol or SLS solutions. The equilibrium absorption amounts ($n°$) were determined for each of the probe compounds with the three MCFs (PDMS, PA, and Wax) in aqueous solutions with different proportion of ethanol or SLS. The partition coefficient of a given compound was calculated from the equilibrium absorption amounts ($n°$) with Equation 3.

Figure 5 shows the effects of ethanol on the log K_{Wax} values of the four compounds. The partition coefficients of the compounds were reduced as the ethanol proportion increased. Linear decreases were observed in the plot of log K_{Wax} versus percentage concentration of ethanol (vol/vol %), while the slopes were slightly varied for different compounds. The quantitative relationships of log K_{Wax} value with ethanol proportion were obtained for all of the probe compounds in the chemical mixture, from which the log K_{Wax} value of a given compound at any ethanol proportion can be obtained. Similarly, the quantitative relationships between log K_{PA} and log K_{PDMS} versus ethanol proportions were also obtained from experimental measured log K_{PA} and log K_{PDMS} at different proportion of ethanol.

Figure 6 shows the effects of SLS surfactant on the partition coefficients of the four compounds by Wax MCFs. The log K_{Wax} values show linear relationship with logarithmic scale of the SLS percentage concentration (wt/vol %) and two linear sections intersecting at the critical micelle concentration (CMC). The CMC of SLS is about 0.25% (Zhao *et al*., 2004). Before the CMC point, the log K_{Wax} values were not significantly affected, while the log K_{Wax} values decreased linearly with increasing SLS concentrations after the CMC. The quantitative

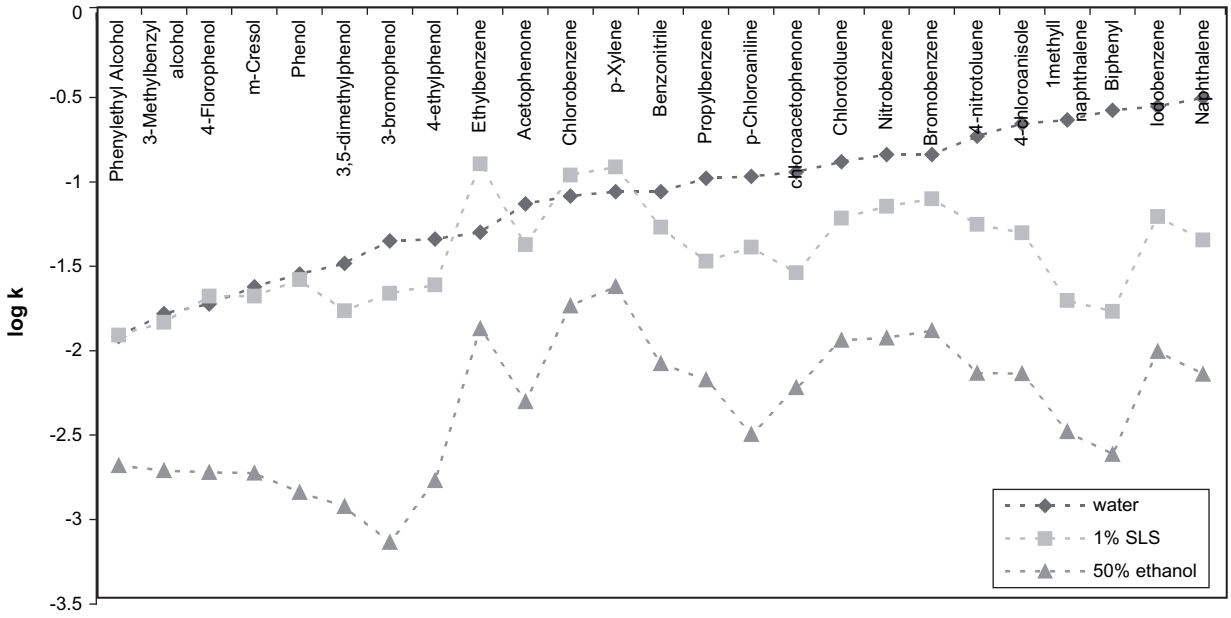

FIG. 3. Porcine skin permeability of diverse compounds from water, 1% SLS, and 50% ethanol.

