Development and Evaluation of Two-Parameter Linear Free Energy Models for the Prediction of Human Skin Permeability Coefficient of Neutral Organic Chemicals

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Graphical Abstract
Abstract

The experimental values of skin permeability coefficients, required for dermal exposure assessment, are not readily available for many chemicals. The existing estimation approaches are either less accurate or require many parameters that are not readily available. Furthermore, current estimation methods are not easy to apply to complex environmental mixtures. We present two models to estimate the skin permeability coefficients of neutral organic chemicals. The first model, referred to here as the 2-parameter partitioning model (PPM), exploits a linear free energy relationship (LFER) of skin permeability coefficient with a linear combination of partition coefficients for octanol-water and air-water systems. The second model is based on the retention time information of nonpolar analytes on comprehensive two-dimensional gas chromatography (GC×GC). The PPM successfully explained variability in the skin permeability data \((n = 175)\) with \(R^2 = 0.82\) and root mean square error \((RMSE) = 0.47\) log unit. In comparison, the US-EPA’s model DERMWIN exhibited an \(RMSE\) of 0.78 log unit. The Zhang model — a 5-parameter LFER equation based on experimental Abraham solute descriptors (ASDs) — performed slightly better with an \(RMSE\) value of 0.44 log unit. However, the Zhang model is limited by the scarcity of experimental ASDs. The GC×GC model successfully explained the variance in skin permeability data of nonpolar chemicals \((n = 79)\) with \(R^2 = 0.90\) and \(RMSE = 0.23\) log unit. The PPM can easily be implemented in US-EPA’s Estimation Program Interface Suite (EPI Suite™). The GC×GC model can be applied to the complex mixtures of nonpolar chemicals.
Key Words: Skin Permeability; Linear Free Energy Relationship (LFER) Modeling; Abraham Solvation Model; GC×GC model, Complex Mixtures, Dermal Permeability Coefficient Program (DERMWIN™).

1. Introduction

The skin, being the largest organ, is prone to exposure of organic chemicals found in environmental media \textsuperscript{1,2}, and occupational settings \textsuperscript{3}, and in consumer products \textsuperscript{4,5}. The permeability coefficient \((K_p)\) is a key parameter for the assessment of dermal exposure to these chemicals. Currently, the experimental data of \(K_p\), available in the public domain, are available for only a few hundred organic chemicals \textsuperscript{6}. Experimental methods based on various \textit{in vivo} and \textit{in vitro} techniques \textsuperscript{7} are expensive, laborious, and have ethical implications of animal-testing. Therefore, there is a growing interest in developing fast and easy estimation methods for skin permeability.

Estimation methods, based on quantitative structure-permeability relationships (QSPeRs), exploit the relationships between the permeability coefficient, and the descriptors of lipophilicity and diffusivity \textsuperscript{7}. Several QSPeRs were developed using octanol-water partition coefficient \((K_{o-w})\) and molecular weight \((MW)\) as the respective descriptors of lipophilicity and diffusivity \textsuperscript{8}. The dermal permeability modeling program (DERMWIN), developed by the United States Environmental Protection Agency (US-EPA), is built on one of such relations. The DERMWIN uses equation 1 for the estimation of skin permeability coefficient \((cm/h)\) for a diverse set of chemicals. This module is freely available in the Estimation Programs Interface (EPI) Suite\textsuperscript{TM} \textsuperscript{9}.

\[
\log K_p = -2.80 + 0.66 \log K_{o-w} - 0.0056 MW
\]  

(1)

The documentation of DERMWIN describes \(R^2 = 0.66\) for this model implying that the
two parameters, \( \log K_{o-w} \) and molecular weight (\( MW \)), are not enough to account for remaining 34\% variability in the skin permeation data. The model based on \( \log K_{o-w} \) and \( MW \) can yield errors up to one to two orders of magnitude compared to experimental data \(^{10}\). The inadequacy of DERMWIN may be attributed to the fact that the octanol is not an exact surrogate phase for the dermal lipid, and it does not reflect all types of interactions that chemicals experience with structural proteins present in the stratum corneum layer of skin. This requires for the improvement of the model by including a descriptor that would take care of the interactions not accounted for by the octanol phase.

