JCIM JOURNAL OF CHEMICAL INFORMATION AND MODELING

Review

Predicting Skin Permeability by means of Computational Approaches: Reliability and Caveats in Pharmaceutical Studies

Beatrice Pecoraro, Marco Tutone, Ewelina Hoffman, Vicky Hutter, Anna Maria Almerico, and Matthew Traynor

J. Chem. Inf. Model., **Just Accepted Manuscript** • DOI: 10.1021/acs.jcim.8b00934 • Publication Date (Web): 18 Jan 2019

ACS Publications

is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Predicting Skin Permeability by means of Computational Approaches: Reliability and Caveats in Pharmaceutical Studies

Beatrice Pecoraro[*a], Marco Tutone[b], Ewelina Hoffman[a], Victoria Hutter[a], Anna Maria Almerico[b], Matthew Traynor[a].

[*]*b.pecoraro@herts.ac.uk*

[a] *Department of Clinical and Pharmaceutical Sciences, University of Hertfordshire, AL10 9AB Hatfield, United Kingdom.*

[b] *Department of Biological Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, 90123 Palermo, Italy.*

Abstract: The skin is the main barrier between the internal body environment and the external one. The characteristics of this barrier and its properties are able to modify and affect drug delivery and chemical toxicity parameters. Therefore, it is not surprising that permeability of many different compounds has been measured through several *in vitro* and *in vivo* techniques. Moreover, many different *in silico* approaches have been used to identify the correlation between the structure of the permeants and their permeability, to reproduce the skin behavior, and to predict the ability of specific chemicals to permeate this barrier. A significant number of issues, like interlaboratory variability, experimental conditions, dataset building rationales, and skin site of origin and hydration, still prevent us from obtaining a definitive predictive skin permeability model. This review wants to show the main advances and the principal approaches in computational methods used to predict this property, to enlighten the main issues arised and to address the challenges to develop in future research.

1. INTRODUCTION

The skin is the largest organ of the body and provides the main barrier between the internal and external environment. It consists of three separate and diverse layers, each one with a distinct cellular composition, characteristic, and function: epidermis, dermis and hypodermis. The outermost layer, epidermis, is formed of the viable epidermis and non-viable stratum corneum, which provides the main barrier to permeation and is considered as the "rate-limiting step of permeation"[1]. The stratum corneum is a specialized tissue type, whose main function is to control the absorption of substances into the skin and to maintain the fluid homeostasis. The structure of this layer has been often compared to a wall built from bricks and mortar, in which the nonpermeable protein-rich corneocytes represent the building blocks, glued with space-filling mortar (intercorneocyte cholesterol, triglycerides, and ceramides). According to Nemes, the barrier function of normal epidermis depends on the quality of its bricks and mortar [2-4].

1.1 Skin permeation

A compound can permeate the stratum corneum by the intercellular, transcellular or appendageal routes, according to its size and its chemico-physical properties [5, 6].

Skin permeability is widely recognized as an essential parameter to be considered for the delivery of active substances [1], but it is also considered important for risk assessment purposes [7]. Guidelines for *in vitro* and *in vivo* skin permeation tests have been drafted by the OECD [8, 9], but no universal protocol has been developed. Measuring skin permeability is generally time consuming due to experimental conditions which need to be optimized for each compound, alongside with a proper analytical method development or adaptation. Moreover, it is impossible to evaluate compounds not yet synthetized. Hence, it is crucial to use an efficient and accurate *in silico* model of human skin permeability in order to reduce product development costs in early stage screening [10], resolve ethical issues, understand the

mechanisms of absorption [1], and reduce measurements variability. In fact, it has been shown that the skin permeability can variate inter- and intra-subject [11]. While inter-variability fits to a normal distribution, intra-variability is non-normally distributed [11]. Furthermore, it has been demonstrated that skin barrier variability is chemical dependent [12] and that it is not correlated with trans epidermal water loss rates (TEWL), especially following physical damage (stripping), commonly performed on skin prior permeability experiments [13].

1.2 Skin Permeability parameters

The passive diffusion process of a chemical from a region of high concentration to low concentration in the skin is described by Fick's first Law of diffusion at steady state:

$$Q = \frac{DAT\Delta C}{h} \text{ (eq.1)}$$

The steady state flux (J_{ss}) through the skin can be described as:

$$J_{ss} = \frac{Q}{AT} = \frac{D\Delta C}{h} \text{ (eq.2)}$$

where **Q** is the amount of solute, **D** is the penetrant diffusivity in the membrane, **A** is the area of the membrane considered, **T** is time, **ΔC** is the penetrant concentration gradient across the membrane, and **h** is the membrane thickness (path length).

The most commonly used parameter to describe and measure permeability is the constant of permeability (or permeability coefficient, $\mathbf{K_p}$), defined as:

$$K_p = \frac{J_{ss}}{\Delta C_v} \text{ (eq.3)}$$

where **ΔCv** is the concentration gradient [14, 15].

Moreover, it is possible to use **Jmax**, the maximum flux of penetrant through the skin when in contact with a saturated solution [16].

1.3 Skin Permeability measurement conditions

The conditions in which permeability experiments can be performed may significantly vary. According to the amount of substance applied to the membrane we can distinguish between finite and infinite dosing. The application of a limited amount of substance, called finite dosing, is closer to the *in vivo* status, but depletion of the donor concentration will occur. In infinite dosing, the compound is applied in large volumes, allowing to consider the donor compartment concentration as constant, but this is unrepresentative of many real-life exposures and may potentially result in changes to the experimental conditions, for example through causing an occlusive effect [17].

Even though human skin is considered the gold standard, other animal models have been used to conduct permeability measurements because of the greater ease in obtaining them and the ability to more tightly control variable parameters such as age, race and donor site. For this reason and because of its structural similarity to human skin [18-20], porcine skin is often used as a surrogate for human skin. Furthermore, many models of skin diseases exist in mice [21-26] and thus mouse skin is another common substitute for human skin. Other kinds of skin, obtained *in vitro*, can be used to conduct permeability experiments. In fact, *in vitro* models of reconstructed human epidermis (RHE) and reconstructed human skin (RHS) are widely used to assess various types of toxicity, such as photo toxicity, corrosion, irritation and sensitization [27]. In the absence of penetration enhancers and controlling the level of skin hydration, it is possible to establish an overall good relationship between values obtained during experiments using RHS models with those using skin from hairless rats or mice, even if for both these models the permeability values are higher compared to human skin [28].

It is also possible to perform permeation experiments using specific skin sections or thicknesses [29]. Full thickness human skin (FT) can be used in *in vitro* permeation experiments and is prepared by mechanically removing the hypodermal connective tissue. In order to further reduce variability between samples, FT skin can be dermatomed to a pre-set thickness [30, 31]. Variation can be further reduced by the use of only stratum corneum or epidermal sheets. These can be prepared by either mechanical or

heat separation [32, 33]. This type of skin section retains the principle barrier function of the stratum corneum, and its use reduces variation due to skin thickness and diffusional pathway length.
Data on the stratum corneum thickness is limited [34]. Even from a given region, the number of cell layers and their thickness can vary [35], and this affects skin permeation measurements [36-39]. The total lipid content and its composition may vary as well, depending on region [40, 41] and species [42]. It has been shown that a change in the lipid composition and organization is characteristic of skin inflammatory diseases, where the skin barrier is compromised [43], and may cause irregular lipid matrix and defective skin permeability function [22, 44-48].

2. COMPUTATIONAL MODELS TO PREDICT PERMEABILITY

The experience of standardizing the conditions of permeation generated challenging and generally unsuccessful experiments [49, 50]. This leads to the need for other approaches that are able to coherently predict permeability values and rank accordingly old and new chemical entities. In this review, the authors have chosen to identify, comment and summarize the main advances and approaches on computational methods used to predict skin permeability.

In order to give a complete and detailed insight over the permeability predictive models, papers covering the period from 1992 to date have been selected and included in this review.

2.1 Linear and nonlinear QSPR models

The fundamental principle of a QSPR (quantitative structure-property relationship) is that the structural and physicochemical characteristics of a compound, codified as descriptors, are correlated to the property of interest (e.g. permeability coefficient) through a mathematical equation.
QSPRs have traditionally been generated for molecules associated with a measurable property [51-53], in this case mainly the Log K_p. But more generically, even binary or categorical responses (e.g. not-permeable or permeable) have been used, applying discriminant analysis, logistic regression and classification methods such as random forest (RF), support vector machine (SVM), and bayesian classifiers [54-58].

2.1.1. Potts and Guy approach and linear QSPR models

In the early 1990s, Potts and Guy [59] developed the first QSPR model, successfully linking the permeability of a compound with its partition coefficient between water and octanol (LogP) and molecular size (in the form of molecular volume or molecular weight), and subsequently adding the Hydrogen Bond Activity [60]. Another similar model was developed by Cronin et al. It showed the diversity between permeation mechanisms with excised human skin compared to polydimethylsiloxane membranes [61]. The compounds melting point was later added by Barratt et al.[62], since this parameter is strongly related to solubility, and their model used molecular volume instead of molecular mass. Using Principal Component Analysis (PCA) techniques, they divided the dataset as either steroids, pharmacologically active compounds, or small molecules. Since the initial QSPR on the pharmacologically active dataset revealed a poor regression correlation, the ultimate QSPR was built with a dataset obtained by means of combining small molecules and steroids, removing from this latter the hydrocortisone subset. It has been shown later that melting point has no statistical significance in this model [63]. Further analysis on a similar basis confirmed that hydrophobicity and molecular size (quantified by molecular weight or volume) provided a good model, when these well-known hydrocortisone derivative outliers were removed [64].
These Potts and Guy and Cronin QSPR models [59, 61] have been further revised, updating the steroid permeability data. The newly generated model showed a high degree of similarity to the previous ones, but it was not statistically necessary to consider the steroids as outliers. This led to the conclusion that the transdermal penetration process of steroids is not significantly different from that for the other molecules considered in the database [65]. Another approach following the Potts and Guy direction was performed by Buchwald and Bodor [66]. Their aim was to decouple two interrelated variables involved

that are used to generate the model: molecular size and acceptor hydrogen bonds formed. Even if these two variables are not correlated from a theoretical point of view, the "drug-like" database used to build the model shows an intercorrelation between them, altering the reliability of the correlation measurements.

Molecular weight (MW) has been found as strictly correlated to J_{max} from a given vehicle [67]. Magnusson et al. analyzed data from aqueous vehicles only, showing that other physicochemical parameters found with stepwise regression, like the solubility in octanol, the number of hydrogen bond acceptor sites, and the melting point, were able to only marginally improve the results. A separate analysis of full and split thickness skin data was then performed, showing that J_{max} is not significantly influenced by the dermal resistance.

Despite the wide use of MW as a descriptor in permeability predictive models, Tayar et al. [68] excluded their correlation. The intracellular route model was then built using Log P_{oct} and ΔLog $P_{oct\text{-}hep}$ (i.e. Log $P_{octanol}$ − Log $P_{heptanol}$), that measures the H-bond donor acidity. Log P_{oct} only was used instead of ΔLog $P_{oct\text{-}hep}$ in the case of transcellular route.

The first attempt of using several kinds of descriptors can be found in the work of Gute et al. [69] using a database of Polycyclic Aromatic Hydrocarbons (PAH). In this hierarchical QSPR, four classes of parameters were used (topostructural, topochemical, geometric, and quantum chemical), alongside with well-established physicochemical properties (e.g. MW and Log P). It has been found that, for the chemicals in the database, the quantum chemical class was not able to make any improvement in the QSPR predictivity.