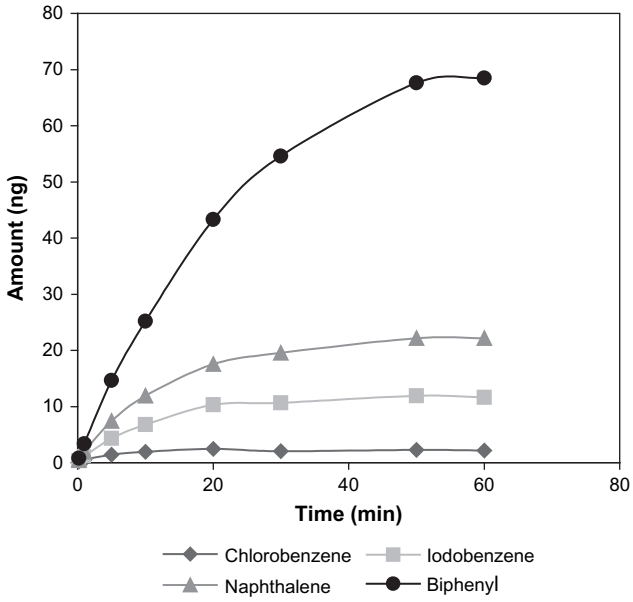

FIG. 4. Absorption kinetics of the Wax MCF.

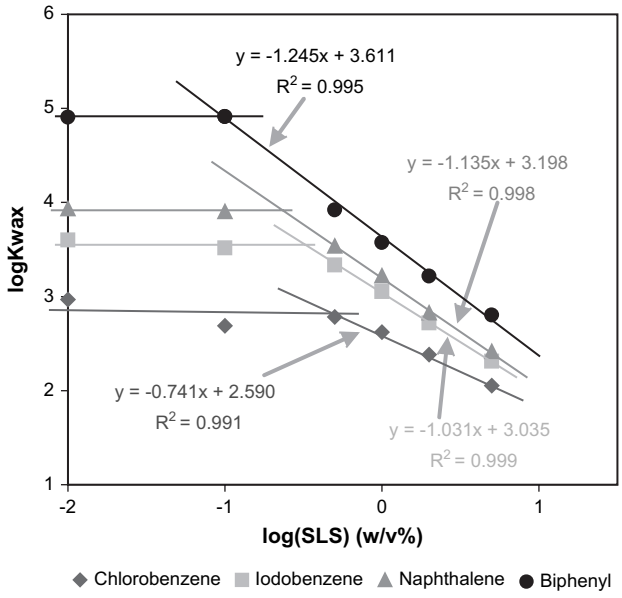

FIG. 6. Effects of SLS on log K_{Wax} values.

relationships of log K_{Wax} values with logarithmic SLS concentrations were obtained for all of the compounds in the chemical mixture, from which the log K_{Wax} value of a given compound at any SLS concentration can be obtained. Similarly, the quantitative relationships between log K_{PA} and log K_{PDMS} versus logarithmic SLS concentrations were also obtained from experimental measured log K_{PA} and log K_{PDMS} at different concentrations of SLS solutions.

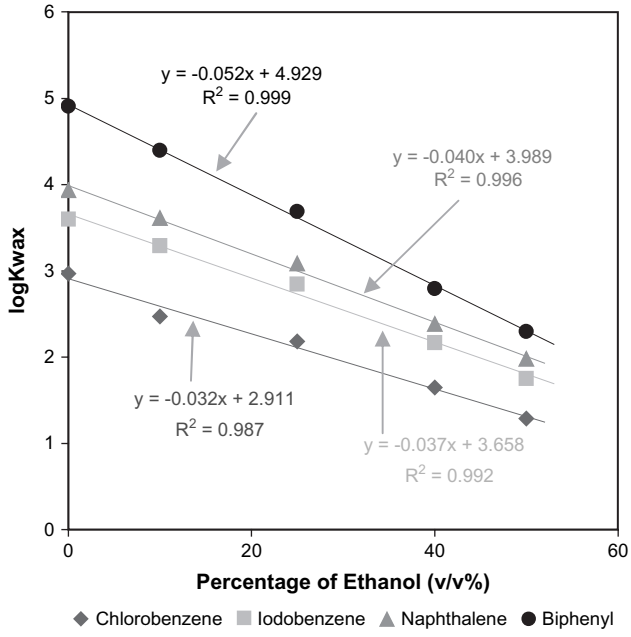

FIG. 5. Effect of ethanol on log K_{Wax} values.

Correlation of Skin Permeability with MCF Partition Coefficients

The receptor solution, composed of 4.5 (wt/vol) % bovine serum albumin in a Krebs–Ringer bicarbonate buffer, was formulated to mimic the microvascular circulation of the skin. The quantitative analysis of phenyl acetate, 3-chlorophenol and benzyl alcohol was interfered by the impurities of the albumin-containing media. The benzoate compounds (methyl, ethyl, and methyl 2-methyl) were metabolized under the experimental conditions. The log k values were not obtained for these compounds. The initial 32 compounds were reduced to 25 that have a complete set of data for permeability and partition coefficients (Fig. 3).

