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Predicting absorption of fragrance chemicals through human skin

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Synopsis

Potts and Guy fitted an equation to the experimental permeation coefficients (Kp) of a variety of molecular structures through human skin (1). This equation was based on molecular weight (MW) and the octanol-water partition coefficient (log P).

In the endeavor to identify a mathematical model predictive of the mostly unexplored skin penetration of fragrance chemicals, the Potts-Guy equation was tested here by correlating the published, measured Kp for 20 such compounds with predicted Kp values. The data, covering a broad range of structures, are well described by that model ($r^2 = 0.86$, $p < 0.001$). Also, calculated octanol-water partition coefficients, an alternative in case of missing measured values, appear well founded when analyzed statistically: comparison of 33 measured partition values with the calculated log Ps show near-perfect correlation for the intermediate lipophilicity range typical of fragrance chemicals ($0.5 < \log P < 3.5$; $r^2 = 0.97$, $p < 0.001$). The Potts-Guy relation thereby recommends itself as a simple and reliable method for the quantitative evaluation of human skin absorption potential of small-molecular-weight nonelectrolytes, and of fragrance chemicals, in particular where such data are largely missing.

INTRODUCTION

Exposure to fragrances is becoming unavoidable in the course of daily activities, as an ever-growing range of consumer goods is perfumed, either for functional or esthetic reasons. Most exposure is olfactory, and thus fleeting, and with the rare exception of individuals suffering from chemical hypersensitivities, without notable adverse effects. Through one category of products, however, exposure to fragrances is immediate, repeated, and significant, and dermatologists as well as toxicologists are becoming increasingly aware of the potential for toxicity. A large number of consumer products, namely the category of cosmetics and toiletries designed for contact with the skin, mostly as so-called stay-on products, contain a relatively high percentage of fragrance materials. Perfumes and colognes consist entirely of highly complex fragrance mixtures,

This paper celebrates the 70th birthday of Bruno P. Vaterlaus, Dr. Sc. Techn. ETHZ, who for many years directed the course of research at Givaudan with great integrity and vision, through good, and sometimes very difficult times.

natural, nature-identical, or fully synthetic. As far as they contain natural oils and essences, they rate a low priority for purposes of human health concerns, as those currently in use have been proven safe based on an age-old history of human exposure without adverse effects of note. However, some 3000 synthetic chemicals are currently also used in fragrance compounding (2). They find increasing application in personal care products, for esthetic as well as economic reasons; yet only a handful have been tested on human or animal skin for permeation, and thus systemic availability assessment. It therefore appeared appropriate to evaluate the relevance of currently developed mathematical models as an expeditious approach for the prediction of the human skin penetration potential by fragrance chemicals.

METHODS

PERMEABILITY COEFFICIENT Kp

In order to avoid or minimize the ethical, economic, and biological difficulties associated with *in vivo* experimentation, *in vitro* studies using human skin are now being used most frequently for estimating dermal absorption. From such studies, where a standard protocol has been observed, a statistically adequate number of data has now been generated that lends itself to mathematical modeling for QSAR purposes. Common parameters that result from such percutaneous absorption experiments are the permeability coefficient Kp, defined as $J_{ss} = KpC$, where J_{ss} and C are the steady-state flux and donor concentration, the latter maintained at an unchanged (or infinite) value. The rate at which a chemical penetrates the skin, in the present paper expressed in cm/hr, is described as the permeability coefficient Kp or as its logarithm, log Kp—a value determined over sufficient time to assure conditions of steady state. When the permeant is applied from aqueous solution, it represents the *in vitro* method by which most compound-specific penetration data currently available in the literature have been generated.

In such studies, a piece of excised human skin is attached to a diffusion cell consisting of a top chamber to receive an adequate volume of the penetrant in solution, an O-ring to secure the skin in place, and a temperature-controlled bottom chamber containing saline and equipped with a sampling port to withdraw fractions at specific time intervals for analysis (2a). Although forearm skin is an ideal choice, most commonly abdominal skin obtained at autopsy is used for reasons of availability. It is now generally accepted that properly conducted *in vitro* tests using human skin yield a reasonably good prediction of the absorption rate for chemicals in humans.