The multilinear regression model developed by Chang et al. [70], using K_p data derived by *in vitro* human skin experiments, identified, with the octanol-water partitioning coefficient, four other properties. Among the 3,224 descriptors calculated by the Dragon software [71], the electrostatic interactions between electric quadrupoles of van der Waals forces, the frequency of carbon-nitrogen bonding at a constant topological distance, and the similarity to antineoplastic compounds in molecular property were selected to predict K_p for dermal hazard assessment. The antineoplastic property similarity (Neoplastic-80 [72]) has been identified in other models as a crucial descriptor, along with molecular cyclicity, topological distances between oxygen and chlorine atoms, and lipophilicity [73].

In an interesting analysis on benzoxazinones regioisomers [74], the descriptors classically used (log P, molecular weight and volume (MV), hydrogen bond donor (H_d) and acceptor (H_a)) were considered along with the molecular refractivity and the solvation enthalpy ($\Delta\Delta H_{solv}$) defined as the difference between formation enthalpies in water and in octanol and represents the energy acquired or transferred during the change of phase from a solvent to another one. It was used as a "correction" of LogP, resulting in an improvement of the correlation coefficient from the Potts and Guy equation.

Surprisingly, lipophilicity and size are not always claimed as the most important properties to build a permeability predictive model. Even though they were present in the pool of descriptors used in the model built using stepwise forward multilinear regression by Lee et al. [75], they were not retrieved as important contributors to the correlation coefficient. The properties involved were indeed the hydrogen bond acceptor and donor activities, followed by the globularity parameter, the PISA (PI carbon-hydrogen component of the solvent-accessible Surface Area), and electron affinity. The absence in the model selected of common descriptors, such as lipophilicity and solute size, could be explained with the vehicle composition, an aqueous solution of PEG 400. This polymer can act like a surfactant, contributing to reducing the difference of surface tension between the vehicle and the stratum corneum [76]. Therefore, in the presence of a vehicle containing a surfactant, lipophilicity may not be considered as an important factor to predict the permeability [75], but it is important to consider that this parameter can be affected by hydrogen bond donor and acceptor activities. The partitioning between water and the barrier phase is expected to show a correlation with lipophilicity values, but these latter could in some cases not be trustworthy[77]. Finally, the solute size is not completely outcast from the model. Even if not considered a major factor, this property can, in some extent, be correlated to the globularity parameter, of significant importance in the model.

Biological parameters can easily be included as QSPR descriptors. Liou et al. [78] developed a multilinear regression on non-steroidal anti-inflammatory drugs (NSAIDs) using, alongside molecular weight, polarity factor (cLogP, Log $K_{o/w}$) and the solubility parameter (δ), other parameters as indicators of the biological state of the skin as a barrier. TEWL, hydration content, lipid content, resonance running time,

and elasticity were successfully used as atypical descriptors, revealing their ability to improve the correlation of the model.

Different approaches were tested by Roberts et al. [79], who compared a model obtained with the use of solvatochromic parameters to six other models: the classical lipophilicity/molecular size approach [59], the molecular group contribution [80], the H-bond donor ability [68] with and without the use of molecular size, a two-phase model and a solubility-based model. This comparison revealed that the group contribution model, the two-phase model and the solvatochromic approach were more predictive compared to the other methods. The solvatochromic approach uses three different parameters (intrinsic volume, polarizability, and a descriptor of H-bond donor and acceptor activity) to describe the transfer of a molecule from an aqueous to an organic solution [81]. This method has been compared to theoretical chemistry-derived structural parameters, molecular connectivity and molecular shape [82]. The hydrogen bonding acceptor activity was recognized as the main limiting factor in skin penetration.

The additive group contribution mentioned previously [80] was based on two sets, an 11-predictor and a 17-predictor, based respectively on empirically determined functional group and on the SMILES method of molecular description. The results shown with this approach were comparable to those retrieved by Potts and Guy [59]. Another fragment based approach, the TOPS-MODE [83], is based on the computation of the spectral moments of the bond matrix [84], and was used to build a QSPR [85], resulting in conclusions similar to other studies previously mentioned [59, 61].

A more statistically rigorous approach was used by Chauhan and Shakya [86], who used a broad variety of descriptors (Dragon descriptors and Abraham descriptors). They divided the training and the test set by the Kennard-Stone algorithm [87, 88], developed a model combining regression methods and Partial Least Square (PLS), and determined an applicability domain for the model obtained. The descriptors retrieved were the octanol-water partition coefficient, the hydrogen bond number, and the Narumi simple topological index (a descriptor related to molecular branching [89]).

A plethora of studies build their models on LFER method of Abraham (or Abraham descriptors). Firstly developed for neutral compounds [90], and later on for ionic species [91-94], this model is based on the calculation of an equilibrium coefficient for a series of solutes in a given system (e.g. in this case, Log K_p) as a multilinear regression of some specific molecular properties (the excess molar refraction, the dipolarity/polarizability coefficient, the hydrogen bond acidity and basicity, the McGowan's characteristic molecular volume, and additional descriptors for ionic species). Models built with this predefined set of descriptors have been widely used [95-99] and show a good prediction for both ionic and non-ionic compounds [100].

Table 1 Linear QSPR models. Multilinear regression (MLR), principal component regression (PCR), linear free-energy regression (LFER), and partial least square (PLS) are the most used methods to develop a linear QSPR.

Computational method	N comp.	Fitness parameters	Robustness and validation parameters	Reference
MLR	93, 42, 23, 19	None	None	59
MLR	37	None	Fischer test	60
Stepwise regression MLR	114	R^2	T-value, least squares regression to identify outliers, Fischer test, σ	61
MLR	91	R^2	q^2 (leave-one-out), t-value, Fischer test, σ	62
Stepwise regression MLR	158	R^2	Fischer test, t-value, σ	64
MLR	119	R^2	q^2 (leave-one-out), t-value, Fischer test, σ	65
MLR	98	R^2	Fischer test, σ	66
Stepwise regression MLR	278	R^2	P-value	67
MLR	22, 8, 11, 18	R^2	σ	68
Hierarchical approach	60	R^2	Fischer test	69
Stepwise regression MLR	158	R^2	None	70

MLR	106	R^2	P-value, Fischer test, σ, sum of squares, mean square, mean square error, absolute maximum error, average absolute error, q^2	[73]
MLR	14	R^2	P-value, Fischer test	[74]
Stepwise regression MLR	61	R^2	Mean absolute error, q^2	[75]
MLR	13	R^2	q^2	[78]
MLR	24	R^2	P-value, Akaike's Information Criterion (AIC)	[79]
MLR	91	R^2	Studentized residual value (t-value), σ, 95% confidence interval, p-value, H leverage	[80]
Stepwise regression MLR	21, 11, 6	R^2	σ, Fischer test	[82]
Stepwise regression MLR	114	R^2	σ, σ_{CV}, q^2 (leave-one-out), p-value, Fischer test	[85]
MLR, PCR, PLS ($RMSE_{CV}$ minimization)	211	R^2	q^2 (leave-one-out), $RMSE_{CV}$	[86]
MLR (LFER)	71	R^2	σ, Fischer test	[95]
MLR (LFER)	112	R^2	σ, t-test	[96]
MLR (LFER)	119	R^2	σ, Fischer test	[97]
MLR (LFER)	47	R^2	σ, Fischer test	[98]
MLR (LFER)	118	R^2	σ, Fischer test, Predictive Standard Deviation leave-one-out (PSD)	[99]
MLR (LFER)	247	R^2	σ, Fischer test, Predictive Standard Deviation leave-one-out (PSD), q^2	[100]

To be described as predictive, a model should have an appropriate measurement of goodness of fit, robustness and predictivity. Among all the model previously described and shown in Table 1, R^2 is unanimously used to represent the goodness of fit measurement. Predictivity and robustness have been measured mostly with Q^2_{LOO} (Leave-One-Out), a Cross Validation parameter. It has been shown that this parameter alone is inadequate and incomplete [101-107], therefore Q^2_{LMO} (Leave-Many-Out) or bootstrap methods should be used instead, and Y-randomization should be calculated in parallel to highlight casual correlations [101, 102, 108]. The Root Mean Square Error and its cross validate counterpart (RMSE and $RMSE_{CV}$) can be useful in case of unevenly distributed data; $RMSE_{CV}$ should be as low as possible and similar to RMSE to show a good standardization [109]. Although, cross validation techniques provide a reasonable measure of the internal predictive power; furthermore, in order to consider the model truly predictive, the internal validation should be supported by external validation [110]. In fact, during the cross validation process runs, the same data are "repeatedly used to build and assess the model" [102, 104]. The external validation should be performed splitting *a priori* the dataset into a training and a test set. The most common external validation, suggested by the OECD [111], is performed through q^2 form parameters [112], R^2_{ext} measurements [106], and other types of metrics [113-115].

2.1.2. Non-linear QSPR models

In the past 30 years QSPR models evolved from simple Multi Linear Regressions with a few thermodynamic variables to non-linear models developed with a wide variety of descriptors [116, 117].
Many different approaches have been used to develop QSPRs models, including artificial neural networks, random forest, gaussian regressions and processes.
The artificial neural network (ANN) is a pattern of computational algorithms that reproduces the functionality of the connection in biological neural clusters [118], simulating in every way a "digital brain" that processes the information [119].
The ANN is compared to MLR by Fatemi and Malekzadeh [120], who built models with descriptors calculated with the software Codessa [121]. A similar comparison has been made by Atobe et al. [122], who use the classical Potts and Guy descriptors, alongside other parameters that take into account the effect of different delivery vehicles. Additionally, Katritzky et al. [123] included fragment-based ISIDA modeling [124, 125] in the comparison. Lim et al. [126] used a feed-forward back-propagation neural network

to correlate skin permeability to descriptors calculated with Molecular Orbital (MO), such as dipole moment, polarizability, sum of charges of nitrogen and oxygen atoms, and sum of charges of hydrogen atoms bonding to nitrogen or oxygen atoms. Artificial Neural Network has also been used with Abraham Descriptors [127-129]. ANN is often considered as superior because it generally performes better for a broad range of chemicals [122]. Although this technique has been applied in building several models [120, 122, 123, 126-129], Chen et al. [73] observed that models with not sufficient number of compounds show a trend of overfitted results [130]. Moreover, it has been shown that ANNs can be trapped in local minima [10] and that the structure of their network is difficult to determine properly [73].

The Random Forest technique is a particular Decision Ensembles of Trees algorithm [131]. After bootstrap sampling, for each sample a certain number of decision trees are grown to the maximum possible size, using the Classification And Regression Trees (CART) algorithm [132]. The boosted CART can be used to obtain permeability classes with an extended set of descriptors. Baert et al. compared this CART model with a MLR model built with the descriptors from the first model [133].

Other ensemble methods used are the Decision Forest [134] and the Random Forest [135]. Among all the non-linear techniques, the tree-based ones show a good set of characteristics. They are able to identify relevant descriptors and to handle high dimensional-data, but they are not able to give a high prediction accuracy [56]. In contrast to the other ensemble algorithms, RF is able to estimate prediction accuracy, descriptor importance in the model and similarity between chemical compounds analyzed [56]. A Random forest approach with these characteristics has been used by Alves et al. [136, 137], to compare human and rodent permeabilities, showing a good predictive performance but a restricted applicability domain compared to other software[138].

A similar model was generated by Baba et al. [10], that, after the calculation of 4803 descriptors with Dragon [71], developed and compared several QSPRs. The models were built through random forest and support vector machine (SVM). The support vector machine uses a kernel transformation, a mathematical function that projects the descriptor matrix in a space with high-dimensionality [139, 140]. The SVM was used to find regression with both a linear or gaussian basis. The models were obtained by stepwise forward selection, using the Potts and Guy model [59] as a baseline. The number of descriptors selected in the RF were 9, in the SVM-Gaussian 11 and 17 in the SVM-Linear, showing that the random forest performed better than all the other models. Furthermore, the support vector regression and the random forest methods were used with greedy stepwise descriptor algorithm selection to predict the solvent effect on human skin permeability [141]. In subsequent work, the SVM–Gaussian and the SVM-Nonlinear were compared to the Potts and Guy model to investigate the permeability of ionic compounds[142]. The results showed the superiority of the nonlinear SVR model and the effectiveness of a new descriptor, Log D, that represents the octanol-water distribution coefficient measured at a specific pH and allows to predict the effects of ionization on the skin permeation process.

Machine learning methods can include the gaussian process regression with automatic resonance detection (GPRARD). This method was compared in different studies [143, 144] to gaussian process regression (GPR), and single linear networks (SLN). The use of GPR, in particular GPRARD, is able to quantify the covariance and the length scale of each descriptor in the model, giving a deeper comprehension of the significance of each feature [144]. GPR methods have been applied to the exploration of skin membranes datasets other than human skin, and to investigate how the nature of the dataset may influence its analysis [145]. Descriptors used to build this latter model are LogP, MW, the number of hydrogen bond donor and acceptor groups and solubility parameter defined by Fedors [146].

An iterative non-linear Gauss–Newton least-squares fit [147] was used by ten Berge [148] to estimate the regression coefficient for aqueous skin permeation, using the logarithm of the water-octanol partition coefficient, molecular weight and water solubility. The model can calculate various parameters, such as the aqueous permeation coefficient, the maximum dermal absorption, the lag time and, finally, the diffusivity in the stratum corneum. The maximum dermal absorption and lag time showed the same order of magnitude of the respective experimental measured properties.

Gaussian processes, along with SLN and k-nearest-neighbour regression (k-NN), were applied to predict the skin permeability coefficient, based on five molecular descriptors (MW, solubility parameter, lipophilicity, the number of Hydrogen acceptor and donor bonds). The results obtained were better than the classical LogP-MW model [149]. k-NN has also been used with ridge regression [150], to predict skin permeability using molecular weight, octanol-water partition coefficient and solvation free energy [130].

As shown in this review, the k-NN algorithm has been widely used in skin permeability predictions. This non-parametric method represents one of the simplest machine learning algorithms [151]. The regression process estimates the value to predict according to a weighted average of the nearest neighbour, weighted by the inverse of their distance [152]. The same method can be used to estimate outliers [153]. A good example of this use of the k-NN methods is delivered by Lindh et al. [154]. After using the RF and SVM regression approaches together with Conformal Prediction (CP), k-NN has been used to estimate the error for each compound.

Conformal Prediction is able to estimate prediction ranges and significance levels for single compounds [155, 156]. Estimating the prediction range [157] and defining the error limits are procedures that quantify the maximum number of errors expected [155, 156] leading to increased confidence in the model.

Ridge regression (RR), also known as Tikhonov regularization or weight decay [150], is used in other models in order to take into account multicollinearity. After reducing the number of descriptors with a modified Gram-Schmidt variable reduction algorithm, Basak et al. [158] compared the RR to principal component regression (PCR) and partial least squares regression (PLS), showing that Ridge Regression outperforms the other approaches.

Skin permeability has also been predicted with other kinds of machine learning algorithms, such as neural fuzzy algorithms. Khajeh and Modarress [159] used Modified Particle Swarm Organization (MPSO) to select the descriptors and Adaptive Neuro-Fuzzy Inference System (ANFIS) [160] to correlate them with skin permeability experimental data. Fuzzy models have been compared to Flynn [161], Potts and Guy [59, 60], and Abraham [162] databases with promising results [163], even if the number of compounds should be increased. Furthermore, a Takagi-Sugeno fuzzy model, able to predict permeability from MW, octanol/water partition coefficient, and temperature, has been compared with a MLR model that used the same descriptors [164], showing the superiority of the first model over the second one.

After measuring the stratum corneum binding property and the extracted lipid partition coefficient of a number of compounds, Wang et al. [165] developed two QSPRs to predict the partition and binding coefficients. The two models built were combined in a two-phase compartmental nonlinear model able to predict the partition coefficient of solutes to the stratum corneum ($K_{sc/w}$).

Given that the greatest part of the QSPR mentioned have been developed using mixtures of different datasets prone to interspecies and interlaboratory variabilities, a novel statistical approach has been used to minimize this error [166]. Fujiwara et al. assumed that each dataset has a relationship between the permeability and the descriptors, considering the different regression coefficients for each dataset as proportional (not identical) to the others. This approach, called “latent membrane permeability”, is a possible explanation of the reason why an absolute permeation rate cannot be predicted, and it is an effective way to compare different databases of skin permeability measurements.

2.1.3. QSPR models for mixtures and enhancers

All of the approaches to predicting skin permeation described above consider only the permeation of single compounds from aqueous solution. It is extremely rare that any chemical actually comes into contact with the skin as a simple aqueous solution, whether medicinal formulations, cosmetic products or accidental exposure to chemicals are being considered. Much more common is that the chemical comes into contact with the skin as a complex mixture of chemicals in a delivery vehicle that often contains aqueous and non-aqueous co-solvents. Therefore, in order to better reflect these real-world scenarios, numerous approaches have been used to describe behavior in situations closer to the actual skin delivery systems, where mixtures and permeability enhancers are commonly present. In fact, the use of permeability enhancers, chemicals that interact with skin constituents to promote drug flux [167], is one of the most commonly used approaches within the pharmaceutical industry to broaden the range of chemicals that can delivered into or across the skin.

To predict skin enhancement for hydrocortisone and hydrocortisone acetate, a Membrane-Interaction Quantitative Structure-Activity Relationship (MI-QSAR) was developed [168], using two enhancer data sets of 61 and 42 molecules. The MI-QSAR technique uses a model membrane, on which a Molecular Dynamics (MD) simulation is conducted, analyzing the permeability enhancer [169-172]. General intramolecular solute descriptors, solute aqueous dissolution and solvation descriptors and solute-membrane intermolecular descriptors were calculated. This latter category is extracted from the

trajectories of the MD simulations, making it possible to build a QSPR model considering the membrane properties.

MLR methods have been adapted for topical preparation mixtures [173] in order to predict ingredient modulation of dermal absorption of caffeine and salicylic acid. Physicochemical descriptors (such as density, vapor pressure, enthalpy of vaporization, flash point, index of refraction, molar refraction, hydrogen bond acceptors and donors, number of freely rotatable bonds, log P, log D, polar surface area, polarizability, surface tension, molar volume, boiling point, melting point, boiling point minus melting point, Henry's Law constant, MW, and water solubility) were calculated and the models were obtained by stepwise forward regression. During a finite-dose diffusion experiment, using porcine skin, Ghafourian et al. [174] tested 12 different penetrants in 34 different solvent mixtures. From the data collected, through a stepwise regression analysis, it was possible to build a QSAR, employing two penetrant descriptors (octanol/water partition coefficient and the ninth order path molecular connectivity index) and one solvent property (the difference between the melting and boiling points). The extremely unusual negative relationship between skin permeability coefficient and Log P was correlated to the high lipophilicity of the compounds of this particular dataset. Then, combining this data with a previous dataset, a new QSAR was obtained, having as descriptors two penetrant properties (Wiener topological index and total dipole moment), the boiling point of the solvent, and the difference between the melting point of the penetrant and the melting point of the solvent [175].

Mixture-related effects were considered by Guth et al. [176], who developed a MLR QSPR to measure the dermal absorption of agrochemical formulations. Along with the five Abraham descriptors, physico-chemical and structural properties were used to calculate the mixture factor (MF). The Abraham descriptors, enriched by a mixture factor (MF), were used by Riviere and Brooks [177], who compared a large number of models, showing the positive impact of a mixture factor on the predictivity of each of them. Adding a sixth term to the Abraham Descriptors, the MF, allowed the same authors, , to take into account the physicochemical properties and quantify the effect of the formulation on the dermal absorption [178] in the further study.

The LFER model of Abraham has been expanded and adapted in different ways to predict the permeability of mixtures. Xu et al. considered an equation for each Metal Working Fluid formulation, and then produced a condensed model [179]. The validation is performed with the "leave-one-solute-out" method (a modified leave-one-out cross-validation) that correlates replicates from the same solute.

The permeability enhancers (PE) analysis still represents a challenge, mainly because of their possible multiple mechanisms of action [180, 181]. Drakulić et al. [182] used four drugs (5-Fluorouracil, Hydrocortisone, Estradiol and Diclofenac Sodium) to evaluate the role of 34 terpenes as permeation enhancers. Molecular modelling showed that the complexation between the PE and the drug could be responsible of the enhancement.

Discriminant analysis (DA) and classical machine learning methods, such as gaussian process regression, K-nearest-neighbour regression, single layer networks, radial basis function networks and SVM classifier algorithms have been successfully applied on permeation enhancers by Moss et al. [183], showing better predictions of GP compared to DA.

Another non-linear QSPR model has been developed by Yerramsetty et al. [184], where the ANN algorithm was used to predict permeability enhancement for insulin when combined with different permeability enhancers.

2.2 Other computational approaches

2.2.1. Molecular Dynamics

Molecular Dynamics is a useful technique to calculate, record, and analyze motions of a many particles-system [185]. In one of the earliest studies in this field, a statistical mechanical theory is used to establish the molecular distribution of a solute on a lipid bilayer, obtained as a function of lateral pressure and solute size and shape [186]. Similarly, Das *et al.* [187] have calculated the diffusivity of water in several lipid bilayers, obtaining permeability values much smaller compared to the experimental ones. On the contrary, measuring the permeation of small molecules through a lipidic matrix, Gupta et al.[188]obtained values a few orders of magnitude higher than the experimental for the hydrophilic molecules.

Molecular dynamics simulations have been used to support results obtained from other kind of *in silico,* or *in vitro* models, confirming the high selectivity of lipid membranes permeation to compound size and shape. In Marrink et al., a molecular dynamics simulation [189-191] was employed to study the transport of small molecules (water, oxygen, ammonia) through a phospholipidic bilayer [192], with the objective of determining if the size, hydrophobicity and asphericity of the permeants were related to their permeability coefficient. They concluded that the shape of the permeation resistance profile was mainly determined by the free energy of solvation.

To add a multi-component diffusion factor, Rim *et al.* proposed a multiscale framework model, considering the microscopic, mesoscopic and macroscopic aspect of transdermal diffusion. Molecular Dynamics in this case is used to find the diffusion coefficient in the lipid bilayers of the stratum corneum [193] for drugs coupled with permeability enhancers.

Another multi-scale approach used multiple constrained molecular dynamics simulations (molecular scale simulation) to calculate the diffusivity coefficient. This was then used to run Finite Model Element (FEM) simulations to calculate the release profile of the compounds in the macroscopic model, the concentration gradient and the amount permeated through the stratum corneum [194]. The study shows a good qualitative match with experimental data, but suffers from some limitations, such as skin lipids composition variability and experimental log K_p values variability.

The barrier properties of human skin have been analyzed *in silico*, simulating electron microscopy patterns on molecular dynamics simulations of a bilayer model. These results have been validated against cryo-electron microscopy data from near native skin [195, 196], showing a thermodynamically stable model and results compatible with values from human skin.

Rocco et al. [197] applied Steered Molecular Dynamics (SMD) of 80 compounds from Flynn's refined database [198] on a SC model, to obtain lipophilicity and diffusion parameters. Further improvements have been obtained considering temperature-related parameters. The variables extracted from the SMD analysis have been correlated with the permeability coefficient measured experimentally. Even if still not enough accurate to represent a valid tool to predict permeability, this model can give useful insight. In fact, the assessment of the behavior of each compound in the different zones of the SC model can lead to the identification of the region that represents the "limiting step during the permeation process" of that compound.

2.2.2. Equation-based models

Other *in silico* methods have been developed with a theoretical approach, producing equations able to predict permeability and dermal absorption [199], mainly from compartmental models.

The multicompartmental spreadsheet-based model developed by Dancik et al. [200] considers the characteristics of skin layers and attaches to each one a corresponding equation, obtained as combination of diffusion principles and experimental coefficients, evaluating several exposure scenarios. Unfortunately, the model shows a great number of discrepancies with the experimental data but attempts to take into account situations not commonly addressed by QSPRs, like finite dose absorption.

Another two-dimensional mathematical multi-scale model was developed by Kattou et al. [201]. Caffeine permeation via the intrafollicular route was predicted using partition properties in sebum collected from literature. The study confirmed the importance of this route for the permeation mechanism of caffeine, providing information difficult to obtain with *in vivo* or *ex vivo* experiments. Even if not exhaustively validated, the model is promising, offering quantitative prediction of intercellular, intracellular and follicular permeation pathways.

More recently, Wen et a. [202] developed a microscopic FEM in order to test different geometries, pathways and hydration levels. Not being derived purely from a theoretical approach, this study argued the unavoidable correlation between parameters experimentally derived and computed SC structures, therefore attributed the significant differences in lag-times and permeabilities predicted in multiple conditions to the lack of verified and standardized experimental transport parameters.

As previously mentioned, it is possible to compare different "skin geometries". What all of these models have in common is a prototype in which the discrete corneocytes cells are embedded in a continuous lipidic matrix. This kind of model is well-known as the brick and mortar-based model [203, 204]. Cuboid

models in two or three dimensions and tetrakaidekahedron in three dimensions are the most commonly investigated methods [205]. Naegel et al. showed that the selected cell shape has a numerical influence on the barrier properties of the SC and that the tetrakaidekahedral shape had a "favorable barrier-to-volume ratio" [205].

Mitragotri developed several theoretical mathematical models considering structural properties of lipid bilayers and molecular properties of the compounds [206, 207]. The Scaled Particle Theory [208] has been used to calculate the diffusion and partition coefficients in bilayers, considering statistical mechanics of lipid chains [206]. It showed that the solute partition coefficients calculated are comparable with the octanol-water partition coefficient, and that the diffusion coefficients of the compounds analyzed decreased with solute cross-sectional area. Other skin permeability analytical expressions based on solute radius and octanol-water partition coefficient have been subsequently developed, considering the importance of lateral lipid diffusion, aqueous pores diffusion, diffusion through shunts and lipid bilayers free-volume diffusion [207].

Other mathematical mechanistic models have been developed in order to understand the microscopic principles of skin penetration. Among these a promising mathematical model validated through published clinical studies on nicotine skin patches, is able to predict solute concentration in the blood, integrating the skin penetration mechanism with the circulation kinetic one [209]; unfortunately, as in other cases, the model still needs a proper validation with an appropriate sized database.

Mathematical models can be used not only to predict permeability, but also disposition of compounds in the skin layers [210]; this latter study confirmed that the disposition and the absorption across the skin are related to the octanol-water partition coefficient, showing a non-linear correlation between these parameters.

3. CONCLUSION

In silico methods have a fundamental role in predicting skin permeability, to minimize product development costs, to address ethical issues, to reduce experimental skin variability, and to gain useful insights on the mechanisms of absorption and distribution on the skin layers. The aim of this review was to highlight the advances of *in silico* predictions of skin permeability. Many different approaches have been used in the past years, such as QSPRs (linear and non-linear), Molecular Dynamics simulations, and various theoretical methods.

Above all these, QPRSs still represent the most widely used technique to predict skin permeability because of its ability to evaluate larger datasets and give faster results. Linear and non-linear models have been developed and validated with different extent of success. The poor correlation obtained in some cases can be attributed to the quality of databases. Many of the studies cited above were developed with databases obtained through experiments performed with mixed animal and human data, different measurement conditions, vehicles, and skin samples regions. As previously mentioned, skin permeability can hugely vary, and even measurements obtained with the same conditions, but in different studies can be affected by this atavist ensemble of errors, that make the data themselves not useful for prediction purposes.

While the QSPRs developers are still struggling with the quality of data, other kind of approaches are trying to become independent from non-standardized parameters. Equation-based models describe the skin layers and the absorption mechanisms not only to give insights into the permeation and the skin barrier properties, but also to measure them. Unfortunately, the only validation of these models is still the experimental data that appears to be particularly lacking for some of the routes of permeation.

Even though MD approaches tend to take many variables into account, rising therefore the chances of errors, they seem to be the next step towards experimentally-independent permeability prediction because of their ability to recreate a model of the skin environment. Furthermore, the improving calculation potency of computing machines is contributing to broaden the limits of this kind of approach.

Despite more than 25 years of computational models' development to predict human skin permeation, this field appears to be continuously expanding. Therefore, the entire spectrum of approaches analyzed in this review has to be considered a necessary and useful step in order to further build a validated satisfactory model of prediction and description of skin permeation pathways.

LIST OF ABBREVIATIONS

- ANFIS, Adaptive Neural Fuzzy Inference System
- ANN, Artificial Neural Network
- CART, Classification And Regression Trees
- CV, Cross Validation
- DA, Discriminant Analysis
- FEM, Finite Element Method
- FT, Full-Thickness
- GA, Genetic Algorithm
- GPR, Gaussian Process Regression
- GPRARD, Gaussian Process Regression with Automatic Resonance Detection
- ISIDA
- k-NN, k-Nearest-Neighbour
- LFER, Linear Free-Energy Relationship
- LMO, Leave Many out
- LOO, Leave One Out
- MD, Molecular Dynamics
- MF, Mixture Factor
- MI-QSAR, Membrane-Interaction Quantitative Structure-Activity Relationship
- MLR, Multi Linear Regression
- MO, Molecular Orbitals
- MPSO, Modified Particle Swarm Organization
- MV, Molecular Volume
- MW, Molecular Weight
- NSAID, Non-Steroidal Anti-Inflammatory Drug
- OECD, Organization for Economic Co-operation and Development
- PAH, Polycyclic Aromatic Hydrocarbons
- PCA, Principal Component Analysis
- PCR, Principal Component Regression
- PE, Permeability Enhancer
- PISA, PI carbon-hydrogen component of the solvent-accessible Surface Area
- PLS, Partial Least Square
- QSAR, Quantitative Structure-Activity Relationship
- QSPR, Quantitative Structure-Property Relationship
- RF, Random Forest
- RHE, Reconstructed Human Epidermis
- RHS, Reconstructed Human Skin
- RMSE, Root Mean Square Error
- RR, Ridge Regression
- SC, Stratum Corneum
- SMD, Steered Molecular Dynamic
- SLN, Single Linear Network
- SMILES, Simplified Molecular Input Line Entry System
- SVM, Support Vector Machine
- SVR, Support Vector Regression
- TEWL, Trans Epidermal Water Loss
- TOPS-MODE, TOPological Sub-Structural MOlecular DEsign

REFERENCES

1. Tsakovska, I.; Pajeva, I.; Al Sharif, M.; Alov, P.; Fioravanzo, E.; Kovarich, S.; Worth, A. P.; Richarz, A.-N.; Yang, C.; Mostrag-Szlichtyng, A.; Cronin, M. T. D., Quantitative structure-skin permeability relationships. *Toxicology* **2017**, 387, 27-42.

2. Elias, P. M.; Menon, G. K. Structural and Lipid Biochemical Correlates of the Epidermal Permeability Barrier. In *Adv. Lipid Res.*, Elias, P. M., Ed.; Elsevier: 1991; Vol. 24, pp 1-26.
3. Benson, H. A.; Watkinson, A. C., *Topical and Transdermal Drug Delivery: Principles and practice*. John Wiley & Sons: 2012.
4. Nemes, Z.; Steinert, P. M., Bricks and mortar of the epidermal barrier. *Mol. Med.* **1999**, 31, 5.
5. Scheuplein, R. J.; Blank, I. H., Permeability of the skin. *Physiol. Rev.* **1971**, 51, 702-747.
6. Lane, M. E., Skin penetration enhancers. *Int. J. Pharm.* **2013**, 447, 12-21.
7. van de Sandt, J. J. M.; Meuling, W. J. A.; Elliott, G. R.; Cnubben, N. H. P.; Hakkert, B. C., Comparative in Vitro–in Vivo Percutaneous Absorption of the Pesticide Propoxur. *Toxicol. Sci.* **2000**, 58, 15-22.
8. OECD, *Test No. 427: Skin Absorption: In Vivo Method*. 2004.
9. OECD, *Test No. 428: Skin Absorption: In Vitro Method*. 2004.
10. Baba, H.; Takahara, J.-i.; Mamitsuka, H., In Silico Predictions of Human Skin Permeability using Nonlinear Quantitative Structure–Property Relationship Models. *Pharm. Res.* **2015**, 32, 2360-2371.
11. Meidan, V. M.; Roper, C. S., Inter- and intra-individual variability in human skin barrier function: A large scale retrospective study. *Toxicol. In Vitro* **2008**, 22, 1062-1069.
12. Monteiro-Riviere, N. A., Anatomical factors affecting barrier function. *Dermatotoxicology* **1996**, 5, 3-17.
13. Chilcott, R. P.; Dalton, C. H.; Emmanuel, A. J.; Allen, C. E.; Bradley, S. T., Transepidermal water loss does not correlate with skin barrier function in vitro. *J. Invest. Dermatol.* **2002**, 118, 871-875.
14. Riviere, J. E.; Xia, X. Quantitative Structure–Permeability Relationships. In *Comparative Pharmacokinetics*; 2011.
15. Mitragotri, S.; Anissimov, Y. G.; Bunge, A. L.; Frasch, H. F.; Guy, R. H.; Hadgraft, J.; Kasting, G. B.; Lane, M. E.; Roberts, M. S., Mathematical models of skin permeability: an overview. *Int. J. Pharm.* **2011**, 418, 115-129.
16. Michaels, A.; Chandrasekaran, S.; Shaw, J., Drug permeation through human skin: Theory and invitro experimental measurement. *AIChE Journal* **1975**, 21, 985-996.
17. Selzer, D.; Abdel-Mottaleb, M. M. A.; Hahn, T.; Schaefer, U. F.; Neumann, D., Finite and infinite dosing: Difficulties in measurements, evaluations and predictions. *Adv. Drug Del. Rev.* **2013**, 65, 278-294.
18. Lademann, J.; Richter, H.; Meinke, M.; Sterry, W.; Patzelt, A., Which skin model is the most appropriate for the investigation of topically applied substances into the hair follicles? *Skin Pharmacol. Physiol.* **2010**, 23, 47-52.
19. Wester, R. C.; Melendres, J.; Sedik, L.; Maibach, H.; Riviere, J. E., Percutaneous absorption of salicylic acid, theophylline, 2, 4-dimethylamine, diethyl hexyl phthalic acid, andp-aminobenzoic acid in the isolated perfused porcine skin flap compared to manin vivo. *Toxicol. Appl. Pharmacol.* **1998**, 151, 159-165.
20. Gray, G.; Yardley, H., Lipid compositions of cells isolated from pig, human, and rat epidermis. *J. Lipid Res.* **1975**, 16, 434-440.
21. Krieg, P.; Rosenberger, S.; de Juanes, S.; Latzko, S.; Hou, J.; Dick, A.; Kloz, U.; van der Hoeven, F.; Hausser, I.; Esposito, I.; Rauh, M.; Schneider, H., Aloxe3 Knockout Mice Reveal a Function of Epidermal Lipoxygenase-3 as Hepoxilin Synthase and Its Pivotal Role in Barrier Formation. *J. Invest. Dermatol.* **2013**, 133, 172-180.
22. Li, W.; Sandhoff, R.; Kono, M.; Zerfas, P.; Hoffmann, V.; Ding, B. C.-H.; Proia, R. L.; Deng, C.-X., Depletion of ceramides with very long chain fatty acids causes defective skin