The permeability coefficients of all 25 probe compounds in a dose vehicle containing 50% ethanol (log $k_{Skin/E50}$) were correlated with the partition coefficients of the compounds measured with three MCFs in 50% ethanol solutions (log $K_{PDMS/E50}$, log $K_{PA/E50}$, and log $K_{Wax/E50}$). A predictive model for the skin permeability of chemicals from 50% ethanol was established via multiple regression analysis of the matrix [log $k_{Skin/E50}$: log $K_{PDMS/E50}$, log $K_{PA/E50}$, log $K_{Wax/E50}$];

$$\log k_{Skin/E50} = -1.18 + 0.36 \log K_{PDMS/E50} + 0.80 \log K_{PA/E50} - 1.32 \log K_{Wax/E50},$$
$$n = 25,\ R^2 = 0.91,\ s = 0.133,\ F = 75. \qquad (4)$$

Figure 7 shows the experimental log k values versus predicted log k values with the three MCFs in 50% ethanol solutions. A linear correlation is observed with a R^2 of 0.91. The standard residuals of the multiple liner regression analysis versus predicted log k values are also depicted in Figure 7. The

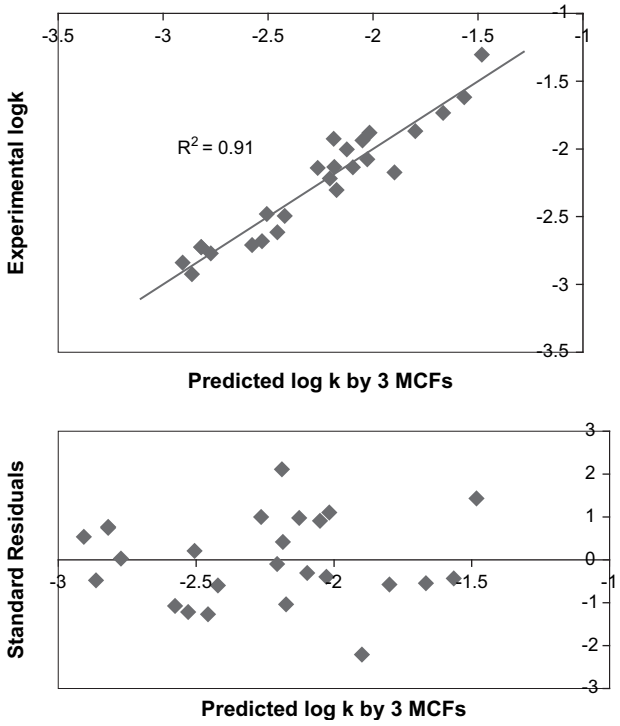

FIG. 7. The predicted log k values by the three MCFs versus the experimental log k values of the 25 probe compounds in 50% ethanol (top), and the predicted log k values versus the standard residuals of the correlation of the experimental log k values with log $K_{PDMS/E50}$, log $K_{PA/E50}$, and log $K_{Wax/E50}$ values (bottom).

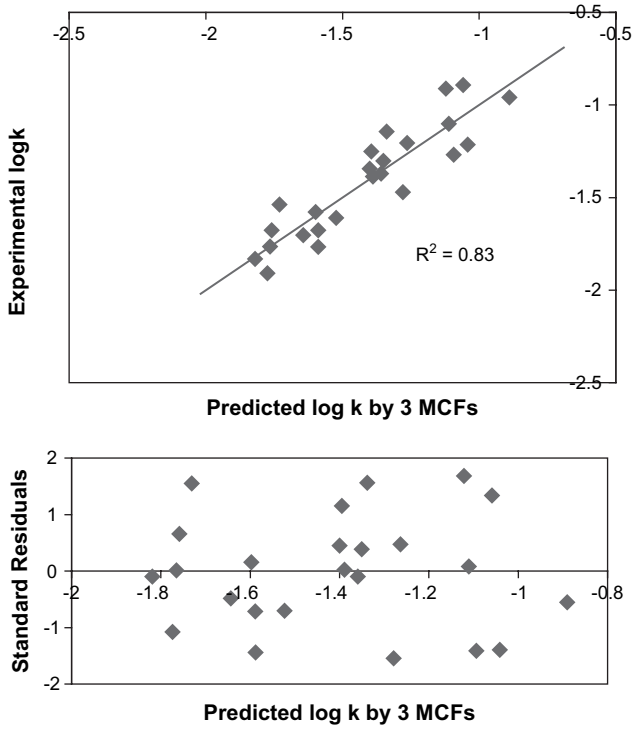

FIG. 8. The predicted log k values by the three MCFs versus the experimental log k values of the probe compounds in 1% SLS (L1) (top), and the predicted log k values versus the standard residuals of the correlation of the experimental log k values with log $K_{PDMS/L1}$, log $K_{PA/L1}$, and log $K_{Wax/L1}$ values (bottom).

