Available online at www.sciencedirect.com
ScienceDirect
Toxicology in Vitro 22 (2008) 1062–1069
Toxicology in Vitro
www.elsevier.com/locate/toxinvit

Inter- and intra-individual variability in human skin barrier function: A large scale retrospective study

Victor M. Meidan [a,*], Clive S. Roper [b]

[a] Division of Pharmaceutical Sciences, Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, 27 Taylor Street, Glasgow G4 0NR, Scotland, UK
[b] In Vitro Sciences, Charles River Laboratories, Tranent, Edinburgh EH33 2NE, Scotland, UK

Received 8 October 2007; accepted 11 January 2008
Available online 26 January 2008

Abstract

In vitro transepidermal tritiated water flux measurements are frequently used to evaluate skin barrier integrity for quality control purposes. However, research in this area to date has been largely based upon small-scale studies, each involving relatively few skin permeation measurements. In order to enhance our understanding in this area, we have conducted a much larger scale retrospective statistical analysis of tritiated water k_p values. These values reflected the permeability of 2400 skin samples that were derived from 112 female volunteers over a 4 year period. It was found that the population of tritiated water k_p values constituted a positively skewed, non-Normal distribution. Mean k_p was 2.04×10^{-3} cm/h while the 95th percentile was 4.50×10^{-3} cm/h. Both values are higher than those reported in previous smaller studies. Hence, our study indicates that previously suggested upper limits for tritiated water flux are too low and that they be revised upwards to a value of 4.5×10^{-3} cm/h. Analysis was also performed on smaller data subsets allowing inter-individual and intra-individual comparisons. For intra-individual k_p variability, site-related differences yielded a non-Normal, positively skewed pattern in most individuals. Inter-individual variability was Normally-distributed and showed scatter that was much smaller in magnitude.

Keywords: Skin; Transdermal drug delivery; Transport; Permeability; Toxicology; Variability; Distribution; Tritiated water flux; Inter-individual; Intra-individual

1. Introduction

Previous human skin studies have indicated that drug permeability coefficient (k_p) distributions do not generally follow a simple Normal configuration (i.e. symmetrical bell shaped curve) but rather tend to follow other more complex patterns. Evidence for this has been attained from both *in vitro* (Williams et al., 1992; Cornwell and Barry, 1995) and *in vivo* studies (Wenkers and Lippold, 1999). Furthermore, *in vitro* measurements of tritiated water flux through human split-thickness epidermal and dermatomed skin membranes suggested that these values were also distributed in a non-Normal fashion (Roper et al., 2000; Fasano et al., 2002). Such water flux measurements are frequently used to evaluate barrier integrity so that any damaged tissues can be eliminated from subsequent *in vitro* studies to assess penetration and dermal delivery of drugs and chemicals. Hence, understanding how tritiated water flux values vary with respect to different variables such as anatomic site, patient age, different individuals etc is crucial within this quality control context.

To shed more light on this issue, we have performed a large scale retrospective statistical analysis of tritiated water k_p values. These values reflected the permeability of 2400 skin samples that were excised from 112 female volunteers over a 4 year period. Those skin penetration studies were originally undertaken to assess barrier integrity for risk assessment purposes. In the current retrospective study,

* Corresponding author. Tel.: +44 (0) 141 548 4274; fax: +44 (0) 141 552 2562.
E-mail address: victor.meidan@strath.ac.uk (V.M. Meidan).

0887-2333/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tiv.2008.01.009

statistical analysis was performed on the entire k_p database as well as on smaller subsets reflecting inter-individual and intra-individual comparisons. The possibility of donor age influencing barrier function was also investigated.

2. Materials and methods

2.1. Receipt and preparation of full-thickness skin

Full-thickness human skin samples were obtained from 112 Caucasian female patients (aged 19–68 years old) that attended the Plastic Surgery Unit of St Johns Hospital (Livingstone, Scotland UK) between 2002 and 2006. All the skin samples were removed from the abdominal or breast regions during the course of routine reduction surgical procedures. Prior to the surgery, each patient gave full informed consent for their skin to be taken for scientific purposes. Moreover, the entire procedure was approved by West Lothian NHS trust. In all cases, excised skin samples were transferred on ice to the Charles River Laboratories (Edinburgh, UK). Initially, the subcutaneous fat and connective tissues were removed from each skin sample by use of a scalpel blade. The skin was washed in cold running water and dried using tissue paper. If necessary, the samples were then cut into smaller pieces. The samples were wrapped in aluminium foil, put into self sealing plastic bags and stored at −20 °C until further use or for a maximum period of 2 years. The age of the donor and site from which the skin was taken were documented.