permeability barrier function, and neonatal lethality in ELOVL4 deficient mice. *Int. J. Biol. Sci.* **2007**, 3, 120.
23. Vasireddy, V.; Uchida, Y.; Salem, J. N.; Kim, S. Y.; Mandal, M. N. A.; Reddy, G. B.; Bodepudi, R.; Alderson, N. L.; Brown, J. C.; Hama, H.; Dlugosz, A.; Elias, P. M.; Holleran, W. M.; Ayyagari, R., Loss of functional ELOVL4 depletes very long-chain fatty acids (≥C28) and the unique ω-O-acylceramides in skin leading to neonatal death. *Hum. Mol. Genet.* **2007**, 16, 471-482.
24. Hadj-Rabia, S.; Baala, L.; Vabres, P.; Hamel-Teillac, D.; Jacquemin, E.; Fabre, M.; Lyonnet, S.; de Prost, Y.; Munnich, A.; Hadchouel, M.; Smahi, A., Claudin-1 gene mutations in neonatal sclerosing cholangitis associated with ichthyosis: A tight junction disease. *Gastroenterology* **2004**, 127, 1386-1390.
25. Plichta, J. K.; Droho, S.; Curtis, B. J.; Patel, P.; Gamelli, R. L.; Radek, K. A., Local Burn Injury Impairs Epithelial Permeability and Antimicrobial Peptide Barrier Function in Distal Unburned Skin*. *Crit. Care Med.* **2014**, 42, e420-e431.
26. Furuse, M.; Hata, M.; Furuse, K.; Yoshida, Y.; Haratake, A.; Sugitani, Y.; Noda, T.; Kubo, A.; Tsukita, S., Claudin-based tight junctions are crucial for the mammalian epidermal barrier. *J. Cell Biol.* **2002**, 156, 1099.
27. Fleischli, F. D.; Morf, F.; Adlhart, C., Skin concentrations of topically applied substances in reconstructed human epidermis (RHE) compared with human skin using in vivo confocal Raman microscopy. *CHIMIA International Journal for Chemistry* **2015**, 69, 147-151.
28. Todo, H., Transdermal Permeation of Drugs in Various Animal Species. *Pharmaceutics* **2017**, 9, 33.
29. Abd, E.; Yousef, S. A.; Pastore, M. N.; Telaprolu, K.; Mohammed, Y. H.; Namjoshi, S.; Grice, J. E.; Roberts, M. S., Skin models for the testing of transdermal drugs. *Clinical Pharmacology : Advances and Applications* **2016**, 8, 163-176.
30. Haigh, J. M.; Smith, E. W., The selection and use of natural and synthetic membranes for in vitro diffusion experiments. *Eur. J. Pharm. Sci.* **1994**, 2, 311-330.
31. Cross, S. E.; Magnusson, B. M.; Winckle, G.; Anissimov, Y.; Roberts, M. S., Determination of the effect of lipophilicity on the in vitro permeability and tissue reservoir characteristics of topically applied solutes in human skin layers. *J. Invest. Dermatol.* **2003**, 120, 759-764.
32. Flynn, G.; Dürrheim, H.; Higuchi, W., Permeation of hairless mouse skin II: membrane sectioning techniques and influence on alkanol permeabilities. *J. Pharm. Sci.* **1981**, 70, 52-56.
33. Kligman, A. M.; Christophers, E., Preparation of isolated sheets of human stratum corneum. *Arch. Dermatol.* **1963**, 88, 702-705.
34. Monteiro-Riviere, N. A., Anatomical factors affecting barrier function. *Dermatotoxicology* **2004**, 5, 43-70.
35. Holbrook, K. A.; Odland, G. F., Regional differences in the thickness (cell layers) of the human stratum corneum: an ultrastructural analysis. *J. Invest. Dermatol.* **1974**, 62, 415-422.
36. Feldmann, R. J.; Maibach, H. I., Regional Variation in Percutaneous Penetration of 14C Cortisol in Man**From the Division of Dermatology, Department of Medicine, University of California School of Medicine, San Francisco, California 94122. *J. Invest. Dermatol.* **1967**, 48, 181-183.
37. Maibach, H. I.; Feldmann, R. J.; Milby, T. H.; Serat, W. F., Regional variation in percutaneous penetration in man. *Arch. Environ. Health* **1971**, 23, 208-211.
38. Wester, R. C.; Noonan, P. K.; Maibach, H. I., Variations in percutaneous absorption of testosterone in the rhesus monkey due to anatomic site of application and frequency of application. *Arch. Dermatol. Res.* **1980**, 267, 229-235.

39. Wester, R. C.; Maibach, H. I., Cutaneous pharmacokinetics: 10 steps to percutaneous absorption. *Drug Metab. Rev.* **1983**, 14, 169-205.
40. Squier, C. A.; Hall, B. K., The permeability of skin and oral mucosa to water and horseradish peroxidase as related to the thickness of the permeability barrier. *J. Invest. Dermatol.* **1985**, 84, 176-179.
41. Lampe, M. A.; Williams, M. L.; Elias, P. M., Human epidermal lipids: characterization and modulations during differentiation. *J. Lipid Res.* **1983**, 24, 131-140.
42. Nicolaides, N.; Fu, H. C.; Rice, G. R., The skin surface lipids of man compared with those of eighteen species of animals. *J. Invest. Dermatol.* **1968**, 51, 83-89.
43. van Smeden, J.; Janssens, M.; Gooris, G. S.; Bouwstra, J. A., The important role of stratum corneum lipids for the cutaneous barrier function. *Biochim. Biophys. Acta* **2014**, 1841, 295-313.
44. Meguro, S.; Arai, Y.; Masukawa, Y.; Uie, K.; Tokimitsu, I., Relationship between covalently bound ceramides and transepidermal water loss (TEWL). *Arch. Dermatol. Res.* **2000**, 292, 463-468.
45. Behne, M.; Uchida, Y.; Seki, T.; de Montellano, P. O.; Elias, P. M.; Holleran, W. M., Omega-hydroxyceramides are required for corneocyte lipid envelope (CLE) formation and normal epidermal permeability barrier function. *J. Invest. Dermatol.* **2000**, 114, 185-192.
46. Downing, D. T., Lipid and protein structures in the permeability barrier of mammalian epidermis. *J. Lipid Res.* **1992**, 33, 301-313.
47. Demerjian, M.; Crumrine, D. A.; Milstone, L. M.; Williams, M. L.; Elias, P. M., Barrier dysfunction and pathogenesis of neutral lipid storage disease with ichthyosis (Chanarin–Dorfman syndrome). *J. Invest. Dermatol.* **2006**, 126, 2032-2038.
48. de Jager, M.; Groenink, W.; van der Spek, J.; Janmaat, C.; Gooris, G.; Ponec, M.; Bouwstra, J., Preparation and characterization of a stratum corneum substitute for in vitro percutaneous penetration studies. *Biochim. Biophys. Acta* **2006**, 1758, 636-644.
49. Johnson, M. E.; Blankschtein, D.; Langer, R., Permeation of steroids through human skin. *J. Pharm. Sci.* **1995**, 84, 1144-1146.
50. van de Sandt, J. J. M.; van Burgsteden, J. A.; Cage, S.; Carmichael, P. L.; Dick, I.; Kenyon, S.; Korinth, G.; Larese, F.; Limasset, J. C.; Maas, W. J. M.; Montomoli, L.; Nielsen, J. B.; Payan, J. P.; Robinson, E.; Sartorelli, P.; Schaller, K. H.; Wilkinson, S. C.; Williams, F. M., In vitro predictions of skin absorption of caffeine, testosterone, and benzoic acid: a multi-centre comparison study. *Regul. Toxicol. Pharm.* **2004**, 39, 271-281.
51. Nantasenamat, C.; Isarankura-Na-Ayudhya, C.; Prachayasittikul, V., Advances in computational methods to predict the biological activity of compounds. *Expert Opin. Drug Discov.* **2010**, 5, 633-654.
52. Nigam, A.; Klein, M. T., A mechanism-oriented lumping strategy for heavy hydrocarbon pyrolysis: imposition of quantitative structure-reactivity relationships for pure components. *Ind. Eng. Chem. Res.* **1993**, 32, 1297-1303.
53. Tutone, M.; Pecoraro, B.; Almerico, A., Investigation on Quantitative Structure-Activity Relationships of 1, 3, 4 Oxadiazole Derivatives as Potential Telomerase Inhibitors. *Curr. Drug Disc. Technol.* **2018**.
54. Cherkasov, A.; Muratov, E. N.; Fourches, D.; Varnek, A.; Baskin, I. I.; Cronin, M.; Dearden, J.; Gramatica, P.; Martin, Y. C.; Todeschini, R.; Consonni, V.; Kuz'min, V. E.; Cramer, R.; Benigni, R.; Yang, C.; Rathman, J.; Terfloth, L.; Gasteiger, J.; Richard, A.; Tropsha, A., QSAR Modeling: Where have you been? Where are you going to? *J. Med. Chem.* **2014**, 57, 4977-5010.
55. Ghasemi, F.; Mehridehnavi, A.; Pérez-Garrido, A.; Pérez-Sánchez, H., Neural network and deep-learning algorithms used in QSAR studies: merits and drawbacks. *Drug Discov. Today* **2018**, 23, 1784-1790.

56. Svetnik, V.; Liaw, A.; Tong, C.; Culberson, J. C.; Sheridan, R. P.; Feuston, B. P., Random Forest: A Classification and Regression Tool for Compound Classification and QSAR Modeling. *J. Chem. Inf. Comput. Sci.* **2003**, 43, 1947-1958.
57. Darnag, R.; Mostapha Mazouz, E. L.; Schmitzer, A.; Villemin, D.; Jarid, A.; Cherqaoui, D., Support vector machines: Development of QSAR models for predicting anti-HIV-1 activity of TIBO derivatives. *Eur. J. Med. Chem.* **2010**, 45, 1590-1597.
58. Singh, N.; Chaudhury, S.; Liu, R.; AbdulHameed, M. D. M.; Tawa, G.; Wallqvist, A., QSAR Classification Model for Antibacterial Compounds and Its Use in Virtual Screening. *J. Chem. Inf. Model.* **2012**, 52, 2559-2569.
59. Potts, R. O.; Guy, R. H., Predicting Skin Permeability. *Pharmaceutical Research* **1992**, 9, 663-669.
60. Potts, R. O.; Guy, R. H., A predictive algorithm for skin permeability: the effects of molecular size and hydrogen bond activity. *Pharm. Res.* **1995**, 12, 1628-1633.
61. Cronin, M.; Dearden, J.; Moss, G.; Murray-Dickson, G., Investigation of the mechanism of flux across human skin in vitro by quantitative structure–permeability relationships. *Eur. J. Pharm. Sci.* **1999**, 7, 325-330.
62. Barratt, M., Quantitative structure-activity relationships for skin permeability. *Toxicol. In Vitro* **1995**, 9, 27-37.
63. Geinoz, S.; Guy, R. H.; Testa, B.; Carrupt, P.-A., Quantitative structure-permeation relationships (QSPeRs) to predict skin permeation: a critical evaluation. *Pharm. Res.* **2004**, 21, 83-92.
64. Patel, H.; Berge, W. t.; Cronin, M. T. D., Quantitative structure–activity relationships (QSARs) for the prediction of skin permeation of exogenous chemicals. *Chemosphere* **2002**, 48, 603-613.
65. Moss, G. P.; Cronin, M. T. D., Quantitative structure–permeability relationships for percutaneous absorption: re-analysis of steroid data. *Int. J. Pharm.* **2002**, 238, 105-109.
66. Buchwald, P.; Bodor, N., A simple, predictive, structure-based skin permeability model. *J. Pharm. Pharmacol.* **2001**, 53, 1087-1098.
67. Magnusson, B. M.; Anissimov, Y. G.; Cross, S. E.; Roberts, M. S., Molecular Size as the Main Determinant of Solute Maximum Flux Across the Skin. *J. Invest. Dermatol.* **2004**, 122, 993-999.
68. El Tayar, N.; Tsai, R.-S.; Testa, B.; Carrupt, P.-A.; Hansch, C.; Leo, A., Percutaneous Penetration of Drugs: A Quantitative Structure–Permeability Relationship Study. *J. Pharm. Sci.* **1991**, 80, 744-749.
69. Gute, B. D.; Grunwald, G. D.; Basak, S. C., Prediction of the Dermal Penetration of Polycyclic Aromatic Hydrocarbons (PAHs): A hierarchical Qsar Approach. *SAR QSAR Environ. Res.* **1999**, 10, 1-15.
70. Chang, Y.-C.; Chen, C.-P.; Chen, C.-C., Predicting Skin Permeability of Chemical Substances using a Quantitative Structure-activity Relationship. *Procedia Engineering* **2012**, 45, 875-879.
71. Talete, S. DRAGON for Windows and Linux. (October 2018),
72. Ghose, A. K.; Viswanadhan, V. N.; Wendoloski, J. J., A knowledge-based approach in designing combinatorial or medicinal chemistry libraries for drug discovery. 1. A qualitative and quantitative characterization of known drug databases. *J. Comb. Chem.* **1999**, 1, 55-68.
73. Chen, C.-P.; Chen, C.-C.; Huang, C.-W.; Chang, Y.-C., Evaluating Molecular Properties Involved in Transport of Small Molecules in Stratum Corneum: A Quantitative Structure-Activity Relationship for Skin Permeability. *Molecules* **2018**, 23, 911.
74. Minghetti, P.; Casiraghi, A.; Cilurzo, F.; Montanari, L.; Valmen Monzani, M.; Bertolini, G.; Zaliani, A., Solvation enthalpies as descriptors of structure – in vitro

percutaneous permeation relationship of benzoxazinones regioisomers. *Il Farmaco* **2000**, 55, 563-568.
75. Lee, P. H.; Conradi, R.; Shanmugasundaram, V., Development of an in silico model for human skin permeation based on a Franz cell skin permeability assay. *Bioorg. Med. Chem. Lett.* **2010**, 20, 69-73.
76. Sinanoğlu, O., Microscopic surface tension down to molecular dimensions and microthermodynamic surface areas of molecules or clusters. *J. Chem. Phys.* **1981**, 75, 463-468.
77. Musiol, R.; Jampilek, J.; Podeszwa, B.; Finster, J.; Tabak, D.; Dohnal, J.; Polanski, J., RP-HPLC determination of lipophilicity in series of quinoline derivatives. *Central European Journal of Chemistry* **2009**, 7, 586-597.
78. Liou, Y.-B.; Ho, H.-O.; Yang, C.-J.; Lin, Y.-K.; Sheu, M.-T., Construction of a quantitative structure-permeability relationship (QSPR) for the transdermal delivery of NSAIDs. *J. Controlled Release* **2009**, 138, 260-267.
79. Roberts, M. S.; Pugh, W. J.; Hadgraft, J.; Watkinson, A. C., Epidermal permeability-penetrant structure relationships: 1. An analysis of methods of predicting penetration of monofunctional solutes from aqueous solutions. *Int. J. Pharm.* **1995**, 126, 219-233.
80. Pugh, W. J.; Hadgraft, J., Ab initio prediction of human skin permeability coefficients. *Int. J. Pharm.* **1994**, 103, 163-178.
81. Kamlet, M. J.; Doherty, R. M.; Abraham, M. H.; Marcus, Y.; Taft, R. W., Linear solvation energy relationship. 46. An improved equation for correlation and prediction of octanol/water partition coefficients of organic nonelectrolytes (including strong hydrogen bond donor solutes). *J. Phys. Chem. A* **1988**, 92, 5244-5255.
82. Ghafourian, T.; Fooladi, S., The effect of structural QSAR parameters on skin penetration. *Int. J. Pharm.* **2001**, 217, 1-11.
83. Gutierrez, Y.; Estrada, E., TOSS-MODE (Topological Sub-Structural Molecular Design) for Windows Version 4.0. *Universidad de Santiago de Compostela, Spain* **1997**.
84. Estrada, E., Edge adjacency relationships and a novel topological index related to molecular volume. *J. Chem. Inf. Comput. Sci.* **1995**, 35, 31-33.
85. González, M. P.; Helguera, A. M. Prediction the human skin permeation through a Topological Substructural approach. In 9th International Electronic Conference on Synthetic Organic, Chemistry (ECSOC-9), 2005; 2005.
86. Chauhan, P.; Shakya, M., Role of physicochemical properties in the estimation of skin permeability: in vitro data assessment by Partial Least-Squares Regression. *SAR QSAR Environ. Res.* **2010**, 21, 481-494.
87. Kennard, R. W.; Stone, L. A., Computer aided design of experiments. *Technometrics* **1969**, 11, 137-148.
88. Lei, B.; Li, J.; Liu, H.; Yao, X., Accurate prediction of aquatic toxicity of aromatic compounds based on genetic algorithm and least squares support vector machines. *Mol. Inform.* **2008**, 27, 850-865.
89. Narumi, H., New topological indices for finite and infinite systems. *MATCH Commun. Math. Comput. Chem* **1987**, 22, 195-207.
90. Abraham, M. H., Scales of solute hydrogen-bonding: their construction and application to physicochemical and biochemical processes. *Chem. Soc. Rev.* **1993**, 22, 73-83.
91. Abraham, M. H.; Acree Jr, W. E., Descriptors for ions and ion-pairs for use in linear free energy relationships. *J. Chromatogr. A* **2016**, 1430, 2-14.
92. Abraham, M. H.; Acree Jr, W. E., The transfer of neutral molecules, ions and ionic species from water to ethylene glycol and to propylene carbonate; descriptors for pyridinium cations. *New J. Chem.* **2010**, 34, 2298-2305.
93. Abraham, M. H.; Acree Jr, W. E., Equations for the transfer of neutral molecules and ionic species from water to organic phases. *J. Org. Chem.* **2010**, 75, 1006-1015.

94. Abraham, M. H., The permeation of neutral molecules, ions, and ionic species through membranes: brain permeation as an example. *J. Pharm. Sci.* **2011**, 100, 1690-1701.
95. Zhang, K.; Chen, M.; Scriba, G. K. E.; Abraham, M. H.; Fahr, A.; Liu, X., Linear Free Energy Relationship Analysis of Retention Factors in Cerasome Electrokinetic Chromatography Intended for Predicting Drug Skin Permeation. *J. Pharm. Sci.* **2011**, 100, 3105-3113.
96. Kupczewska-Dobecka, M.; Jakubowski, M.; Czerczak, S., Calculating the dermal flux of chemicals with OELs based on their molecular structure: An attempt to assign the skin notation. *Environ. Toxicol. Pharmacol.* **2010**, 30, 95-102.
97. Abraham, M. H.; Martins, F., Human Skin Permeation and Partition: General Linear Free-Energy Relationship Analyses. *J. Pharm. Sci.* **2004**, 93, 1508-1523.
98. Abraham, M. H.; Chadha, H. S.; Martins, F.; Mitchell, R. C.; Bradbury, M. W.; Gratton, J. A., 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.* **1999**, 55, 78-88.
99. Zhang, K.; Chen, M.; Scriba, G. K. E.; Abraham, M. H.; Fahr, A.; Liu, X., Human Skin Permeation of Neutral Species and Ionic Species: Extended Linear Free Energy Relationship Analyses. *J. Pharm. Sci.* **2012**, 101, 2034-2044.
100. Zhang, K.; Abraham, M. H.; Liu, X., An equation for the prediction of human skin permeability of neutral molecules, ions and ionic species. *Int. J. Pharm.* **2017**, 521, 259-266.
101. Tropsha, A.; Gramatica, P.; Gombar, V. K., The Importance of Being Earnest: Validation is the Absolute Essential for Successful Application and Interpretation of QSPR Models. *QSAR Comb. Sci.* **2003**, 22, 69-77.
102. Baumann, K.; Stiefl, N., Validation Tools for Variable Subset Regression. *J. Comput. Aided Mol. Des.* **2004**, 18, 549-562.
103. Golbraikh, A.; Tropsha, A., Predictive QSAR Modeling Based on Diversity Sampling of Experimental Datasets for the Training and Test Set Selection. *Mol. Divers.* **2000**, 5, 231-243.
104. Baumann, K., Cross-validation as the objective function for variable-selection techniques. *Trends Analyt. Chem.* **2003**, 22, 395-406.
105. Kubinyi, H., From narcosis to hyperspace: the history of QSAR. *Quantitative Structure-Activity Relationships* **2002**, 21, 348-356.
106. Golbraikh, A.; Tropsha, A., Beware of q2! *J. Mol. Graphics Modell.* **2002**, 20, 269-276.
107. Shao, J., Linear model selection by cross-validation. *J. Am. Stat. Assoc.* **1993**, 88, 486-494.
108. Eriksson, L.; Jaworska, J.; Worth, A. P.; Cronin, M. T.; McDowell, R. M.; Gramatica, P., Methods for reliability and uncertainty assessment and for applicability evaluations of classification-and regression-based QSARs. *Environ. Health Perspect.* **2003**, 111, 1361.
109. Guha, R.; Serra, J. R.; Jurs, P. C., Generation of QSAR sets with a self-organizing map. *J. Mol. Graphics Modell.* **2004**, 23, 1-14.
110. Gramatica, P., Principles of QSAR models validation: internal and external. *QSAR Comb. Sci.* **2007**, 26, 694-701.
111. Co-operation, O. f. E.; Development, *Guidance document on the validation of (quantitative) structure-activity relationship [(Q) SAR] models*. OECD Publishing: 2014.
112. Chirico, N.; Gramatica, P., Real External Predictivity of QSAR Models: How To Evaluate It? Comparison of Different Validation Criteria and Proposal of Using the Concordance Correlation Coefficient. *J. Chem. Inf. Model.* **2011**, 51, 2320-2335.
113. Roy, K., On some aspects of validation of predictive quantitative structure–activity relationship models. *Expert Opin. Drug Discov.* **2007**, 2, 1567-1577.

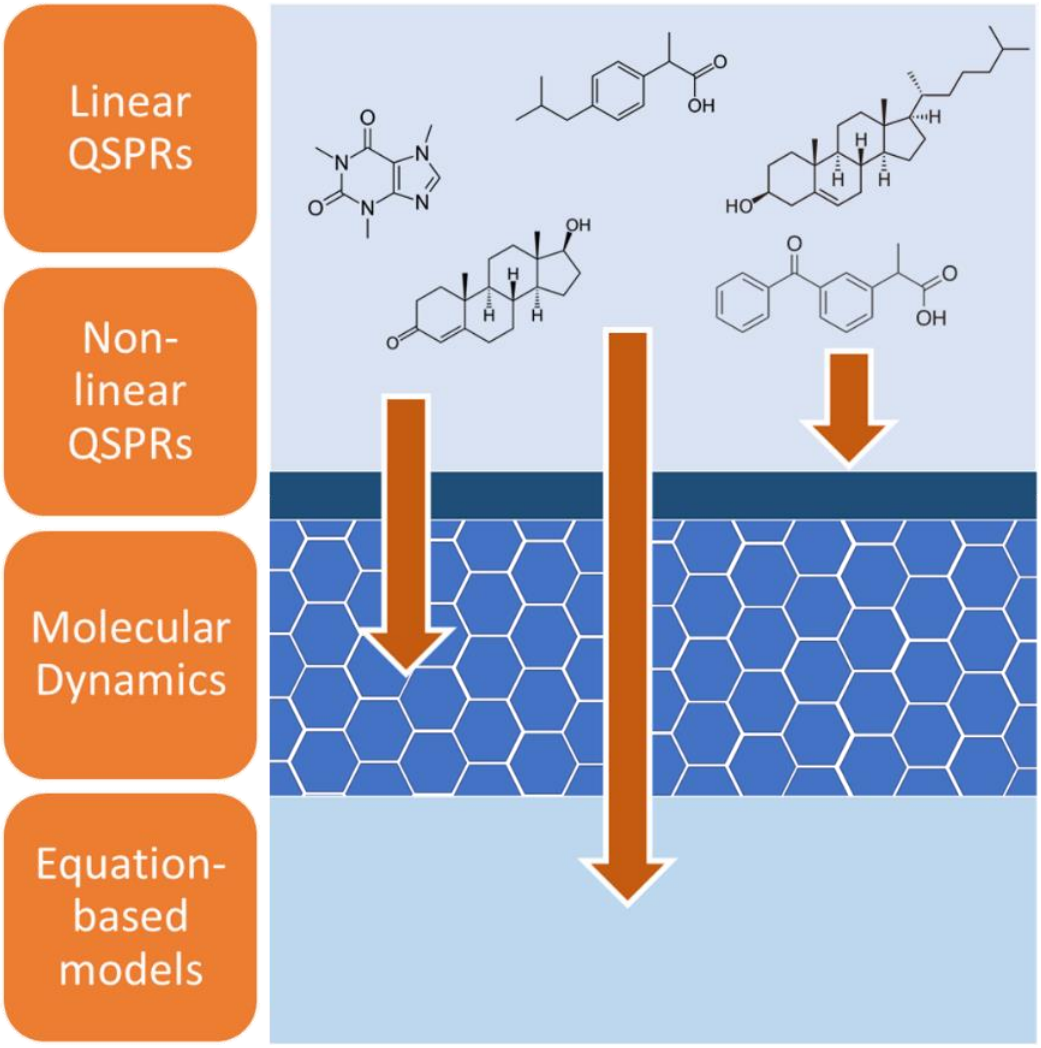
114. Consonni, V.; Ballabio, D.; Todeschini, R., Comments on the Definition of the Q2 Parameter for QSAR Validation. *J. Chem. Inf. Model.* **2009**, 49, 1669-1678.
115. Aptula, A. O.; Jeliazkova, N. G.; Schultz, T. W.; Cronin, M. T., The better predictive model: high q2 for the training set or low root mean square error of prediction for the test set? *QSAR Comb. Sci.* **2005**, 24, 385-396.
116. Lučić, B.; Trinajstić, N., Multivariate Regression Outperforms Several Robust Architectures of Neural Networks in QSAR Modeling. *J. Chem. Inf. Comput. Sci.* **1999**, 39, 121-132.
117. So, S.-S.; Karplus, M., Evolutionary optimization in quantitative structure− activity relationship: an application of genetic neural networks. *J. Med. Chem.* **1996**, 39, 1521-1530.
118. Patel, J.; Patel, L., Artificial neural networks and their applications in pharmaceutical research. *Pharmabuzz* **2007**, 2, 8-17.
119. Patel, J., Science of the science, drug discovery and artificial neural networks. *Curr. Drug Disc. Technol.* **2013**, 10, 2-7.
120. Fatemi, M. H.; Malekzadeh, H., In silico prediction of dermal penetration rate of chemicals from their molecular structural descriptors. *Environ. Toxicol. Pharmacol.* **2012**, 34, 297-306.
121. Katritzky, A.; Lobanov, V.; Karelson, M., CODESSA: training manual. *University of Florida, Gainesville, FL* **1995**.
122. Atobe, T.; Mori, M.; Yamashita, F.; Hashida, M.; Kouzuki, H., Artificial neural network analysis for predicting human percutaneous absorption taking account of vehicle properties. *J. Toxicol. Sci.* **2015**, 40, 277-294.
123. Katritzky, A. R.; Dobchev, D. A.; Fara, D. C.; Hür, E.; Tämm, K.; Kurunczi, L.; Karelson, M.; Varnek, A.; Solov'ev, V. P., Skin Permeation Rate as a Function of Chemical Structure. *J. Med. Chem.* **2006**, 49, 3305-3314.
124. Benson, S. W.; Buss, J. H., Additivity rules for the estimation of molecular properties. Thermodynamic properties. *J. Chem. Phys.* **1958**, 29, 546-572.
125. Varnek, A.; Fourches, D.; Horvath, D.; Klimchuk, O.; Gaudin, C.; Vayer, P.; Solov'ev, V.; Hoonakker, F.; Tetko, I. V.; Marcou, G., ISIDA-Platform for virtual screening based on fragment and pharmacophoric descriptors. *Curr. Comput. Aided Drug Des.* **2008**, 4, 191.
126. Lim, C. W.; Fujiwara, S.-i.; Yamashita, F.; Hashida, M., Prediction of human skin permeability using a combination of molecular orbital calculations and artificial neural network. *Biol. Pharm. Bull.* **2002**, 25, 361-366.
127. Chen, L.-j.; Lian, G.-p.; Han, L.-j., Prediction of human skin permeability using artificial neural network (ANN) modeling. *Acta Pharmacol. Sin.* **2007**, 28, 591.
128. Saini, S.; Singh, S.; Garg, A.; Khanna, K.; Shandil, A.; Mishra, D., Prediction of skin penetration using artificial neural network. *Int. J. Eng. Sci. Technol* **2010**, 2, 1526-1531.
129. Değim, T.; Hadgraft, J.; İlbasmiş, S.; Özkan, Y., Prediction of Skin Penetration Using Artificial Neural Network (ANN) Modeling. *J. Pharm. Sci.* **2003**, 92, 656-664.
130. Neumann, D.; Kohlbacher, O.; Merkwirth, C.; Lengauer, T., A Fully Computational Model for Predicting Percutaneous Drug Absorption. *J. Chem. Inf. Model.* **2006**, 46, 424-429.
131. Breiman, L., Random Forests. *Machine Learning* **2001**, 45, 5-32.
132. Praagman, J., In; North-Holland: 1985.
133. Baert, B.; Deconinck, E.; Van Gele, M.; Slodicka, M.; Stoppie, P.; Bodé, S.; Slegers, G.; Vander Heyden, Y.; Lambert, J.; Beetens, J.; De Spiegeleer, B., Transdermal penetration behaviour of drugs: CART-clustering, QSPR and selection of model compounds. *Biorg. Med. Chem.* **2007**, 15, 6943-6955.
134. Tong, W.; Hong, H.; Fang, H.; Xie, Q.; Perkins, R., Decision forest: combining the predictions of multiple independent decision tree models. *J. Chem. Inf. Comput. Sci.* **2003**, 43, 525-531.

135. Hawkins, D.; Musser, B., One tree or a forest? Alternative dendrographic models. *Computing Science and Statistics* **1998**, 534-542.
136. Alves, V. M.; Muratov, E.; Fourches, D.; Strickland, J.; Kleinstreuer, N.; Andrade, C. H.; Tropsha, A., Predicting chemically-induced skin reactions. Part II: QSAR models of skin permeability and the relationships between skin permeability and skin sensitization. *Toxicol. Appl. Pharmacol.* **2015**, 284, 273-280.
137. Alves, V. M.; Muratov, E.; Fourches, D.; Strickland, J.; Kleinstreuer, N.; Andrade, C. H.; Tropsha, A., Predicting chemically-induced skin reactions. Part I: QSAR models of skin sensitization and their application to identify potentially hazardous compounds. *Toxicol. Appl. Pharmacol.* **2015**, 284, 262-272.
138. EPA, A. *Risk Assessment Guidance for Superfund. Volume I: Human Health Evaluation Manual (Part E, Supplemental Guidance for Dermal Risk Assessment)*; EPA/540/R/99: 2004.
139. Cortes, C.; Vapnik, V., Support-vector networks. *Machine Learning* **1995**, 20, 273-297.
140. Vapnik, V., Statistical learning theory new york. *NY: Wiley* **1998**.
141. Baba, H.; Takahara, J.-i.; Yamashita, F.; Hashida, M., Modeling and Prediction of Solvent Effect on Human Skin Permeability using Support Vector Regression and Random Forest. *Pharm. Res.* **2015**, 32, 3604-3617.
142. Baba, H.; Ueno, Y.; Hashida, M.; Yamashita, F., Quantitative prediction of ionization effect on human skin permeability. *Int. J. Pharm.* **2017**, 522, 222-233.
143. Lam, L. T.; Sun, Y.; Davey, N.; Adams, R.; Prapopoulou, M.; Brown, M. B.; Moss, G. P., The application of feature selection to the development of Gaussian process models for percutaneous absorption. *J. Pharm. Pharmacol.* **2010**, 62, 738-749.
144. Moss, G. P.; Sun, Y.; Prapopoulou, M.; Davey, N.; Adams, R.; Pugh, W. J.; Brown, M. B., The application of Gaussian processes in the prediction of percutaneous absorption. *J. Pharm. Pharmacol.* **2009**, 61, 1147-1153.
145. Moss, G. P.; Sun, Y.; Wilkinson, S. C.; Davey, N.; Adams, R.; Martin, G. P.; Prapopopolou, M.; Brown, M. B., The application and limitations of mathematical modelling in the prediction of permeability across mammalian skin and polydimethylsiloxane membranes. *J. Pharm. Pharmacol.* **2011**, 63, 1411-1427.
146. Fedors, R. F., A method for estimating both the solubility parameters and molar volumes of liquids. *Polymer Engineering & Science* **1974**, 14, 147-154.
147. Wilschut, A.; ten Berge, W. F.; Robinson, P. J.; McKone, T. E., Estimating skin permeation. The validation of five mathematical skin permeation models. *Chemosphere* **1995**, 30, 1275-1296.
148. ten Berge, W., A simple dermal absorption model: Derivation and application. *Chemosphere* **2009**, 75, 1440-1445.
149. Sun, Y.; Brown, M. B.; Prapopoulou, M.; Davey, N.; Adams, R.; Moss, G. P., The application of stochastic machine learning methods in the prediction of skin penetration. *Appl. Soft Comp.* **2011**, 11, 2367-2375.
150. Friedman, J.; Hastie, T.; Tibshirani, R., *The elements of statistical learning*. Springer series in statistics New York, NY, USA:: 2001; Vol. 1.
151. Hu, L.-Y.; Huang, M.-W.; Ke, S.-W.; Tsai, C.-F., The distance function effect on k-nearest neighbor classification for medical datasets. *SpringerPlus* **2016**, 5, 1304.
152. Altman, N. S., An introduction to kernel and nearest-neighbor nonparametric regression. *Am. Stat.* **1992**, 46, 175-185.
153. Ramaswamy, S.; Rastogi, R.; Shim, K. Efficient algorithms for mining outliers from large data sets. In ACM Sigmod Record, 2000; ACM: 2000; Vol. 29; pp 427-438.

154. Lindh, M.; Karlén, A.; Norinder, U., Predicting the Rate of Skin Penetration Using an Aggregated Conformal Prediction Framework. *Molecular Pharmaceutics* **2017**, 14, 1571-1576.
155. Shafer, G.; Vovk, V., A tutorial on conformal prediction. *Journal of Machine Learning Research* **2008**, 9, 371-421.
156. Vovk, V.; Gammerman, A.; Shafer, G., *Conformal prediction*. Springer: 2005.
157. Norinder, U.; Carlsson, L.; Boyer, S.; Eklund, M., Introducing conformal prediction in predictive modeling. A transparent and flexible alternative to applicability domain determination. *J. Chem. Inf. Model.* **2014**, 54, 1596-1603.
158. Basak, S. C.; Mills, D.; Mumtaz, M. M., A quantitative structure–activity relationship (QSAR) study of dermal absorption using theoretical molecular descriptors. *SAR QSAR Environ. Res.* **2007**, 18, 45-55.
159. Khajeh, A.; Modarress, H., Linear and nonlinear quantitative structure-property relationship modelling of skin permeability. *SAR QSAR Environ. Res.* **2014**, 25, 35-50.
160. Jang, J.-S., ANFIS: adaptive-network-based fuzzy inference system. *IEEE transactions on systems, man, and cybernetics* **1993**, 23, 665-685.
161. Flynn, G., Physiochemical determinants of skin absorption. *Principles of route-to-route extrapolation for risk assessment* **1990**, 93-127.
162. Abraham, M. H.; Martins, F.; Mitchell, R. C., Algorithms for skin permeability using hydrogen bond descriptors: the problem of steroids. *J. Pharm. Pharmacol.* **1997**, 49, 858-865.
163. Pannier, A. K.; Brand, R. M.; Jones, D. D., Fuzzy Modeling of Skin Permeability Coefficients. *Pharm. Res.* **2003**, 20, 143-148.
164. Keshwani, D. R.; Jones, D. D.; Brand, R. M., Review: Takagi–Sugeno Fuzzy Modeling of Skin Permeability. *Cutaneous and Ocular Toxicology* **2005**, 24, 149-163.
165. Wang, L.; Chen, L.; Lian, G.; Han, L., Determination of partition and binding properties of solutes to stratum corneum. *Int. J. Pharm.* **2010**, 398, 114-122.
166. Fujiwara, S. I.; Yamashita, F.; Hashida, M., QSAR Analysis of Interstudy Variable Skin Permeability Based on the latent Membrane Permeability Concept. *J. Pharm. Sci.* **2003**, 92, 1939-1946.
167. Williams, A. C.; Barry, B. W., Penetration enhancers. *Adv. Drug Del. Rev.* **2004**, 56, 603-618.
168. Zheng, T.; Hopfinger, A. J.; Esposito, E. X.; Liu, J.; Tseng, Y. J., Membrane-Interaction Quantitative Structure−Activity Relationship (MI-QSAR) Analyses of Skin Penetration Enhancers. *J. Chem. Inf. Model.* **2008**, 48, 1238-1256.
169. Kulkarni, A.; Hopfinger, A.; Osborne, R.; Bruner, L. H.; Thompson, E. D., Prediction of eye irritation from organic chemicals using membrane-interaction QSAR analysis. *Toxicol. Sci.* **2001**, 59, 335-345.
170. Kulkarni, A. S.; Hopfinger, A., Membrane-interaction QSAR analysis: application to the estimation of eye irritation by organic compounds. *Pharm. Res.* **1999**, 16, 1245-1253.
171. Kulkarni, A.; Han, Y.; Hopfinger, A. J., Predicting Caco-2 cell permeation coefficients of organic molecules using membrane-interaction QSAR analysis. *J. Chem. Inf. Comput. Sci.* **2002**, 42, 331-342.
172. Iyer, M.; Mishra, R.; Han, Y.; Hopfinger, A., Predicting blood–brain barrier partitioning of organic molecules using membrane–interaction QSAR analysis. *Pharm. Res.* **2002**, 19, 1611-1621.
173. Muhammad, F.; Jaberi-Douraki, M.; de Sousa, D. P.; Riviere, J. E., Modulation of chemical dermal absorption by 14 natural products: a quantitative structure permeation analysis of components often found in topical preparations. *Cutaneous and Ocular Toxicology* **2017**, 36, 237-252.

174. Ghafourian, T.; Samaras, E. G.; Brooks, J. D.; Riviere, J. E., Modelling the effect of mixture components on permeation through skin. *Int. J. Pharm.* **2010**, 398, 28-32.
175. Ghafourian, T.; Samaras, E. G.; Brooks, J. D.; Riviere, J. E., Validated models for predicting skin penetration from different vehicles. *Eur. J. Pharm. Sci.* **2010**, 41, 612-616.
176. Guth, K.; Riviere, J. E.; Brooks, J. D.; Dammann, M.; Fabian, E.; van Ravenzwaay, B.; Schäfer-Korting, M.; Landsiedel, R., In silico models to predict dermal absorption from complex agrochemical formulations. *SAR QSAR Environ. Res.* **2014**, 25, 565-588.
177. Riviere, J. E.; Brooks, J. D., Prediction of dermal absorption from complex chemical mixtures: incorporation of vehicle effects and interactions into a QSPR framework. *SAR QSAR Environ. Res.* **2007**, 18, 31-44.
178. Riviere, J. E.; Brooks, J. D., Predicting Skin Permeability from Complex Chemical Mixtures: Dependency of Quantitative Structure Permeation Relationships on Biology of Skin Model Used. *Toxicol. Sci.* **2011**, 119, 224-232.
179. Xu, G.; Hughes-Oliver, J. M.; Brooks, J. D.; Baynes, R. E., Predicting skin permeability from complex chemical mixtures: incorporation of an expanded QSAR model. *SAR QSAR Environ. Res.* **2013**, 24, 711-731.
180. Hadgraft, J., Skin deep. *Eur. J. Pharm. Biopharm.* **2004**, 58, 291-299.
181. Hadgraft, J.; Walters, K., Skin penetration enhancement. *Journal of dermatological treatment* **1994**, 5, 43-47.
182. Drakulić, B. J.; Juranić, I. O.; Erić, S.; Zloh, M., Role of complexes formation between drugs and penetration enhancers in transdermal delivery. *Int. J. Pharm.* **2008**, 363, 40-49.
183. Moss, G. P.; Shah, A. J.; Adams, R. G.; Davey, N.; Wilkinson, S. C.; Pugh, W. J.; Sun, Y., The application of discriminant analysis and Machine Learning methods as tools to identify and classify compounds with potential as transdermal enhancers. *Eur. J. Pharm. Sci.* **2012**, 45, 116-127.
184. Yerramsetty, K. M.; Neely, B. J.; Madihally, S. V.; Gasem, K. A. M., A skin permeability model of insulin in the presence of chemical penetration enhancer. *Int. J. Pharm.* **2010**, 388, 13-23.
185. Alder, B. J.; Wainwright, T. E., *Studies in Molecular Dynamics. I. General Method*. 1959; Vol. 31, p 459.
186. Xiang, T. X.; Anderson, B. D., Molecular distributions in interphases: statistical mechanical theory combined with molecular dynamics simulation of a model lipid bilayer. *Biophys. J.* **1994**, 66, 561-572.
187. Das, C.; Olmsted, P. D.; Noro, M. G., Water permeation through stratum corneum lipid bilayers from atomistic simulations. *Soft Matter* **2009**, 5, 4549-4555.
188. Gupta, R.; Sridhar, D. B.; Rai, B., Molecular Dynamics Simulation Study of Permeation of Molecules through Skin Lipid Bilayer. *The Journal of Physical Chemistry B* **2016**, 120, 8987-8996.
189. Marrink, S. J. Permeation of Small Molecules Across Lipid Membranes: A Molecular Dynamics Study. Thesis, University of Groningen, 1994.
190. Egberts, E.; Marrink, S.-J.; Berendsen, H. J., Molecular dynamics simulation of a phospholipid membrane. *Eur. Biophys. J.* **1994**, 22, 423-436.
191. Marrink, S.-J.; Berendsen, H. J., Simulation of water transport through a lipid membrane. *J. Phys. Chem. A* **1994**, 98, 4155-4168.
192. Marrink, S. J.; Berendsen, H. J. C., Permeation Process of Small Molecules across Lipid Membranes Studied by Molecular Dynamics Simulations. *The Journal of Physical Chemistry* **1996**, 100, 16729-16738.
193. Rim, J. E.; Pinsky, P. M.; van Osdol, W. W., Multiscale Modeling Framework of Transdermal Drug Delivery. *Ann. Biomed. Eng.* **2009**, 37, 1217-1229.

194. Gajula, K.; Gupta, R.; Sridhar, D. B.; Rai, B., In-Silico Skin Model: A Multiscale Simulation Study of Drug Transport. *J. Chem. Inf. Model.* **2017**, 57, 2027-2034.
195. Lundborg, M.; Narangifard, A.; Wennberg, C. L.; Lindahl, E.; Daneholt, B.; Norlén, L., Human skin barrier structure and function analyzed by cryo-EM and molecular dynamics simulation. *J. Struct. Biol.* **2018**, 203, 149-161.
196. Lundborg, M.; Wennberg, C. L.; Narangifard, A.; Lindahl, E.; Norlén, L., Predicting drug permeability through skin using molecular dynamics simulation. *J. Controlled Release* **2018**, 283, 269-279.
197. Rocco, P.; Cilurzo, F.; Minghetti, P.; Vistoli, G.; Pedretti, A., Molecular Dynamics as a tool for in silico screening of skin permeability. *Eur. J. Pharm. Sci.* **2017**, 106, 328-335.
198. Vecchia, B. E.; Bunge, A. L., Skin absorption databases and predictive equations. *Transdermal Drug Delivery Systems. Marcel Dekker, New York* **2002**, 57-141.
199. Selzer, D.; Neumann, D.; Schaefer, U. F., Mathematical models for dermal drug absorption. *Expert Opin. Drug Metab. Toxicol.* **2015**, 11, 1567-1583.
200. Dancik, Y.; Miller, M. A.; Jaworska, J.; Kasting, G. B., Design and performance of a spreadsheet-based model for estimating bioavailability of chemicals from dermal exposure. *Adv. Drug Del. Rev.* **2013**, 65, 221-236.
201. Kattou, P.; Lian, G.; Glavin, S.; Sorrell, I.; Chen, T., *Development of a Two-Dimensional Model for Predicting Transdermal Permeation with the Follicular Pathway: Demonstration with a Caffeine Study*. 2017; Vol. 34.
202. Wen, J.; Koo, S. M.; Lape, N., How Sensitive Are Transdermal Transport Predictions by Microscopic Stratum Corneum Models to Geometric and Transport Parameter Input? *J. Pharm. Sci.* **2018**, 107, 612-623.
203. Chen, L.; Lian, G.; Han, L., Modeling transdermal permeation. Part I. Predicting skin permeability of both hydrophobic and hydrophilic solutes. *AIChE Journal* **2010**, 56, 1136-1146.
204. Chen, L.; Lian, G.; Han, L., Use of "bricks and mortar" model to predict transdermal permeation: model development and initial validation. *Ind. Eng. Chem. Res.* **2008**, 47, 6465-6472.
205. Naegel, A.; Heisig, M.; Wittum, G. Computational Modeling of the Skin Barrier. In *Permeability Barrier: Methods and Protocols*, Turksen, K., Ed.; Humana Press: Totowa, NJ, 2011, pp 1-32.
206. Mitragotri, S., A theoretical analysis of permeation of small hydrophobic solutes across the stratum corneum based on scaled particle theory. *J. Pharm. Sci.* **2002**, 91, 744-752.
207. Mitragotri, S., Modeling skin permeability to hydrophilic and hydrophobic solutes based on four permeation pathways. *J. Controlled Release* **2003**, 86, 69-92.
208. Lebowitz, J. L.; Helfand, E.; Praestgaard, E., Scaled particle theory of fluid mixtures. *J. Chem. Phys.* **1965**, 43, 774-779.
209. Chen, T.; Lian, G.; Kattou, P., In Silico Modelling of Transdermal and Systemic Kinetics of Topically Applied Solutes: Model Development and Initial Validation for Transdermal Nicotine. *Pharm. Res.* **2016**, 33, 1602-1614.
210. Chen, L.; Han, L.; Saib, O.; Lian, G., In Silico Prediction of Percutaneous Absorption and Disposition Kinetics of Chemicals. *Pharm. Res.* **2015**, 32, 1779-1793.


Linear QSPRs
Non-linear QSPRs
Molecular Dynamics
Equation-based models