random distribution of residuals revealed that the model adequately fitted the experimental data (Equation 4).

The permeability coefficients of the probe compounds in a dose vehicle containing 1% SLS (log $k_{Skin/L1}$) were correlated with the partition coefficients of the compounds measured with three MCFs in 1% SLS solutions (log $K_{PDMS/L1}$, log $K_{PA/L1}$, and log $K_{Wax/L1}$). The quantitative analysis of toluene was interfered in SLS dose solutions, so that only data for 24 probe compounds were used for regression analysis. A predictive model for the skin permeability of chemicals from 1% SLS was established via multiple linear regression analysis of the data matrix [log k: log $K_{PDMS/L1}$, log $K_{PA/L1}$, log $K_{Wax/L1}$):

$$\log k_{Skin/L1} = -0.56 + 0.38 \log K_{PDMS/L1} + 1.35 \log K_{PA/L1} - 1.48 \log K_{Wax/L1},$$
$$n = 24,\ R^2 = 0.83,\ s = 0.133,\ F = 31. \quad (5)$$

Figure 8 shows the experimental log k values versus predicted log k values with the three MCFs in 1% SLS solutions. A linear correlation is observed with a R^2 of 0.83. The standard residuals of the multiple liner regression analysis versus predicted log k values are also depicted in Figure 8. The random distribution of residuals revealed that the model adequately fitted the experimental data (Equation 5).

A predictive model for the skin permeability of chemicals from water with the MCF partition coefficients of the chemical in water was obtained as described previously (Xia *et al.*, 2007);

$$\log k_{Skin/W} = -2.34 - 0.124 \log K_{PDMS/W} + 1.91 \log K_{PA/W} - 1.17 \log K_{Wax/W},$$
$$n = 25,\ R^2 = 0.93,\ s = 0.101,\ F = 93. \quad (6)$$

DISCUSSION

MCF Array for Vehicle Effects

The skin permeability of the 25 compounds was well correlated with the three MCF partition coefficients in water (Equation 6); and good correlations were also observed in 50% ethanol and 1% SLS dose vehicles (Equations 4 and 5). Comparing Equations 4 and 5 with Equation 6, it is noted that the system coefficients were considerably altered due to the addition of 50% ethanol or 1% SLS, respectively; but the linear correlation of log k with log K_F values was maintained under various conditions. These results support the hypothesis that the skin permeability is correlated with the MCF partition

coefficients in complex chemical mixtures with different vehicles; and the formulation and vehicle effects were reflected by the changes in the system coefficients. Thus, the MCF array approach can be used to predict skin permeability from chemical mixtures in different vehicles.

The skin permeability was reduced considerably when ethanol or SLS was added into the dose vehicle (Fig. 3). The polar or less lipophilic compounds (on left-side of benzonitrile) were not significantly affected by SLS. The MCF partition coefficients were also altered by ethanol or SLS; for a given compound, the log K values were linearly decreased with the ethanol percentage concentration (Fig. 5) or logarithmic SLS concentration (Fig. 6), but the slopes varied for different compounds. Similar complex effects of vehicle and formulations have been described in the literature, but quantitative assessment of the effects is challenging (Van der Merwe and Riviere, 2005). Most of the existing predictive models were developed for individual chemicals in water solution or single solvent vehicles; complex formulation and vehicle effects were not considered (Geinoz *et al*., 2004; Moss *et al*., 2002). Riviere and Brooks (2005, 2007) have introduced a mixture factor to represent the mixture effects in their hybrid QSPR approach.