2.2. Preparation of split-thickness skin membranes

The full-thickness skin samples were removed from frozen storage and allowed to thaw at ambient temperature. The thickness of these skins was measured with a micrometer. Split-thickness membranes were prepared by pinning each section of full-thickness skin, stratum corneum uppermost, onto a raised cork board. An electric dermatome (Zimmer®, Swindon, UK) was used to section each skin to a depth equivalent of 200–400 μm. These membranes were then laid out onto aluminium foil and the thickness was measured using a micrometer to confirm the actual thickness of the membranes cut. The split-thickness skin membranes were then stored in a frozen state at *ca* −20 °C for a duration not exceeding 1 month. This procedure of using split-thickness human skin samples and refreezing them followed current guidelines for regulatory testing (OECD, 2004b).

Prior to their use in the permeation studies, the skin membranes were removed from the freezer and allowed to thaw to room temperature. The membranes were then cut into sections of approximate dimensions 1.5 cm × 1.5 cm. The amount of skin obtained from each patient varied tremendously. Indeed, the number of sections (1.5 cm × 1.5 cm) derived per donor ranged from 1 to 74. Out of the 112 patients, a total of 2400 sections were harvested.

2.3. Diffusion cell equipment

The Scott-Dick diffusion cells and automated flow-through system were manufactured at the University of Newcastle (Newcastle, UK). Each diffusion cell had an exposure area of 0.64 cm^2 and a receptor compartment volume of 0.25 ml. The diffusion cells were incorporated into a steel manifold that was heated via a circulating water bath so that skin surface temperatures were maintained at 32 ± 1 °C. The cells were connected to multi-channel peristaltic pumps from their afferent ports with the receptor fluid effluent dropping *via* fine bore tubing into scintillation vials on a fraction collector. The peristaltic pumps were adjusted to maintain a flow-rate of *ca* 1.5 ml/h.

2.4. Tritiated water permeability assessment

Each split-thickness skin section (*ca* 1.5 cm × 1.5 cm) was positioned onto the receptor chamber containing a small magnetic stirring bar. The donor chamber was then placed on to the skin and sealed with screws. The diffusion cells were placed in the heated manifold and connected to the peristaltic pump. The receptor fluid consisted of either physiological saline, tissue culture medium containing bovine serum albumin or polyoxyethylene glycol ether. The precise composition was chosen subject to the lipophilicity of the test item. Therefore, the only variable not controlled in this study was the choice of receptor fluid. Yet OECD Guidance Document No. 28 (OECD, 2004b) approves the use of all these receptor fluids as they fulfill the stated criterion that "barrier integrity of skin must not be damaged". Hence, we can be confident that the receptor fluid variable did not affect tritiated water flux. Solvent based receptor fluids, such as ethanol:water (1:1, v/v) were not used for the tritiated water barrier test. However, in a limited number of studies, physiological saline was used as the receptor fluid for the tritiated water test and then the receptor fluid was changed to ethanol:water (1:1, v/v) for the test item absorption test.

In all cases, the underside of the skin was continuously stirred using a Telesystem HP15 device (Variomag®, Daytona Beach, FL). An equilibration period of *ca* 15 min was allowed while receptor fluid was pumped through the receptor chambers. The effluent was then collected for *ca* 30 min and retained as blank samples for use in the tritiated water permeability assessment. Subsequently, a single 250 μl aliquot of tritiated water (*ca* 0.045 μCi) was applied to the skin surface.

Research in our labs over the years has shown that steady state water flux is achieved extremely rapidly. Steady state water flux values derived from a 2 h experiment are identical to that derived from a 6 h experiment (Simpson et al., 1998). Hence, fractions of receptor fluid were collected hourly for 2 h. The samples were analysed by addition of 10 ml of Aquasafe 500 or Quickzint scintillation fluid (Zinsser Analytic, Maidenhead, UK) to each scintillation vial. All samples were counted for 1 min

together with representative blanks using a Packard 2100-TR liquid scintillation analyser (Perkin–Elmer, Beaconsfield, UK) with automatic quench correction by external standard. Representative blank sample values were subtracted from sample count rates to give net dpm per sample. Prior to analysis, samples were allowed to stabilise with regard to light and temperature.

2.5. Determination of k_p values

Microsoft Excel software on an IBM-compatible computer was employed in order to convert the measured activity levels into tritiated water amounts. Linear regression of the gradient for each sample was calculated. The tritiated water permeability coefficient was then calculated from the rate of absorption and the applied dose. Since the aim of this study was to investigate barrier function variability, we did not seek to exclude data outliers. Nevertheless, out of a total of 2400 tested skin sections, 10 sections were found to exhibit a tritiated water k_p value $>20 \times 10^{-3}$ cm/h. This was larger than the mean value by approximately an order of magnitude and probably represented samples that incurred gross damage at some point before testing (e.g. on the patient, during surgery, during transport, preparation, storage or processing into the cells). These 10 extreme outliers were removed from all subsequent data analysis.