Zhang and coworkers developed a poly-parameter Linear Free Energy Relationship (LFER) model (equation 2) based on Abraham solute descriptors to estimate skin – water permeability coefficients \(^{11}\). The Zhang model shows that intermolecular interaction parameters such as solute size, polarity/polarizability, hydrogen-bond interactions and ionizability of chemical play a significant role in the estimation of \( K_p \).

\[
\log K_p = -5.328(\pm 0.071) + 0.137(\pm 0.082)E - 0.604(\pm 0.057)S
-0.338(\pm 0.094)A - 2.428(\pm 0.090)B + 1.797(\pm 0.079)V - 1.485(\pm 0.121)J^+ + 2.471(\pm 0.113)J^- 
\]

\( n = 274, \quad R^2 = 0.866, \quad Q^2 = 0.858, \quad RMSE = 0.432 \)

In equation (2), \( E \) describes the polarizability of molecule, \( S \) shows the mix of polarity/polarizability interaction of the solute, \( A \) describes the hydrogen bond donating capacity, \( B \) denotes the hydrogen bond accepting capacity, \( V \) expresses the volume of a solute in McGowan characterization (\( \text{cm}^3/\text{mol} \)) /10, and \( J^+ \), \( J^- \) are descriptors which are specific for anions and cations respectively \(^{11-20}\). In equation (2), \( K_p \) is given in unit of
cm/s. The explanatory power of equation (2) is higher than that of DERMWIN but at the cost of expensive experimental input parameters. Experimental data of Abraham solute descriptors (ASDs) comprises of ≤ 8000 chemicals. This calls for a model that can accurately estimate $K_p$ for the chemicals for which the ASDs are not available.

Previous studies demonstrated the potential of chromatographic techniques such as liquid chromatography and micellar chromatography for the estimation of skin permeation. However, these techniques are not easy to apply on the complex mixtures. Comprehensive two-dimensional gas chromatography (GC×GC) is a powerful technique that is capable of separating hundreds of thousands of chemicals in complex mixtures. Scientists were able to identify known skin penetrants in environmental samples such as the household dust using comprehensive two-dimensional liquid chromatography coupled with time-of-flight mass spectrometry. In addition to its separation power, recent studies demonstrated the potential of GC×GC in chemical risk assessment. Several environmental partitioning and diffusion-related properties ($\log K$) of nonpolar complex organic mixtures were successfully estimated using LFER based on two solute parameters ($u_{1,i}$ and $u_{2,i}$), which were derived from the first- and second-dimension retention times of analytes on GC×GC chromatogram. The GC×GC model (equation 3) was first calibrated for 32 properties using a set of 79 nonpolar model chemicals, and then validated with a set of 52 nonpolar chemicals analyzed on the GC×GC instrument.

$$\log K = \lambda_1 u_{1,i} + \lambda_2 u_{2,i} + \lambda_3$$  \hspace{1cm} (3)

Where, $\lambda_1$, $\lambda_2$, $\lambda_3$ are specific to partitioning system. The power of GC×GC model is that the estimates of properties can be applied directly on to the detected nonpolar chemicals in environmental mixtures.
The skin permeability coefficient of a chemical through stratum corneum is related to the partition coefficient and diffusivity via equation 4.

\[ K_p = \frac{K_m D}{h} \]  

Equation 4

Where \( K_m \) is the partition coefficient between the stratum corneum and the vehicle, \( D \) is the effective diffusion coefficient of the chemical through the stratum corneum, and \( h \) is the apparent thickness of the stratum corneum. Previously, equation 3 was quite successful in predicting the aqueous diffusivity, and the partitioning of nonpolar chemicals from lipid and protein (important phases of stratum corneum) to water. Therefore, we expect that equation 3 can explain the variability in skin permeability of nonpolar chemicals.

In this study, we hypothesized following: 1) a linear combination of \( K_{o-w} \) and \( K_{a-w} \) better explains the variability of skin permeation data as compared to DERMWIN equation because \( K_{a-w} \) brings in significant information about hydrogen-bonding interaction, which is not sufficiently provided by the combination of \( K_{o-w} \) and \( MW \). 2) Given success of the GC\times GC model with rate-related properties in previous studies, the GC\times GC instrument provides suitable solute descriptors to model skin permeability of nonpolar complex mixtures.