Selection of the MCFs

The skin permeability was well predicted with the three MCFs under various dose conditions (Equations 4–6, Figs. 7 and 8). These results suggest that the three MCFs reflected the molecular interactions relevant to skin absorption. Several types of molecular interactions (lipophilic, hydrogen bonding, and polarizable π*-electron interactions) are the primary forces governing the skin absorption of chemicals (Moss *et al*., 2002). The three MCFs were selected to represent these primary molecular interaction forces: PDMS is a lipophilic membrane widely used as a substitution for skin to study skin absorption of chemicals (Flynn and Yalkowsky, 1972; Geinoz *et al*., 2002); it has high thermal stability so that the absorbed compounds to be thermally desorbed in a GC injector for quantitative analysis (Xia *et al*., 2003). PA is characterized by heavy π*-electrons for polarizable interactions (Xia *et al*., 2004) and was used for biomimetic applications for measuring nonselective absorption of organic chemicals (Leslie *et al*., 2002). CarboWax is a polyethylene glycol polymer membrane having characteristic hydrogen bonding donor and acceptor interactions.

Criteria for the Probe Compounds

The probe compounds were used to detect the relative molecular interaction strengths of the absorption system, which provides a quantitative linkage between skin permeability and MCF partition coefficients in the MCF array approach. The selection of the probe compounds is critical for developing a robust predictive model. (1) The probe compounds should cover wide strength-ranges of the molecular interactions, which will eventually determine the application range of the system coefficients. For a specific application, the strength-ranges of the molecular interactions should cover the chemicals of interest. (2) The probe compounds should be added into the chemical mixture in trace or minor concentrations not affecting the system coefficients. Analytical methods should be available for their quantitative analysis. (3) The probe compounds should be chemically stable; metabolism and specific biological interactions are negligible during the absorption processes. (4) Passive diffusion is the main driving force in the absorption processes (Wester and Maibach, 1983).

The probe compounds used in this study were selected for diverse physicochemical properties, which were recommended as calibration compounds for determining the selectivity of micelle electrokinetic chromatography (Poole *et al*., 1998). Although the MCF approach was successfully demonstrated using these probe compounds, these compounds may not be suitable for other applications, for example, limited range of hydrophobicity or molecular weight. An appropriate set of probe compounds is required to meet the criteria for specific applications using the MCF array approach.

The MCF Array Approach

The MCF array approach is an experimental-based methodology for developing predictive model of skin permeability from chemical mixtures. No literature data or molecular structure information is required, in contrast to most of the existing QSAR predictive models based on compiled literature data. This eliminated the serious error sources from interlaboratory data due to different experimental protocols and skin from different sources and anatomic locations. The MCF partition coefficients of chemicals can be measured with high throughput and reproducibility, which allows higher prediction accuracy to be achieved. In the present study, the correlation coefficients (R^2) of skin permeability with the MCF partition coefficients from water, 50% ethanol and 1% SLS were 93, 91, and 83, respectively (Equations 4–6); of which most of the errors could be originated from the skin permeability measurement of the probe compounds using conventional diffusion experiments (Fig. 1). The difficulties in measuring the skin permeability were discussed in a review paper (Moss *et al*., 2002). It should be noted that the correlation coefficient (R^2) was 0.66 in the refined model for skin permeability estimation in the final version of the Supplementary Guidance for Dermal Risk Assessment (USEPA, 2004), which is empirical model based on compiled data from 15 literature sources over three decades (Moss *et al*., 2002).

In the model development, the skin permeability and MCF partition coefficients were measured in chemical mixtures, i.e., all of the probe compounds were measured simultaneously in a same solution. Errors due to batch-to-batch experiments using individual chemicals were eliminated. This is one of the advantages of the MCF array approach that all of the probe

compounds were measured under identical experimental conditions. The log k or log K_F values were only dependent on the relative molecular interaction strengths of the chemical with the vehicle and the stratum corneum or the MCF membranes. Subtle differences in molecular interactions could be differentiated by using such experimental methods (Kong *et al*., 2005). The subtle changes in system coefficients for varying concentrations of ethanol or SLS surfactant were detected with the MCF approach (Xia *et al*., 2005).

The present study demonstrated that the MCF array may be a useful laboratory-based tool to estimate skin absorption from chemical mixtures in risk assessment or pharmaceutical formulation studies. The MCF array approach is based on the principle that passive diffusion is the primary barrier of chemical transport across skin, which has been demonstrated to be the case for many organic compounds (Moss *et al*., 2002). For some compounds, passive diffusion and active transport could coexist. When the passive diffusion flux is predicted by the MCF array approach, the active transport could be known by subtracting the passive diffusion flux from the total flux measured experimentally. Some conditions such as skin binding of the test compounds or ion transport through appendages may also confound interpretation of the MCF prediction. However, the MCF array provides an experimental method to assess specific formulation additives or mixture components modulate dermal absorption of chemicals of toxicological or pharmacological interests.

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