2.6. Statistical analysis

The statistical tests were performed using IBM-compatible software, specifically a Prism version 2 spreadsheet (GraphPad Software, San Diego, CA). A suitable version (Dallal and Wilkinson, 1986) of the well-established Kolmogirov-Smirnov (KS) test (Lilliefors, 1967) for normality was used to analyse the distribution of selected k_p databases. This test has been previously used to analyse the variability of drug transport across both skin and synthetic membranes (Khan et al., 2005; Frum et al., 2007a,b).

For analysis of the entire database, all 2390 k_p values were pooled together and subjected to the KS test.

For the analysis of inter-individual variability, it was first necessary to calculate a mean (i.e. anatomic site-averaged) k_p value for each of the 112 donor individuals. In order to determine if age influenced skin barrier function, these mean k_p values were initially plotted as a function of the individual's age and linear regression analysis was undertaken. Subsequently, these 112 mean k_p values were combined and subjected to the KS test.

For measurements of intra-individual variability, our analysis was limited to those skin samples were a large quantity of skin had been excised leading to at least 40 skin sections (1.5 cm × 1.5 cm). This is because it is only permissible to apply the KS test when the number of samples (n) is at least 12. Yet the test remains weak as long as n is relatively small. Hence, it was decided to select 40 as a minimal sample size and this meant that intra-individual skin barrier function could be assessed in 15 donors.

3. Results

3.1. Variability of the entire k_p database

All 2390 k_p values were pooled together and plotted as a frequency histogram. This is presented in Fig. 1 where the frequency is simply the number of skin samples exhibiting tritiated water k_p values in the class range indicated on the x-axis. This gave a subjective visual representation of the data distribution but of course it was necessary to determine the quantitative parameters. The mean tritiated water k_p was 2.041×10^{-3} cm/h with a standard deviation of $\pm 1.614 \times 10^{-3}$ cm/h. This yielded a coefficient of variation (CV) of 79.1%. Our mean value was somewhat higher than those reported by others. For example, mean k_p values of 1.55×10^{-3} cm/h (Bronaugh et al., 1986), 1.5×10^{-3} cm/h (Davies et al., 2004) and 1.6×10^{-3} cm/h (Buist et al., 2005) have been documented for *in vitro* water flux through

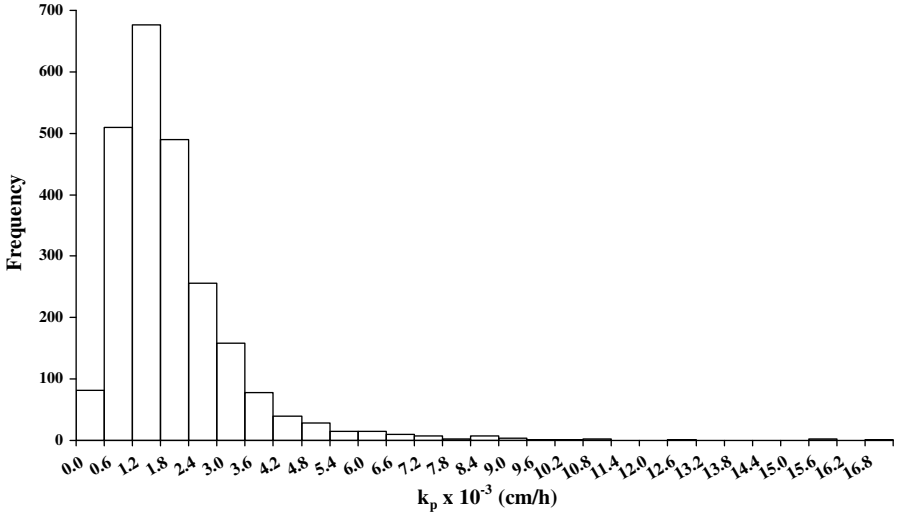

Fig. 1. Frequency histogram of all k_p values.

human epidermis. The discrepancy is explainable by the fact that the previous studies involved a relatively small number of skin samples from which the researchers excluded a relatively large fraction of high permeability outliers thought to represent compromised membranes. For example, Bronaugh et al. (1986) assigned a k_p value of 2.5×10^{-3} cm/h as a cut-off between damaged and undamaged membrane. In that study, out of a total of 49 epidermal membranes, 16 were omitted from the mean k_p calculation as their permeability exceeded the cut-off value. Yet Fig. 1 shows that a value of 2.5×10^{-3} cm/h is well within the distribution of our 2390 data points. The other two studies (Davies et al., 2004; Buist et al., 2005) also excluded high permeability measurements although they did not report numerical details. Thus, over-exclusion of high permeability k_p values probably explains the slightly (*ca* 24%) lower mean k_p values obtained by others.