OCTANOL/WATER PARTITION COEFFICIENT P_{oct}

Polarity was found empirically to be a critical, if not the most important, physicochemical parameter determining the ability of chemical compounds to penetrate the skin barrier. It describes the partitioning behavior of a solute between a lipid and a polar phase, typically n-octanol and water. Expressed as the partition coefficient P_{oct} or its logarithm log P_{oct}, it represents the solvent system most frequently used to describe the polarity of chemicals.

Log P_{oct} is the only experimentally determined component in the Potts-Guy algorithm predictive of skin penetration. As a measure of lipophilicity, it is thereby confirmed as a critical factor determining the diffusion of solutes through biological membranes in general. A large body of octanol-water partitioning values has been compiled by Hansch and Leo in the Pomona College Medicinal Chemistry Project (PCMCP) log P database over a period of 25 years (3). In the process, more than 30,000 published experiments have been reviewed and also tested for validity. While partitioning values between water and a variety of lipophilic solvents, and also with soil, are included in that database to describe polarity, the core of that information system consists of octanol-water partition behavior. Among the latter, the numbers found to be the best after review of the experimental methods are highlighted as "star-values," and at this time over 9000 such "star-values" make up the so-called starlist of measured log P_{oct}, which is constantly under review and expansion.

The approximately 30,000 measured partition values, however, represent only a small portion of the total inventory of hundreds of thousands of chemicals presently available for industrial or commercial use. Those log P_{oct} values on record have been experimentally determined on a priority basis, focusing primarily upon the most prominent pharmaceuticals, pesticides, and frequently used solvents. Log P_{oct} for fragrance chemicals, on the other hand, are only by chance included in that list. For compounds for which no experimental data exist, an accurate method had been elaborated at the PCMCP for calculating log P by summation of fragment values, combined with factors that take into account any fragment interaction. Including an estimate of error, they are accessible alongside the measured values, and thus data required for the description of polarity of virtually all chemicals have become readily retrievable.

In order to ascertain the validity of log P_{oct} values so calculated, they have been correlated here with the corresponding measured values published in the literature for 33 fragrance compounds, in order to justify their use in the Potts-Guy algorithm.

THE POTTS-GUY SKIN PENETRATION MODEL

In recent time a number of mathematical models have been developed that are mechanistically and biologically founded, in the endeavor to render the penetrability of the skin by chemicals predictable, without requiring laborious and expensive experimentation. In a review and evaluation of such models, the U.S. E.P.A. selected an equation by Potts and Guy (1) as the most appropriate for predicting skin penetration of priority pollutants (4). It is based upon physicochemical interpretation of molecular transport across biological membranes, and adequately accounts for the percutaneous absorption of compounds of the most diverse structures. Using measured permeability coefficients, Kp, from the literature for a wide selection of nonelectrolytes, from aqueous solution through human epidermis, as a basis, Potts and Guy have identified, through linear regression analysis, the two basic physicochemical parameters that determine transport through the skin: molecular size, expressed as the molecular weight MW, and the octanol-water partition coefficient log P_{oct}, a measure of lipophilicity. The derived predictive equation

$$\log Kp\ (cm/hr) = -2.72 + 0.71 \log P_{oct} - 0.0061\ MW \qquad (1)$$

was shown to be applicable to chemicals of diverse structures and functionality in

general, ranging in molecular weight from 18 to 750, and in lipophilicity with a log P_{oct} between -3 to $+6$.

To test the validity of the Potts-Guy algorithm when it is applied to fragrance chemicals, the values calculated according to Eq. (1) for 20 structures commonly encountered in fragrance compositions have been correlated with experimental Kps from the literature, determined through human skin *in vitro* from aqueous solution under steady-state conditions.

The multiple regression analysis by Potts and Guy, correlating the predicted with experimental log Kps, was also performed on the 20 fragrance chemicals, for direct comparison with the fit obtained for Flynn's much wider assortment of 93 chemicals (1).

SAMPLE SELECTION

The 20 compounds chosen for analysis of the predictive power of the Potts-Guy algorithm were selected based on the criteria that

- The measured log P_{oct} value does not exceed 4
- An experimental permeability coefficient determined with human skin under steady-state conditions from aqueous solution is available from the literature
- The octanol-water partition coefficient has been determined experimentally
- They are listed in the standard reference on perfumery chemicals by S. Arctander (5).