2. Materials and Methods

2.1. Data Source and Analysis

The experimental values of skin permeability coefficient (\( K_p \)) comprising 247 chemicals were taken from compilation given in the previous work. We excluded ionized species from this data because our proposed models, PPM and GC\times GC model, can theoretically account for the intermolecular interactions for neutral organic chemicals only. This resulted
into a data size of 175 neutral organic chemicals, and is shown in Table S1 of Supporting Information (SI) along with the values of ASDs.

For calibration and evaluation of the PPM, the experimental $K_{o-w}$ and $K_{a-w}$ values were available only for 68 chemicals in $K_p$ dataset. Therefore, we calibrated the PPM with the $\log K_{o-w}$ and $\log K_{a-w}$ values estimated using Abraham Solvation Model (ASM) equations\textsuperscript{30,31}. The PPM was also evaluated with the input of experimental and EPI Suite\textsuperscript{TM} (KOWWIN ver 1.68 and HenryWin ver 3.2)\textsuperscript{9} estimated $K_{o-w}$ and $K_{a-w}$ values. The experimental and estimated values of $K_{o-w}$ and $K_{a-w}$ for the final datasets are shown in Table S2 of the SI. Besides $K_{o-w}$ and $K_{a-w}$, we included other descriptors such as molecular weight ($MW$), organic carbon to water partition coefficient ($K_{oc-w}$), bioaccumulation factor ($BCF$), diffusion constant for water ($D_w$) and for ethanol ($D_{ew}$) to inspect their explanatory power for the $K_p$ data.

The experimental dataset of $K_p$, used to develop the PPM model, was diverse and spanned 7 orders of magnitude (Table S1). The dataset contains chemicals with diverse structures and comprises of chemical families such as steroids, alcohol, acids, amines, amides, carbonyls, esters, urea, carboxylic acids, ether, halides, nitriles, nitro compounds and nonpolar organic compounds. The partition coefficients, $K_{o-w}$ and $K_{a-w}$, used for the calibration of the PPM, traversed diversified ranges.

For the GC×GC model, a two-parameter LFER equation was calibrated using retention parameters ($u_1, u_2$) that were estimated for a set of 79 model nonpolar chemicals following the approach developed elsewhere\textsuperscript{26,27}. Briefly, the model training set (Table S3) is formulated to span several nonpolar families in a balanced way. The solute parameters, $u_1$ and $u_2$ were obtained by applying the singular value decomposition (SVD) algorithm
on the Abraham solute parameters of 79 model nonpolar chemicals. These two new orthogonal solute parameters, $u_1$ and $u_2$, were used to develop 2-parameter linear free energy relationship (GC×GC model) for the estimation of $\log K_p$. Then, the GC×GC model was validated independently using a set of 52 nonpolar chemicals (Table S4), which were analyzed on the GC×GC instrument in previous studies 26,27. The experimental values of $K_p$ for the nonpolar chemicals in the training and validation sets for the GC×GC model were not available. Therefore, the values of $K_p$ were estimated using the ASM equation developed by Zhang et al 32 (Table S3 and S4 in SI). The approach used to develop the GC×GC model is further elaborated in Figure S1.

The training and validation datasets for the GC×GC model comprise of nonpolar chemicals only, which includes representatives of chemical families such as $n$-alkanes, cycloalkanes, cycloalkenes, halogenated alkanes, halogenated alkenes, benzene, linear alkylbenzenes, halogenated benzenes, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and polychlorinated naphthalenes (PCNs), and organochlorine pesticides.

2.2. Statistical Analysis

The statistical tests such as Multiple Linear Regression, Cross Validation Tests, Principle Component Analysis (PCA) were carried out using R – computational environment (3.5.3) 33 and XLSTAT (2018) 34. The selection of significant and optimum number of descriptors was done using step-wise multiple linear regression based on the statistical criteria such as Student's t-test, Akaike information criteria, Variance Inflation Factor. To delineate the domain of applicability, and to identify the influential values in the training datasets, the
regression diagnostics such as Studentized Residuals, Hat Values and Cook’s Distance were applied to each model (Table S5, S6, Figure S2, S3). Standard errors of beta-coefficients in all models were estimated using the bootstrapping technique (Table S7, S8). Cross-validation tests such as K-Nearest Neighbors, K-Fold, Repeated K-Fold \((r = 10)\), Leave One-Out and bootstrapping \((n = 1000)\) were performed for each model to evaluate the robustness (Table S9, S10). The PCA test was used to identify the contribution of all variables in the principal components.