In our present study, having determined the mean and standard deviation of the tritiated water k_p values, a KS test was performed in order to determine whether this data were Normally-distributed. If the scatter of k_p values followed a perfect Normal distribution, the KS distance would be zero. Larger values of the KS distance correspond to larger deviations from an ideal Normal distribution. By taking into account the KS distance and the sample size, it was possible to calculate a specific P value. This value was designed to answer the question: If the parent population ($n = \infty$) was really Normally-distributed, what was the chance that our smaller sample ($n = 2390$) would have a KS distance as large as or larger than observed? Calculations showed that the KS distance was 0.168 and that $P < 0.0001$, strongly indicated that the values were not Normally-distributed. This was visually apparent from Fig. 1, where it was clear that there were many highly permeable skin samples producing a "tail" on the right hand side of the distribution. Importantly, a similarly skewed distribution of tritiated water k_p values was also reported by Fasano et al. (2002). In their study using human epidermal membranes, a similar retrospective assessment was made, albeit on a much smaller scale than the present study.

3.2. Inter-individual variability in epidermal barrier function

Initially, each donor's site-averaged k_p value was plotted as a function of that donor's age. Crucially, it was determined that there was no correlation ($r^2 = 0.081$) between age and tritiated water flux. This result fitted in with previous studies that also showed that ageing in adults had no effect on *in vitro* skin barrier function (Fenske and Lober, 1986; Roskos et al., 1989; Oriba et al., 1996; Lawrence, 1997; Williams, 2003). However, a comprehensive consensus on the effects of adult ageing on skin permeability has still not been agreed upon (Waller and Maibach, 2005).

Having eliminated age as a confounding variable, all 112 body site-averaged donor k_p values were combined and plotted as a frequency histogram (see Fig. 2). It was calculated that among all 112 donors, mean tritiated water k_p was 2.21×10^{-3} cm/h with a standard deviation of $\pm 0.83 \times 10^{-3}$ cm/h. Hence, the CV was 37.6%. Statistical analysis indicated that the KS distance was 0.107 and that $P > 0.100$ (when the calculated P value was greater than 0.1, the software did not give a precise value, but just stated that this was the case). This means that inter-individual variability in skin barrier function was Normally-distributed.

3.3. Intra-individual variability in skin barrier function

As explained previously, assessments of intra-individual variability in skin permeability were limited to those 15 individual patients that had donated a sufficient quantity of skin. The results of the statistical analysis performed on each of these 15 donors (denoted A–O) are presented in Table 1. Intra-individual variability, as quantified by

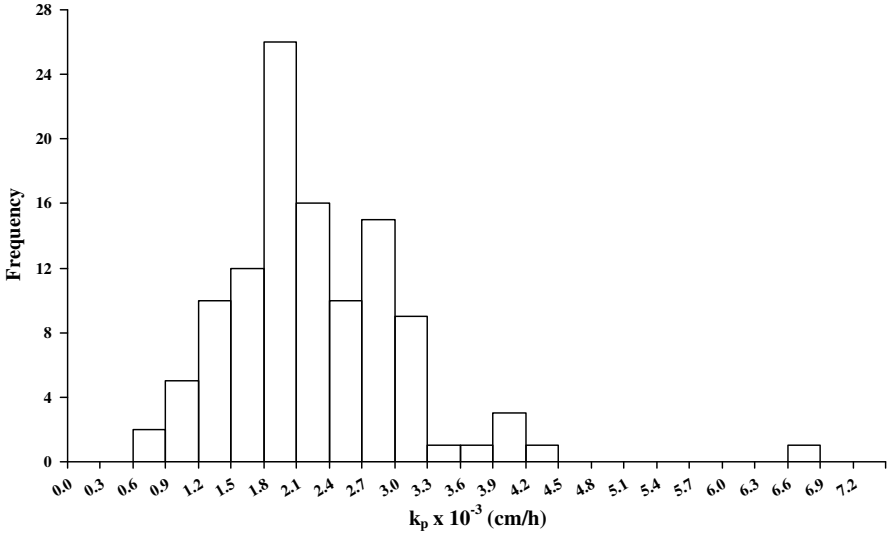

Fig. 2. Frequency histogram of site-averaged k_p values amongst 112 donors.

Table 1
Table of statistical parameters describing the tritiated water k_p data for 15 donors

Donor (age)	No. of skin samples	Mean $k_p \pm$ SD ($\times 10^{-3}$ cm/h)	% CV	KS distance	P	Passed Normality test
A (66y)	50	0.99 ± 0.67	67.6	0.196	0.043	No
B (22y)	47	1.78 ± 0.68	38.3	0.163	>0.100	Yes
C (68y)	42	1.54 ± 1.79	115.7	0.261	0.006	No
D (39y)	58	1.27 ± 1.04	81.6	0.310	~0.000	No
E (45y)	42	2.36 ± 2.08	88.0	0.245	0.013	No
F (40y)	74	0.94 ± 0.70	75.3	0.206	0.004	No
G (38y)	41	2.33 ± 0.91	39.2	0.171	>0.100	Yes
H (32y)	53	1.90 ± 1.19	62.7	0.193	0.039	No
I (39y)	55	1.34 ± 1.07	79.8	0.260	0.001	No
J (41y)	74	2.00 ± 1.16	58.2	0.161	0.043	No
K (58y)	57	1.28 ± 0.76	59.7	0.190	0.033	No
L (19y)	52	2.39 ± 1.60	66.8	0.244	0.004	No
M (33y)	56	1.44 ± 0.62	43.3	0.167	0.087	Yes
N (51y)	58	2.45 ± 1.81	73.9	0.216	0.009	No
O (57y)	45	2.59 ± 1.23	47.6	0.176	>0.100	Yes

CV, ranged between 38.3% and 115.7%. Notably, intra-individual variability did not correlate with donor age ($r^2 = 0.20$) or with the number of skin samples derived from the donor ($r^2 = 0.001$). KS tests showed that in 4 donors, skin barrier variability could be fitted to a Normal distribution. In contrast, in 11 donors, epidermal barrier variability could not be fitted to a Normal distribution. This was generally due to excessive positive skewing i.e. an excess of highly permeable skin samples causing "tailing" on the right hand side of the frequency distribution. Fig. 3 shows the k_p histogram for a single donor (I), whose skin permeability data was reasonably typical of the larger group of 11 donors.

4. Discussion

4.1. Implications for skin barrier assessments

The use of human skin for percutaneous penetration studies invariably involves storage, handling and technical preparation of the tissues prior to use. Hence, barrier assessment tests such as tritiated water flux measurements constitute a key quality control tool, applicable for both regulatory testing or for academic purposes. The tests are designed to identify any damaged tissue specimens. Any such atypical samples can then be rejected prior to application of the test item to the skin (OECD, 2004a). The findings presented in this paper have the greatest relevance within this context. Barrier integrity testing generally involves pooling together the measurements of all the samples regardless of their anatomic site and donor origins. A cut-off point is then assigned and samples exhibiting a test permeability exceeding this limit are then rejected.

In the current study, human skin tritiated water k_p data were shown to be non-Normally distributed and skewed towards the right hand side. Given the very large number of samples involved (2390), the appreciable subset of membranes in the "shoulder" and "tail" were not necessarily damaged per se but were probably genuinely part of the distribution of intact human skin membranes. There are

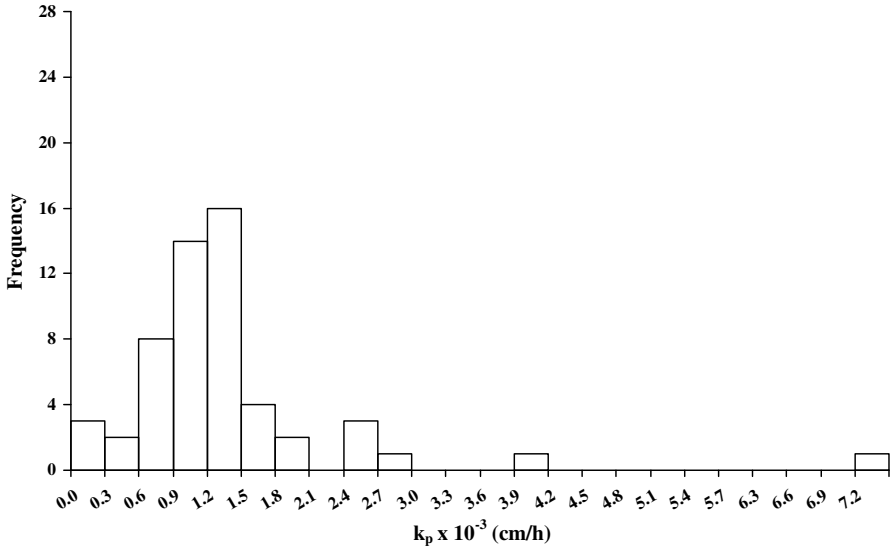

Fig. 3. Frequency histogram of site-dependent k_p values in a single, relatively typical donor (I).

a couple ways of treating such a distribution in order to derive a suitable cut-off value for quality control purposes. One option is to designate a limit given by the "mean ± 3 times the standard error of the mean" rule (Lawrence, 1997). However, this method is statistically disallowed in the case of non-Normal distributions. Another approach is to simply designate the 95th percentile of the data as a limit (Fasano et al., 2002). For our current study this yielded a tritiated water k_p cut-off value of exactly 4.50×10^{-3} cm/h. From Fig. 1, it can be seen that this limit seems intuitively reasonable as it excludes k_p values that are located far into the "tail", but not those merely within the right hand "shoulder" of the distribution. The value of 4.50×10^{-3} cm/h is appreciably higher than the previously proposed limits of 1.5×10^{-3} cm/h (Scott et al., 1987), 2.0×10^{-3} cm/h (Van de Sandt et al., 2000) and 2.5×10^{-3} cm/h (Bronaugh et al., 1986). However, it is more similar to that suggested most recently of 4.0×10^{-3} cm/h (Buist et al., 2005). Again, this is explainable by the fact that previous studies involved much fewer samples from which a substantial fraction were excluded as highly permeable outliers. For risk assessment purposes, it is important to remove samples that are damaged, but also accept intact samples with high permeability since these samples relate to the population that are most at risk to absorption. Hence, the results of our large scale study indicated that the previously suggested upper limits for tritiated water flux are probably too low and that they be revised upwards to a figure nearer the 4.50×10^{-3} cm/h value.

From Fig. 1, it was observed that there were some skin samples exhibiting k_p values within the $0–0.6 \times 10^{-3}$ cm/h range. However, the majority of these were located towards the higher end of this range. There were only 7 skin samples that exhibited k_p values of 0.2×10^{-3} cm/h or below, representing less than 0.3% of the entire data set. There is negligible published data on such highly impermeable skin samples, probably because of their relative scarcity and reduced significance for risk assessment purposes. Skin with a very low water k_p would be considered to be from donors who were at a lower risk to any adverse effects of the test chemical as they have a better skin barrier function allowing less of the test chemical through the skin. Additionally, unlike the right hand side of the distribution, there was no significant 'shouldering' and 'tailing' effect on the left hand side. Thus, there was no equivalent need to consider imposing cut-off thresholds.

Since the entire k_p database is not Normally-distributed, this leads to another important statistical consequence. Specifically, use of parametric tests such as the *t*-test to evaluate significance assumes a Normally-distributed population. As this was not the case for tritiated water k_p values, then ideally, nonparametric tests such as the Wilcoxan's signed rank test or the Mann-Whitney *U*-test must be used.

This study was confined to skins derived from only female donors. Previous reports have indicated that skin gender does not significantly influence both mean and standard deviation values of tritiated water flux (Bronaugh et al., 1986; Lawrence, 1997). In general, the relevant literature seems to mostly ignore gender when assigning upper tritiated water flux limits to skin samples. Despite this, it is just conceivable that the data distributions may differ somewhat between female and male skin. Hence, a study similar to the current one but performed on male skin would be necessary in order to eliminate this potential caveat.

4.2. Inter-individual versus intra-individual variability

A further aim of this paper was to separate out inter-subject variability from intra-subject variability in skin barrier function. It was found that inter-individual differences in skin barrier function (CV = 37.6%) were smaller than intra-individual ie. regional site-dependent differences ($38.3\% \leqslant CV \leqslant 115.7\%$). This seemed to contradict most of the percutaneous absorption relationships documented in the literature. For example, it was reported (Southwell et al., 1984) that for various chemicals permeating human epidermal membranes *in vitro*, inter-individual CV values averaged *ca* 70% whilst intra-individual CV values averaged *ca* 40%. More recently, fentanyl permeation across full-thickness human skin *in vitro* was studied (Larsen et al., 2003). Inter-individual differences were described as 'extensive' but intra-individual differences were 'limited'. Brown's group (Akomeah et al., 2007) examined the *in vitro* flux of caffeine, methyl paraben and butyl paraben through human epidermal samples. It was determined that inter-subject variability was greater than intra-subject variability. Similarly, *in vivo*, inter-individual differences in the percutaneous absorption of aromatic hydrocarbons were much greater than intra-individual differences (VanRooij et al., 1993). Another team (Pinnagoda et al. 1989) conducted TEWL measurements on the forearms of 30 individuals at 8 different sites. Analysis of variance indicated that inter-individual effects contributed to 84.5% of all variability while anatomic site-related effects contributed to only 15.5% of all variability.

There are several potential reasons explaining the discrepancy between the results of the present study and those of other studies. Water is a small molecule that interacts with and accumulates within the stratum corneum, extensively modifying its properties. Thus, it is quite different to other larger molecules, such as caffeine, fentanyl, methyl paraben, aromatic hydrocarbons etc. Indeed, it has already been proposed that skin barrier variability is chemical-specific (Monteiro-Riviere, 1996). With respect to TEWL, there is evidence that the extent of *in vitro* skin barrier integrity differs significantly depending upon whether tritiated water flux or TEWL are used as probes (Heylings et al., 2001; Chilcott et al., 2002). Furthermore, the present study involved a large number of subjects (112) and skin samples (2400) from which only 10 extreme sample outliers were removed. This contrasts with the generally much smaller sample sizes analysed by others where excluded

outliers may have possibly represented a much larger fraction of the data. This constitutes another potential confounding variable.

The inter-individual variability in tritiated water flux could be easily fitted to a Normal distribution. To our knowledge, this is the first time that this has been reported. In contrast, intra-individual variability could not be fitted to a Normal distribution in most cases (11 out of 15 donors) due to an excess of skin samples exhibiting a much higher than average permeability. Again, all this has statistical implications for the analysis of tritiated water data. If the skin samples are harvested from different anatomical regions of a single donor, *t*-tests may be invalid. It would first be necessary to determine whether the flux values could be fitted to a Normal distribution. If not, nonparametric tests would be required to analyse the data. Yet *t*-tests would be valid for comparing site-averaged water flux data between donors.

5. Conclusions

Our analysis of 2390 skin samples showed that tritiated water k_p values were non-Normally distributed and exhibited substantial positive skewing. The mean k_p was 2.04×10^{-3} cm/h and the data's 95th percentile was at 4.50×10^{-3} cm/h. Both these values are appreciably higher than figures derived from previous similar, but much smaller, studies. Therefore, it is recommended that those using such measurements as a quality control tool use a more conservative cut-off value of 4.5×10^{-3} cm/h. As far as intra-individual k_p variability, regional site-related differences yielded a skewed, non-Normal pattern in most individuals. In contrast, inter-individual variability was Normally-distributed and showed scatter that was much smaller in magnitude.

Acknowledgements

The authors wish to thank Leanne Stupart, Frank Toner and their teams (Charles River Laboratories, Edinburgh, UK) for their efforts in helping to generate the 2400 skin penetration measurements.

References

Akomeah, F.K., Martin, G.P., Brown, M.B., 2007. Variability in human skin permeability in vitro: Comparing penetrants with different physicochemical properties. Journal of Pharmaceutical Sciences. 96, 824–833.

Bronaugh, R.L., Stewart, R.F., Simon, M., 1986. Methods for in vitro percutaneous absorption studies VII. Use of excised human skin. Journal of Pharmaceutical Sciences 75, 1094–1097.

Buist, H.E., van de Sandt, J.J., van Burgsteden, J.A., de Heer, C., 2005. Effects of single and repeated exposure to biocidal active substances on the barrier function of the skin in vitro. Regulatory Pharmacology and Toxicology 43, 76–84.

Chilcott, R., Dalton, C., Emmanuel, A., Allen, C., Bradley, S., 2002. Transepidermal water loss does not correlate with skin barrier function in vitro. Journal of Investigative Dermatology 118, 871–875.

Cornwell, P.A., Barry, B.W., 1995. Effects of penetration enhancer treatment on the statistical distribution of human skin permeabilities. International Journal of Pharmaceutics 117, 101–112.

Dallal, G.E., Wilkinson, L., 1986. An analytic approximation to the distribution of Lilliefors' test for normality. American Statistics 40, 294–296.

Davies, D.J., Ward, R.J., Heylings, J.R., 2004. Multi-species assessment of electrical resistance as a skin integrity marker for in vitro percutaneous absorption studies. Toxicology In Vitro 18, 351–358.

Fasano, W.J., Manning, L.A., Green, J.W., 2002. Rapid integrity assessment of rat and human epidermal membranes for in vitro dermal regulatory testing: correlation of electrical resistance with tritiated water permeability. Toxicology In Vitro 16, 731–740.

Fenske, N.A., Lober, C.W., 1986. Structural and functional changes of normal ageing skin. Journal of the American Academy of Dermatology 15, 571–585.

Frum, Y., Eccleston, G.M., Meidan, V.M., 2007a. Evidence that drug flux across synthetic membranes is described by normally distributed permeability coefficients. European Journal of Pharmaceutics and Biopharmaceutics 67, 434–439.

Frum, Y., Khan, G.M., Sefcik, J., Rouse, J., Eccleston, G.M., Meidan, V.M., 2007b. Towards a correlation between drug properties and in vitro transdermal flux variability. International Journal of Pharmaceutics 336, 140–147.

Heylings, J.R., Clowes, H.M., Hughes, L., 2001. Comparison of tissue sources for the skin integrity function test (SIFT). Toxicology In Vitro 15, 597–600.

Khan, G.M., Frum, Y., Sarheed, O., Eccleston, G.M., Meidan, V.M., 2005. Assessment of drug permeability distributions in two different model skins. International Journal of Pharmaceutics 303, 81–87.

Larsen, R.H., Nielsen, F., Sorensen, J.A., Nielsen, J.B., 2003. Dermal penetration of fentanyl: inter and intra individual variations. Journal of Pharmacology and Toxicology 93, 244–248.

Lawrence, J.N., 1997. Electrical resistance and tritiated water permeability as indicators of barrier integrity of in vitro human skin. Toxicology In Vitro 11, 241–249.

Lilliefors, H.W., 1967. On the Kolmogorov-Smirnov tests for normality with mean and variance unknown. Journal of the American Statistical Association 62, 399–402.

Monteiro-Riviere, N.A., 1996. Anatomical factors affecting barrier function. In: Marzulli, F.N., Maibach, H.I. (Eds.), Dermatotoxicity, Fifth ed. Taylor & Francis, London, pp. 3–13.

OECD (2004a) Guideline for Testing of Chemicals, Guideline 428: Skin Absorption: In Vitro Method.

OECD (2004b). OECD Environmental Health and Safety Publications Series on Testing and Assessment No. 28. Guidance Document for the Conduct of Skin Absorption Studies.

Oriba, H.A., Bucks, D.A.W., Maibach, H.I., 1996. Percutaneous absorption of hydrocortisone and testosterone on the vulva and forearm: effect of the menopause and site. British Journal of Dermatology 134, 1365–2133.

Pinnagoda, J., Tupker, R.A., Smit, J.A., Coenraads, P.J., Nater, J.P., 1989. The intra-and inter-individual variability and reliability of transepidermal water loss measurements. Contact Dermatitis 21, 255–259.

Roper, C.S., Simpson, A.G., Madden, S., Cameron, B.D., 2000. Tritiated water permeability coefficient assessment and rejection criteria for barrier function of human skin. In: Brain, K.R., Walters, K.A. (Eds.), Perspectives in Percutaneous Penetration, vol. 7A. STS Publishing, Cardiff, p. 61.

Roskos, K.V., Maibach, H.I., Guy, R.H., 1989. The effect of ageing on percutaneous absorption in man. Journal of Pharmacokinetics and Biopharmaceutics 17, 617–630.

Scott, R.C., Dugard, P.H., Ramsey, J.D., Rhodes, C., 1987. In vitro absorption of some o-phalate diesters through human and rat skin. Environmental Health Perspectives 74, 223–227.

Simpson, A.G., Roper, C.S., Cameron, B.D., 1998. Comparison of tritiated water penetration coefficients as predictors for human skin

barrier integrity. In: Brain, K.R., Walters, K.A. (Eds.), Perspectives in Percutaneous Penetration, vol. 6A. STS Publishing, Cardiff, p. 61.

Southwell, D., Barry, B.W., Woodford, R., 1984. Variations in permeability of human skin within and between specimens. International Journal of Pharmaceutics 18, 299–309.

Van de Sandt, J.J., Meuling, W.J., Elliott, G.R., Cnubben, N.H., Hakkert, B.C., 2000. Comparative in vitro-in vivo percutaneous absorption of the pesticide propoxur. Toxicological Sciences 58, 15–22.

VanRooij, J.G., De Roos, J.H., Bodelier-Bade, M.M., Jongeneelen, F.J., 1993. Absorption of polycyclic aromatic hydrocarbons through human skin: differences between anatomical sites and individuals. Journal of Toxicology and Environmental Health 38, 355–368.

Waller, J.M., Maibach, H.I., 2005. Age and skin structure and function, a quantitative approach (I): blood flow, pH, thickness, and ultrasound echogenicity. Skin Research and Technology 11, 221–235.

Wenkers, B.P., Lippold, B.C., 1999. The statistical distribution of in-vivo skin permeability. Pharmacy and Pharmacology Communications 5, 425–427.

Williams, A.C., 2003. Transdermal and Topical Drug Delivery: From theory to clinical practice. