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Determination of the effective dermal penetration barrier pH of porcine skin

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The prediction of drug and chemical absorption across the skin is largely based on mathematical models that assume passive Fickian diffusion as the primary mechanism for transdermal delivery. This assumption, coupled to a significant historic database of drug and chemical absorption, suggests that only noncharged neutral chemicals pass through the intercellular lipid channels of the stratum corneum that comprises the epidermal penetration barrier (Elias, 1983; Riviere, 2006). Quantitative structure permeability (QSPeR) models used to quantitate passive chemical diffusion usually incorporate parameters such as the octanol–water partition coefficient (log $K_{o/w}$) to estimate stratum corneum partitioning (Hansch & Dunn, 1972; Potts & Guy, 1992). However, when aqueous-based formulations or complex chemical mixtures including weak acids or bases are exposed topically to skin, only the noncharged moiety will partition into the stratum corneum. A major determinant of this nonionized fraction is the pH of the effective stratum corneum barrier that restricts diffusion relative to the pKa of the chemical making log $K_{o/w}$ potentially biased for compounds with a significant ionized fraction unless this fraction is determined (Geinoz *et al.*, 2002). This can be accounted for by using log D_{pH}, operationally the log $K_{o/w}$ at different pHs, to adjust for different pH. The question however is what pH should be employed to calculate this value?

The literature places the pH of the surface of the skin determined by a multitude of methods as ranging from 4.8 to 6.5 (Ehlers *et al.*, 2001; Fluhr *et al.*, 2001; Sznitowska *et al.*, 2001; Kamal *et al.*, 2005; Lambers *et al.*, 2006; Schmid-Wendtner & Korting, 2006), with most animal species falling into this range (Meyer & Neurand, 1991). Differences in body site, gender, temperature, measuring techniques as well as numerous other variables generate considerable variance in these estimates. However, the surface may not be the optimal location for assessing the pH that modulates compound flux through the stratum corneum, as a pH gradient exists between the acid stratum corneum and the normal physiological pH 7.4 of the dermis.

Previous studies in our laboratory have used various QSPeR models to estimate chemical absorption from complex chemical mixtures (Riviere & Brooks, 2005, 2007). These experiments provide a rich dataset to explore the effective skin pH modulating dermal absorption as a number of different vehicles were employed. The purpose of the present analysis was to determine the operational or effective pH of the skin relative to dermal penetration by using an existing QSPeR model and assessing its best model fit as a function of penetrant's log D_{pH}.

These analyses employed this previously published dataset (Riviere & Brooks, 2005, 2007) on the dermal permeability constants (log k_p) of 12 marker compounds (Table 1) in 24 mixture combinations (Table 2) resulting in a full factorial experimental design of 288 unique chemical-mixture treatments (n = 4–5 replicates per treatment). Log k_p was obtained from experimental studies using finite-dosed dermatomed porcine skin

Table 1. Identity of 12 marker compounds investigated in *in vitro* porcine skin diffusion cells

Substituted phenols	*Organophosphates*	*Triazine herbicides*
Nonylphenol	Chlorpyrifos	Atrazine
Pentachlorophenol*	Ethylparathion	Propazine
p-Nitrophenol*	Methylparathion	Simazine
Phenol	Fenthion	Triazine*

*Compounds with log D_{pH} values that vary over the studied pH range.

Table 2. Composition of 24 mixtures investigated in *in vitro* porcine skin diffusion cells

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

MNA, Methylnicotinic acid; PG, Propylene glycol (1,2-propanediol); SLS, Sodium lauryl sulphate.

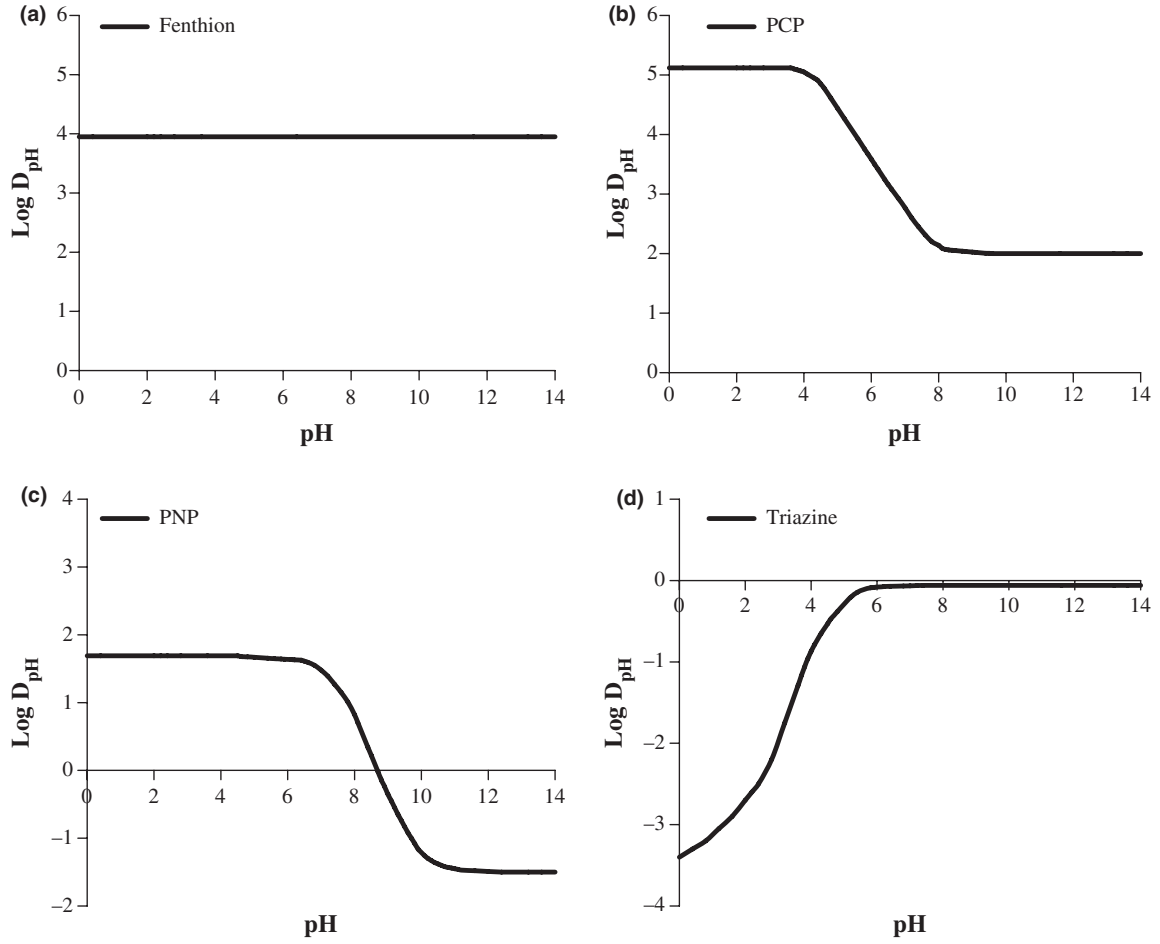

Fig. 1. log D_{pH} vs. pH for (a) Fenthion, (b) Pentachlorophenol, (c) *p*-Nitrophenol, and (d) Triazine. (Adapted from Pharma Algorithms, Inc. ADME Boxes 4.1).

in flow-through Bronaugh diffusion cells fully described elsewhere (Bronaugh & Stewart, 1985; Chang & Riviere, 1991; Riviere & Brooks, 2005).

We utilized the Potts and Guy QSPeR model [log $K_{o/w}$, Molecular Weight(MW)] for predicting log k_p with and without mixture factors (MF) to assess the effect of substituting log $K_{o/w}$ with log D_{pH} as a function of pH ranging from 4.6 to 8.0. Briefly, the MF is a mixture-component weighted covariate that accounts for vehicle effects and interactions on dermal permeability. A MF is determined by optimizing r^2 and examining the relationship between the residual plot of the QSPeR against concentration-weighted physical/chemical properties of the mixtures or solvents in which they were dosed. This approach has been shown to work for multiple QSPeR models (Riviere & Brooks, 2007). Each QSPeR model has an optimal MF with Topical Polar Surface Area (TPSA) being previously determined appropriate for the Potts–Guy equation for our dataset. Calculated QSPeR parameters (log $K_{o/w}$, Log D_{pH}, MW, TPSA) were obtained from ADME Boxes 4.1 (Pharma Algorithms, Inc., Toronto, ON, Canada).

For the purpose of probing effective barrier pH, the selection of a more complex and hence, a more predictive QSPeR model is not necessary as they are as sensitive to pH effects as the simpler two-termed equation. The simplest QSPeR model is always preferred (Cronin & Schultz, 2003). Therefore, the four equations utilized were

$$\log k_p = a\log K_{o/w} + b\mathrm{MW}$$

$$\log k_p = a\log D_{pH} + b\mathrm{MW}$$

$$\log k_p = a\log K_{o/w} + b\mathrm{MW} + m\mathrm{TPSA}$$

$$\log k_p = a\log D_{pH} + b\mathrm{MW} + m\mathrm{TPSA}$$

Goodness of fit was judged by changes in statistical estimates of correlation coefficients (r^2, q^2_{LOO} and $q^2_{25\%}$) of model predicted vs. observed log *k*p. Tabulated statistics include the square of the correlation coefficients adjusted to the number of degrees of freedom (r^2), the cross-validation square of the correlation coefficients using the 'leave-one-out' (q^2_{LOO}) and the 'leave-a-random-25%-out' ($q^2_{25\%}$) techniques, the standard deviation (*s*) and the Fischer's statistical test (*F*) for each equation. r^2 was

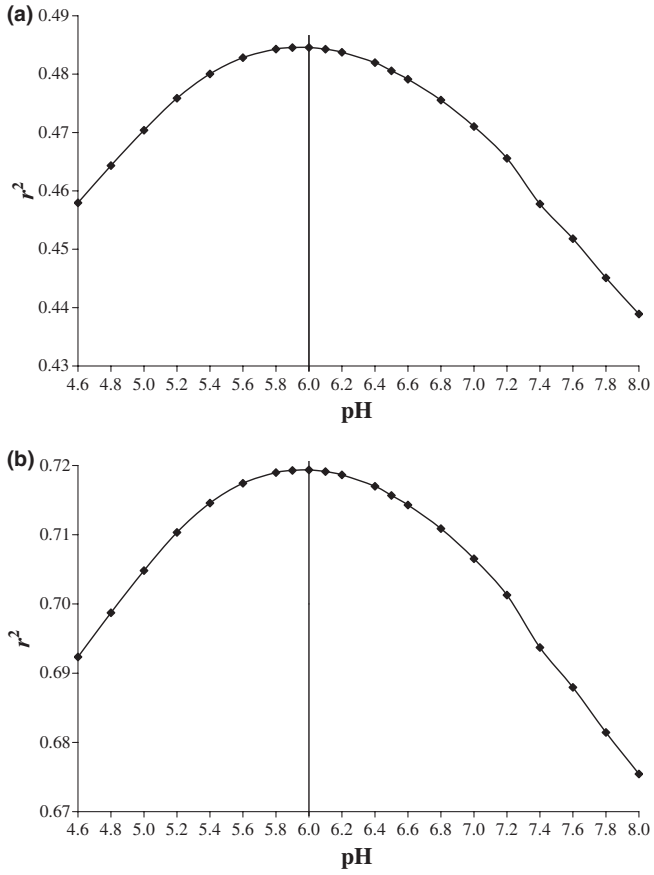

Fig. 2. r^2 (correlation) vs. log D_{pH} at the indicated pH value for (a) without a mixture factor and (b) utilizing Topical Polar Surface Area (TPSA) as the mixture factor.

then plotted as a function of pH using models with log D_{pH} to determine 'effective' skin barrier pH.

Figure 1 illustrates the dependency of partition coefficient on pH for the weak acids pentachlorophenol (PCP), *p*-Nitrophenol (PNP) and Triazine compared to the nonionic Fenthion, demonstrating the importance of knowing an 'effective' skin barrier pH. Using the two QSPeR equations parameterized with log D_{pH} (with and without MF), r^2 for model fits as a function of pH were plotted in Fig. 2. Both of these plots clearly demonstrate optimal correlations at pH of 5.7 to 6.3, with an average of 6 being representative. Figure 3 and Table 3 show the improvement in predictability using log D_{pH} = 6 compared with using log $K_{o/w}$ in models with and without a MF. For example, the experimental value for log $K_{o/w}$ for PCP is 5.12, a value determined at a pH of 1.4 in hydrochloric acid (Hansch & Leo, 1979). This is not representative of what happens on the surface of the skin. Note also the dramatic improvement in predictability for the basic model when a MF is added (Fig. 3a–d), which accounts for vehicle effects on solubility and partition coefficient.

A skin pH of 6 agrees favourably with other studies that have directly measured surface or stratum corneum pH ranging from 4.8 to 6.5 (Meyer & Neurand, 1991; Ehlers *et al.*, 2001; Fluhr *et al.*, 2001; Sznitowska *et al.*, 2001; Kamal *et al.*, 2005; Lambers *et al.*, 2006; Schmid-Wendtner & Korting, 2006). The approach used in our study is actually assessing an 'effective stratum corneum barrier pH' that impacts molecular diffusion through the barrier. In all probability, this barrier is not on the surface of skin, but rather is within the stratum corneum intercellular lipid pathway and thus is intermediate between the well-known 'acid corneum' and the well buffered physiological

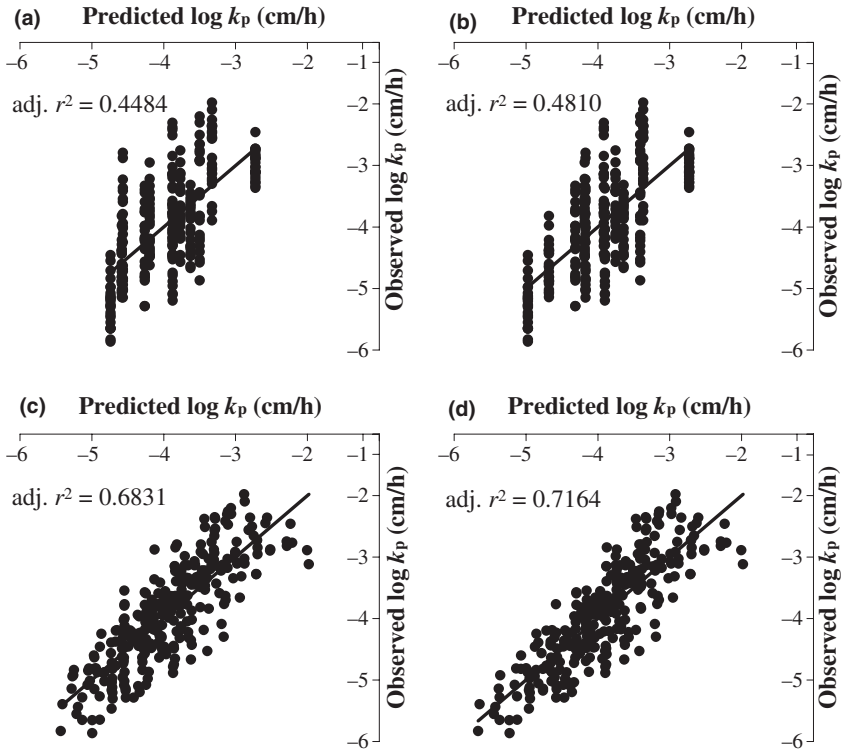

Fig. 3. Multiple linear regression analysis of 288 PSFT doses utilizing the Potts and Guy (1992) Model (a) log $K_{o/w}$ + Molecular Weight, (b) log $D_{(pH\ 6.0)}$ + Molecular Weight, (c) log $K_{o/w}$ + Molecular Weight + TPSA, and (d) log $D_{(pH\ 6.0)}$ + Molecular Weight + TPSA. (TPSA, Topical Polar Surface Area).

Table 3. log k_p QSPeR parameter values and goodness of fit criteria for *in vitro* porcine skin diffusion cell predictions

r^2	q^2_{LOO}	$q^2_{25\%}$	s	F	i	m MF	a	b
0.45	0.44	0.44	0.62	118	−2.81(0.13)	No MF	−0.30(0.03) log *Ko/w*	−0.00074(0.0007) MW
0.48	0.48	0.48	0.60	134	−2.77(0.13)	No MF	−0.40(0.03) log *D*(pH 6.0)	0.00010(0.0007) MW
0.68	0.68	0.68	0.47	207	−2.05(0.11)	−0.04(0.003) TPSA	−0.30(0.02) log *Ko/w*	−0.00079(0.0006) MW
0.72	0.71	0.71	0.45	243	−2.01(0.11)	−0.04(0.002) TPSA	−0.40(0.02) log *D*(pH 6.0)	0.000054(0.0005) MW

Standard error in parentheses (n = 288 chemical-mixture combinations).
TPSA, topical polar surface area.

pH of 7.4 in the dermis making a pH of 6 a reasonable estimate of effective barrier pH. Finally, this study also demonstrates the utility of mathematical models such as these simple QSPeR relationships to probe physiological processes where direct measurements may be disruptive of physiological processes.

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