3. Results and Discussion

3.1 Justification of 2P-LFER

As a starting point for developing a parsimonious LFER model, we propose that skin permeation of neutral organic chemicals may be adequately estimated by the use of only two parameters, \(K_{o-w}\) and \(K_{a-w}\). To explore this hypothesis, we analyzed the information content contained in Abraham solute descriptors (ASDs) of the training set used to develop the Zhang Model. For neutral organic chemicals, the Zhang model shows that five dimensions of information are needed to successfully explain the variability in the skin permeability data. However, the PCA on 175 × 5 matrix, \([E \ S \ A \ B \ V]\), of ASDs of the training set of the Zhang Model shows that the first two of total five dimensions encode 89.65 % of information (Figure 1a). The first dimension (principal component) is found to be formed by the linear combination of \(E, S, B,\) and \(V,\) with negligible contributions from \(A\) descriptor. The second dimension is represented mainly by \(A\) descriptor with very minor contribution from other ASDs (Figure 1b). This indicates the possibility for the
development of a parsimonious model based on two parameters without much loss of information.

Dimensionality analysis of the Zhang model set led us to the next important question of the study: what could be the two appropriate descriptors that would correspond to the first two dimensions of the PCA? The search for the appropriate descriptors started with the following considerations: these descriptors (i) should be easily accessible, (ii) have either large experimental database available or can easily be estimated using computationally-inexpensive but accurate methods, (iii) should sufficiently account for the changes in free energy due to transfer of molecule from water to skin. As shown below, the partition coefficients for octanol-water and air-water systems qualified for these considerations. To find the answer, we inspected the information loading resulting from the PCA of $175 \times 8$ matrix, $[E \ S \ A \ B \ V \ logK_{o-w} \ logK_{a-w} \ logK_p]$, in the principal components (Figure 1). The first two dimensions correspond to 80.9% variability of the dataset (Figure 1a). Skin permeability coefficient was partitioned almost entirely between the first two dimensions. The partition coefficients for octanol-water and air-water ($logK_{o-w}$ and $logK_{a-w}$) were also apportioned almost entirely in the first two dimensions, respectively (Figure 1c).
Figure 1. Dimensionality analysis for the PPM training set. Top panels show the results obtained by the Principal Component Analysis (PCA) ran on \(175 \times 5\) matrix, \([E S A B V]\), of Abraham solute descriptors for the training set of the Zhang Model in the form of (a) Scree Plot of eigenvalues (i.e., the amount of variation retained by each principal component), and (b) the correlation circle showing the relationship and quality of representation, square cosine (cos2), of variables in first two dimensions. Lower panels show (d) the distribution of quality of representation, Cos2, into the first five dimensions obtained by the PCA, and (e) the correlogram of the correlation matrix obtained respectively by the PCA and Pearson correlation analysis of \(175 \times 8\) matrix, \([E S A B V \log K_{o-w} \log K_{a-w} \log K_p]\). In Panel (b), the length of arrowed line from the origin shows the quality of representation of variable. Angles between the arrowed lines show
the degree of correlations: Descriptor A is almost orthogonal to E, S, B and V descriptors, which are mutually positively correlated. In Panel (c), color intensity and size of the circle are proportional to the quality of presentation of a variable. In Panel (d), Blue and Red color respectively show positive and negative correlations between the pair. The value of correlation coefficient for each pair of variables is shown in each square. All correlations, shown here, were statistically significant ($p < 0.05$). In Panel c and d, Dim. stands for the dimension.

The correlation plot of all variables ($K_p$, ASDs, $K_{o-w}$ and $K_{a-w}$) indicates that $K_{o-w}$ and $K_{a-w}$ captures the important intermolecular interactions, otherwise coded in the ASDs, to describe the $K_p$ (Figure 1d). Further, $\log K_{o-w}$ and $\log K_{a-w}$ are almost mutually orthogonal (Pearson correlation coefficient, $r = 0.09$), implying that both descriptors would deliver independent information to build a robust model for the skin permeability. Both descriptors, $\log K_{o-w}$ and $\log K_{a-w}$, shows strong correlations ($r = 0.61$ and $r = 0.72$, respectively) with $\log K_p$. Practically, the suitability of $K_{o-w}$ and $K_{a-w}$ is desirable because these properties have a wider experimental database and quicker estimation approaches than those available for the ASDs$^{9,30,31}$. Taken together, above results indicate that $K_{o-w}$ and $K_{a-w}$ are appropriate alternative parameters to describe the permeability variability for neutral organic molecules.

### 3.1.1 Two - Parameter Partitioning Model

The PPM, based on a relationship of $\log K_p$ with a linear combination of $\log K_{o-w}$ and $\log K_{a-w}$, successfully described 82% of variation in the $\log K_p$ data (equation 8 and Figure 2a).
\[ \log K_p = -5.41(\pm 0.08) + 0.46(\pm 0.03) \log K_{o-w} + 0.14(\pm 0.007) \log K_{a-w} \]  
(5)

\[ n = 175, \quad R^2 = 0.82, \quad \text{Adj.} R^2 = 0.82, \quad Q^2 = 0.81, \quad \text{RMSE} = 0.47 \]

\[ \text{PRESS RMSE} = 0.48 \]

Here, the values of \( K_{o-w} \) and \( K_{a-w} \), used to train equation 5, were estimated by the respective ASM equations \(^{30,31}\). Where, \( n \), \( R^2 \), \( \text{Adj.} R^2 \), \( Q^2 \), \( \text{RMSE} \) and \( \text{PRESS RMSE} \) respectively denote number of experimental values of \( \log K_p \), coefficient of determination, adjusted coefficient of determination, leave-one-out cross-validated \( R^2 \), root mean squared error and predicted residual error sum of squares, respectively.

Results of four independent cross-validation tests indicate that model is internally valid for predictive purpose (Table S8, S9). With the input of the limited experimental data of \( K_{o-w} \) and \( K_{a-w} \) values \((n = 68)\), equation 5 exhibited good agreement between the experimental and predicted values of \( \log K_p \) \((\text{RMSE} = 0.36 \log \text{units})\). Finally, we tested the performance of the PPM by inputting \( K_{o-w} \) and \( K_{a-w} \) values \((n = 175)\) that were estimated respectively from the KOWWIN 1.68 and HenryWin 3.2 modules of EPI Suite\(^{\text{Tm}}\) 4.1. This yielded an \( \text{RMSE} = 0.60 \log \text{unit} \), which is better than the one \((\text{RMSE} = 0.82)\) observed for the DERMWIN when compared with the same experimental data \((n = 175)\).

These statistics suggest that the PPM can be integrated in the EPI Suite\(^{\text{Tm}}\) as a better alternative to DERMWIN (Figure 2c).
Figure 2. Linear regression plot for (a) Two-parameter Partitioning Model (PPM), and (b) GC×GC Model. Upper and lower green lines bound 95% confidence interval around the regression line (dotted black line in the middle). Lower panels show (c) scatterplot obtained by comparing the prediction of \( \log K_p \) from three models, Zhang model (green triangles), DERMWIN (red square) and PPM (purple crosses), with the experimental values. Panel (d) shows the result of independent validation of the GC×GC Model obtained by comparing the predictions (green circles) for 52 nonpolar chemicals — which were analyzed on the GC×GC — with the predictions of the Zhang model. Predictions of DERMWIN (red squares) also shown for comparative purpose. In the lower panels, the dotted line in the middle shows 1:1 agreement, and upper and lower dotted lines indicate 1:2 agreement between the reference and predicted values.
For external validation, the PPM full dataset (n = 175) was split randomly into a training set (n = 140, Table S11) and a validation set (n = 35, Table S12). Equation 6 was derived using the training set of 140 compounds.

\[
\log K_p = -5.46 (\pm 0.09) + 0.47(\pm 0.03) \log K_{o-w} + 0.13 (\pm 0.008) \log K_{a-w} \quad (6)
\]

\[n = 140, \quad R^2 = 0.82, \quad \text{Adj.} R^2 = 0.82, \quad Q^2 = 0.81, \quad \text{RMSE} = 0.47\]

\[\text{PRESS RMSE} = 0.47, \quad n_{\text{external}} = 35, \quad R^2_{\text{external}} = 0.81, \quad \text{RMSE}_{\text{external}} = 0.48\]

The fitting coefficients and regression statistics of equation 6 are statistically similar to equation 5. Predictions of equation 6 compared favorably with the experimental data for the external validation set (\(R^2_{\text{external}} = 0.81, \quad \text{RMSE}_{\text{external}} = 0.48\)) (Figure S4).

Finally, we compared the explanatory power of \(K_{o-w}\) and \(K_{a-w}\) with that of other common physicochemical properties for describing the variance in \(K_p\) data. When stepwise regression algorithm was applied on all descriptors \((K_{o-w}, K_{a-w}, K_{oc-w}, BCF, D_w, D_{ethanol})\), as the explanatory variables of \(K_p\), only \(K_{o-w}\) and \(K_{a-w}\) were retained as statistically significant variables (Table S13). Two models, based on the linear combinations of \(\log K_{o-w}\) and \(\log D_w\), and of \(\log K_{o-w}\) and \(\log D_{ethanol}\), were identified with \(R^2 = 0.81\) and 0.79, and \(\text{RMSE} = 0.47\) and 0.50, respectively (Table S13). These models are not discussed further, since \(D_w\) and \(D_{ethanol}\) are not as widely accessible as are the \(K_{o-w}\) and \(K_{a-w}\).

The PPM shows that skin permeability increases with increase in \(K_{o-w}\) and \(K_{a-w}\). This is expected as octanol is considered as a good surrogate medium of lipid \(^{29}\). However, stratum corneum is not exclusively comprised of lipids but also contain structural proteins (keratins) among other biotic phases \(^{35}\), which play an important role in permeability \(^{36}\),
especially for the compounds exhibiting significant hydrogen bonding interactions\textsuperscript{37}. The octanol-water system is not as sensitive to hydrogen bonding interactions as is the air-water system. This is evident from Pearson’s correlations (Figure 1d) of $log K_{a-w}$ with $A$ ($r = -0.36$) and $B$ ($r = -0.91$), which are higher in magnitude than the ones observed for the $log K_{o-w}$ with $A$ ($r = -0.11$) and $B$ ($r = -0.10$). Chemicals with higher value of $K_{a-w}$ would be more volatile and less-soluble in water phase\textsuperscript{29}. The magnitude of $K_{a-w}$ increases with the increase in the dispersive interactions and decrease in polarity/polarizability, and hydrogen-bonding interactions\textsuperscript{38}. Hence, the greater is the value of $K_{a-w}$ of the chemicals, the faster is the skin absorption of chemicals. Taken together, the PPM model sheds light on the propensity of chemical permeability in terms of widely used partitioning properties.

3.1.2 GC\texttimes{}GC Model

The GC\texttimes{}GC model (equation 7 and Figure 2b) successfully explained the variance in the $log K_p$ data of nonpolar organic chemicals. Here, $log K_p$ values of training set were estimated using the Zhang model due to lack of experimental $K_p$ values (Table S3 and S4).

$$
log K_p = -5.35 (\pm 0.07) + 0.58(\pm 0.02)u_1 - 3.51 (\pm 0.19)u_2 \quad (7)
$$

$n = 79$, $R^2 = 0.90$, $Adj. R^2 = 0.89$, $Q^2 = 0.89$

$RMSE = 0.23$, $PRESS \ RMSE = 0.24$

The RMSE shown for equation 7 is calculated by comparing equation 7’s predicted values of $log K_p$ with the Zhang model-predicted values. For the same model set, DERMWIN exhibited an RMSE of 0.93 $log$ unit. The comparison of experimental values of $log K_p$, 

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which were available only for 7 nonpolar chemicals, with equation 7’s predicted values yielded an RMSE of 0.48 log unit.

For external validation, the full dataset of model nonpolar chemicals \((n = 79)\) was split randomly with a ratio of 1:4 into a training set \((n = 64, \text{Table S14})\) and an external validation set \((n = 15, \text{Table S15})\). Equation 8 was derived using the training set of 64 compounds.

\[
\log K_p = -5.34 (\pm 0.08) + 0.58(\pm 0.03)u_1 - 3.56 (\pm 0.22)u_2
\]

\(n = 64, \quad R^2 = 0.89, \quad \text{Adj.}R^2 = 0.89, \quad Q^2 = 0.89, \text{RMSE} = 0.24
\)

\(PRESS \text{ RMSE} = 0.25, \quad n_{\text{external}} = 15, \quad R_{\text{external}}^2 = 0.85, \quad \text{RMSE}_{\text{external}} = 0.22
\)

The fitting coefficients and regression statistics of equation 8 are statistically similar to equation 7. There was a good agreement (Figure S5) between predictions of equation 8 and the predictions of the Zhang model (equation 2) for external validation set \((R_{\text{external}}^2 = 0.85, \quad \text{RMSE}_{\text{external}} = 0.22)\).

Since the external validation approach can be sensitive to the partitioning of data into training set and validation set for the small datasets \(^{39,40}\) such as the GC\(\times\)GC model set \((n = 79)\), we performed four independent cross-validation tests, which indicated that the GC\(\times\)GC model is valid for predictive purpose (Table S8).

Finally, we validated the GC\(\times\)GC model using the following independent approach. The experimental retention parameters, \(u_1\) and \(u_2\) — obtained by analyzing 52 nonpolar chemicals on GC\(\times\)GC instrument in a previous study \(^{26}\) — were inputted in equation 7 to calculate \(K_p\) values of nonpolar analytes. The calculated \(K_p\) values by this means compared favorably with the \(K_p\) values estimated by the Zhang model with \(\text{RMSE} = 0.39\) (Table S4).
By the virtue of equation 7, analysts can overlay the estimates of skin permeability coefficients on the GC×GC chromatograms of complex mixtures of nonpolar chemicals — akin to cases shown previously for the GC×GC chromatogram of polychlorinated alkane mixtures having several thousand congeners.\textsuperscript{26,27}

4. Limitations and Outlook

The PPM model developed here works only for the neutral organic molecules. The model is not appropriate for the ionized species, which follows different partitioning \textsuperscript{29} and permeation \textsuperscript{41} behavior than is shown by neutral species. The PPM model can work only under the conditions where the permeants, if they have general acidic or basic functional groups such as carboxylic acids, phenols, or amines, are neutral. However, the partitioning behavior of ionized species may sufficiently be accounted for by considering descriptors such as pKa (acid dissociation constant) at a given pH of the system of interest \textsuperscript{42}. Inclusion of the descriptors of ionizability in the model might extend the domain of its applicability to ionized species, which may be evaluated in a future study.

The GC×GC model, in its current form, is calibrated only for nonpolar chemicals, and is not considered suitable for the polar contaminants. This is because the combination of stationary phases (polydimethylsiloxane and phenylmethylpolysiloxane) used in developing the GC×GC model does not capture the hydrogen-bonding interactions adequately \textsuperscript{43,44}. However, the ionic liquid (IL) stationary phases may offer the opportunity to capture such interactions \textsuperscript{45,46}, which may be evaluated in future studies to extend the application domain of the GC×GC model to polar contaminants.

The values of $\log K_{o-w}$ and $\log K_{a-w}$, used to train the PPM, were estimated using the
ASM equations\textsuperscript{30,31} due to the scarcity of experimental data. Though the respective ASM equations are known to provide accurate estimates of $log K_{o-w}$ and $log K_{a-w}$\textsuperscript{30,31}, the predictive performance of the PPM is expected to improve if trained on the experimental data. In the same vein, the GC\times GC Model, which is currently trained on the $log K_p$ values estimated by the Zhang model (equation 2), is expected to perform better when trained on the experimental data of $log K_p$. However, the training of models on the thin experimental data would lead to inflated errors around the regression coefficients for both models. The advantage of our approach is that we can estimate $K_p$ of neutral organic chemicals for which Abraham solute descriptors are not available.

In summary, the PPM performs better than the DERMWIN and similarly to the Zhang model. The DERMWIN model in EPI-Suite\textsuperscript{TM} may be replaced easily with parsimonious PPM, as $K_{o-w}$ and $K_{a-w}$ values can be estimated with reasonable accuracy from EPI-Suite\textsuperscript{TM}. The GC\times GC model predicts skin permeability of nonpolar chemicals with adequate accuracy, and can be applied to thousands of nonpolar analytes detected in complex environmental and technical mixtures. Thus, this study overcomes some of the limitations of existing models and illuminates a pathway for accurate and rapid risk assessment of neutral organic chemicals for their tendencies to penetrate human skin.

5. Declarations

Availability of data and materials
The Supporting Information for this manuscript is available on the Springer Nature Publications website.

Competing interests
The authors declare that they have no competing interests.
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Authors’ contributions
DN supervised the project. SN worked on the project as part of Master Thesis. YZ collaborated on the on comprehensive two-dimensional gas chromatography (GC×GC) modelling. All authors contributed to writing the paper. All authors read and approved the final manuscript.

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