The list of chemicals selected for the validation of calculated versus measured log P values, on the other hand, was expanded to include 33 entries, as an experimental Kp is no longer a requirement there. All chemicals are listed in Table I.

STATISTICS

Statistical analysis was conducted using the Minitab™ statistical package (Minitab Release 6.1.1., Minitab Inc., State College, PA, 1987).

RESULTS

RELATIONSHIP BETWEEN EXPERIMENTAL AND PREDICTED Log P

Linear regression analysis (Figure 1) shows near-perfect concordance between measured and calculated partition coefficients obtained from the PCMCP database for 33 common fragrance compounds ($r^2 = 0.97$, $p < 0.001$), thereby legitimizing the use of calculated values from that database in the Potts-Guy algorithm where measured values are not available.

PREDICTIVE VALUE OF THE POTTS-GUY ALGORITHM

Validity of the Potts-Guy algorithm predictive of skin permeability described in Eq. (1) was demonstrated when applied to fragrance chemicals in particular. Using measured log P values, the correlation of estimated permeability constants Kp with experimental values for 20 compounds, through human skin from an aqueous medium for steady-state

Table I
Measured and Predicted Log P_{oct} and Kp (cm/hr) Values for Fragrance Chemicals

Chemical	MW	M log P(a) (PCMCP)	C log P(b) (PCMCP)	M Kp(c) (P-G)	log M Kp (P-G)
3,4-Xylenol	122.17	2.23	2.42	1.31e-02	−1.88
3-Cresol	108.14	1.96	1.97	1.03e-02	−1.99
4-Chloro-3-xylenol	156.62	3.39	3.48	5.39e-02	−1.27
4-Cresol	108.14	1.94	1.97	9.95e-03	−2.00
4-Ethylphenol	122.17	2.58	2.50	2.33e-02	−1.63
4-Methoxy acetophenone	150.17	1.74	1.80	3.98e-03	−2.40
4-Methoxybenzyl alcohol	138.17	1.10	1.02	1.65e-03	−2.78
4-Nitrophenol	139.11	1.91	1.85	6.13e-03	−2.21
Benzophenone	182.22	3.18	3.18	2.67e-02	−1.57
Benzyl acetate	150.17	1.96	1.96	5.70e-03	−2.24
Benzyl alcohol	108.14	1.10	1.10	2.52e-03	−2.60
Benzyl benzoate	212.25	3.97	3.88	6.37e-02	−1.20
Cinnamic acid	148.16	2.13	2.09	7.74e-03	−2.11
Cinnamic alcohol	134.18	1.95	1.41	7.01e-03	−2.15
Cinnamic aldehyde	132.15	1.90	1.90	6.65e-03	−2.18
Coumarin	146.15	1.39	1.41	2.37e-03	−2.62
Cumene	120.20	3.66	3.57	1.40e-01	−0.85
Heptanoic acid	130.19	2.50	2.41	1.82e-02	−1.74
Heptanol	116.21	2.72	2.41	3.18e-02	−1.50
Hexanoic acid	116.16	1.92	1.88	8.60e-03	−2.07
Hexanol	102.18	2.03	1.88	1.25e-02	−1.90
Indole	117.15	2.14	2.13	1.22e-02	−1.92
Isoquinoline	129.16	2.08	1.82	9.31e-03	−2.03
Methyl-4-OH benzoate	152.14	1.96	1.98	5.54e-03	−2.26
Methylsalicylate	152.14	2.34	2.44	1.03e-02	−1.99
Nitrobenzene	123.11	1.85	1.88	6.96e-03	−2.16
Octanoic acid	144.17	2.94	3.05	3.08e-02	−1.51
Octanol	130.23	3.00	2.94	4.13e-02	−1.38
Pentanoic acid	102.14	1.39	1.35	4.40e-03	−2.36
Pentanol	88.15	1.56	1.35	7.08e-03	−2.15
Phenylethyl alcohol	122.17	1.18	1.36	2.36e-02	−2.63
Resorcinol	110.11	0.80	0.81	1.50e-03	−2.82
Thymol	150.22	3.30	3.40	5.09e-02	−1.29

Chemical	C Kp(d) (P-G)	log CKp (P-G)	Lit. Kp(e)	log lit. Kp	Ref.
3,4-Xylenol	1.79e-02	−1.75	3.60e-02	−1.44	6
3-Cresol	1.04e-02	−1.98	1.50e-02	−1.82	6
4-Chloro-3-xylenol	6.24e-02	−1.20	5.30e-02	−1.28	6
4-Cresol	1.04e-02	−1.98	1.80e-02	−1.74	6
4-Ethylphenol	2.04e-02	−1.69	3.50e-02	−1.46	6
4-Methoxy acetophenone	4.38e-03	−2.36			
4-Methoxybenzyl alcohol	1.45e-03	−2.84			
4-Nitrophenol	5.56e-03	−2.26	5.60e-03	−2.25	6
Benzophenone	2.67e-02	−1.57			
Benzyl acetate	5.70e-03	−2.24			
Benzyl alcohol	2.52e-03	−2.60	6.00e-03	−2.22	7
Benzyl benzoate	5.50e-02	−1.26			
Cinnamic acid	7.25e-03	−2.14			
Cinnamic alcohol	2.90e-03	−2.54			

Table I (continued)

Chemical	C Kp(d) (P-G)	log CKp (P-G)	Lit. Kp(e)	log lit. Kp	Ref.
Cinnamic aldehyde	6.65e-03	−2.18			
Coumarin	2.45e-03	−2.61	9.10e-03	−2.04	8
Cumene	1.21e-01	−0.92			
Heptanoic acid	1.57e-02	−1.80	2.00e-02	−1.70	7
Heptanol	1.92e-02	−1.72	3.20e-02	−1.49	7
Hexanoic acid	8.06e-03	−2.09	1.40e-02	−1.85	7
Hexanol	9.81e-03	−2.01	1.30e-02	−1.89	9
Indole	1.20e-02	−1.92			
Isoquinoline	6.09e-03	−2.22	1.70e-02	−1.77	10
Methyl-4-OH benzoate	5.72e-03	−2.24	9.12e-03	−2.04	6
Methylsalicylate	1.21e-02	−1.92			
Nitrobenzene	7.31e-03	−2.14			
Octanoic acid	3.68e-02	−1.43	2.50e-02	−1.60	7
Octanol	3.74e-02	−1.43	5.20e-02	−1.28	7
Pentanoic acid	4.13e-03	−2.38	2.00e-03	−2.70	7
Pentanol	5.02e-03	−2.30	6.02e-03	−2.22	9
Phenylethyl alcohol	3.16e-03	−2.50			
Resorcinol	1.53e-03	−2.82	2.40e-04	−3.62	6
Thymol	5.99e-02	−1.22	5.30e-02	−1.28	6

Physicochemical parameters for 33 fragrance chemicals used in the correlation of log P_{oct} and Kp values, experimental Kp values, and respective sources. a) log of measured o/w partition coefficients P_{oct} from PCMCP; b) log of calculated P_{oct} from PCMCP; c) permeation constants kP after Potts/Guy based on a); d) Kp after Potts/Guy based on b); e) experimental literature values.

conditions, provides an excellent fit ($r^2 = 0.86$, $p < 0.001$) (Figure 2). When calculated log P values are used instead, the correlation is still highly satisfactory, with an $r^2 = 0.83$ ($p < 0.001$) (figure not shown).

A log/log correlation between predicted and experimental Kps for the same 20 fragrance compounds was plotted for comparison with the original Potts-Guy correlation obtained for Flynn's 93 chemicals (1) (figure not shown). This was done in order to ascertain a possible benefit achieved by bracketing the polarity boundaries more tightly ($0.5 < \log P_{oct} < +4$ versus $-3 < \log P_{oct} < +6$). The resulting r^2 values are 0.72 ($p < 0.001$) for the fragrance compounds, versus 0.67 seen by Potts and Guy for Flynn's set.

DISCUSSION

Mathematical models predictive of skin permeability mainly focus on the stratum corneum (SC) as the rate-limiting barrier against penetration, and imply that the SC lipids alone can account for the penetration data observed (1). This could certainly be confirmed by the good correlations obtained here between observed and predicted Kps in the polarity range of fragrance compounds. In earlier investigations it was shown that, at high partition coefficients, i.e., at high log P, tissue strata other than the SC assume rate control, as the viable epidermis contributes significant resistance to chemical pen-

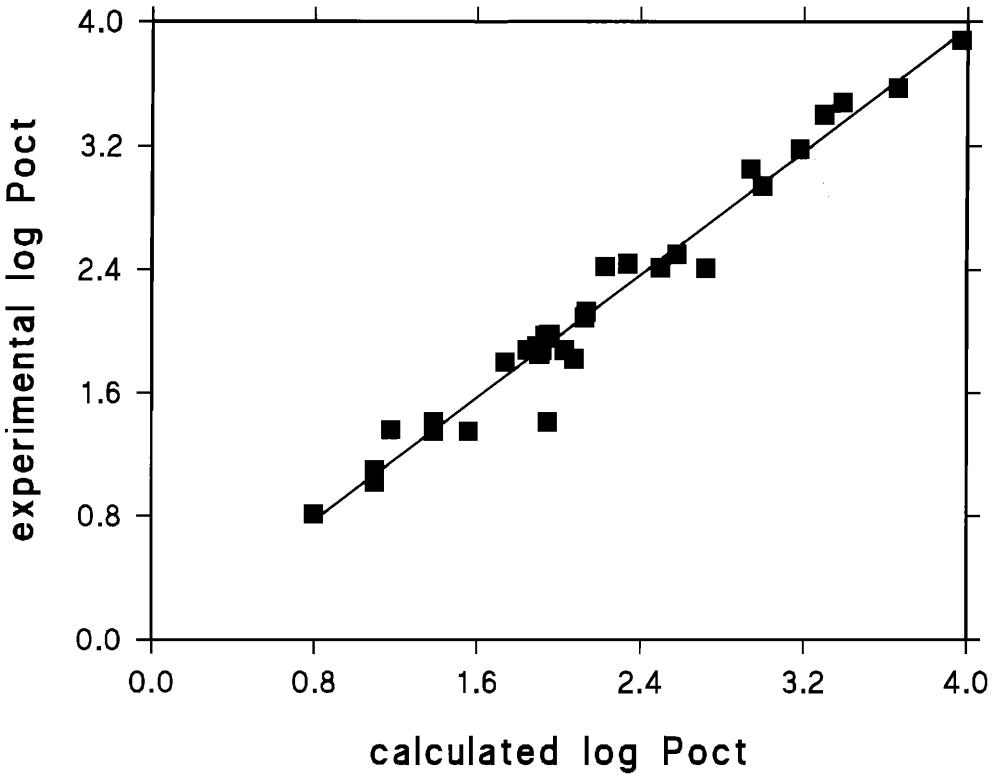

Figure 1. Log/log plot of experimental versus calculated n-octanol/water partition coefficients (P_{oct}) for 33 fragrance chemicals ($r^2 = 0.97$, $p < 0.001$).

etration of the skin barrier. When Kp values of a homologous series of n-alkanols from an aqueous medium through human skin *in vitro* were plotted against log P_{oct}, the permeability coefficient was seen to rise linearly with increasing chain length. As lipophilicity increased for homologues beyond octanol, however, i.e., at log P values of 4.26 (nonanol) and 4.57 (decanol), Kp levels off, and the correlation curve assumes a sigmoidal character (11). Linearity thus appears to end where intercellular stratum corneum lipids alone are the limiting factor for passive diffusion, a requisite on which the Potts-Guy model is also based. Towards the high end of log P values, the Potts-Guy model also nears the limit of its predictive accuracy for the diffusivity of permeants. It is for that reason in the selection of compounds for this study that those with high measured lipophilicity values (log $P > 4$) were omitted albeit available.

This apparently arbitrary cut-off based on lipophilicity is further justified by the physicochemical properties characteristic of fragrance chemicals. The polarity range into which the preponderant majority of these chemicals is seen to fit spans log P_{oct} values of -1 and $+4$, and structures that exceed that level of lipophilicity are exceptional (12).

With fragrance compounds in particular as objective for this discussion, the limited list of compounds with available measured penetration values in Table I was expanded, covering more diverse structures, as they are used in that specialty, so that C log P values could be matched with measured data. That expanded list includes some of the most

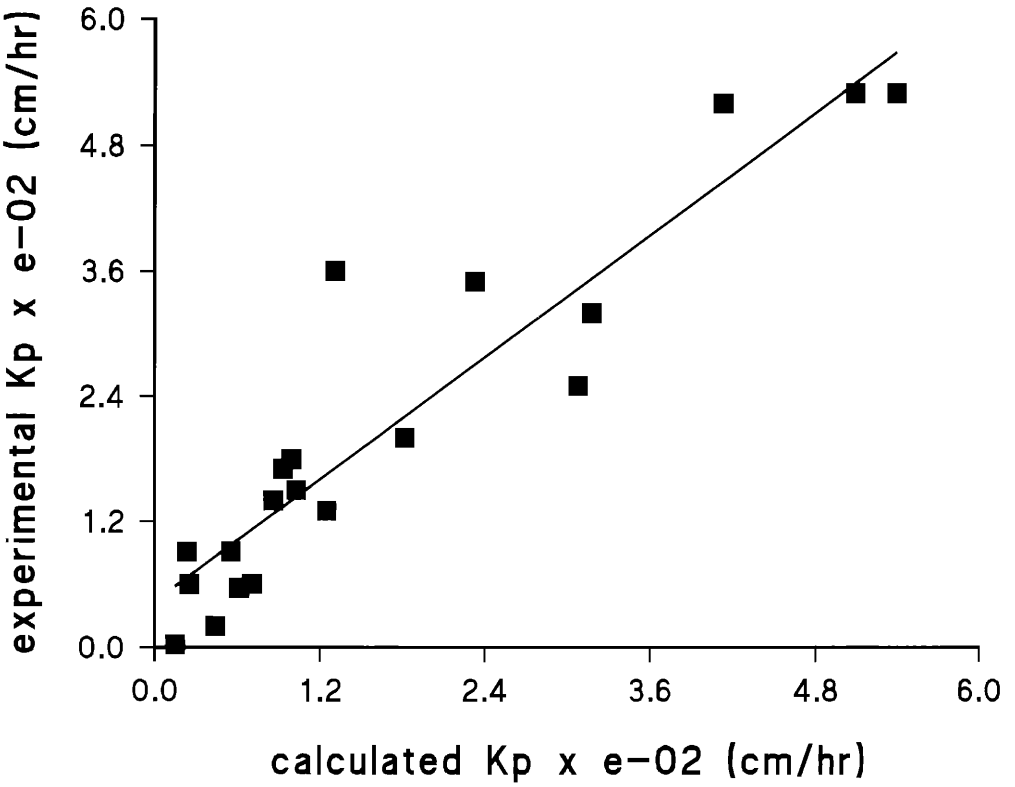

Figure 2. Correlation of experimental versus predicted permeation coefficients, Kp, for 20 fragrance compounds through human epidermis. Predicted values are based on the Potts-Guy algorithm using measured log P_{oct} data ($r^2 = 0.86$, $p < 0.001$).

important fragrance chemicals, used frequently and in large amounts, e.g., benzyl benzoate, phenylethyl alcohol, and cinnamaldehyde, making the list more representative of materials used in fragrance compositions (12).

CONCLUSIONS

It is demonstrated that the values for the steady-state permeability coefficient Kp of 20 fragrance chemicals across human skin predicted by the Potts-Guy algorithm correlate well with the corresponding experimental data ($r^2 = 0.86$, $p < 0.001$). Also the calculated octanol-water partition coefficients log P_{oct} for 33 chemical structures of intermediate polarity typical of fragrance chemicals ($0.8 < \log P < 4.0$) show remarkably good correlation with measured values ($r^2 = 0.97$, $p < 0.001$). Since log P_{oct} values demonstrated as reliable are also readily accessible, the prediction method developed by Potts and Guy, based on octanol/water partition and molecular weight for permeation coefficients of otherwise untested compounds through human skin, becomes the obvious method of choice, particularly in the range of polarity typical for fragrance compounds.

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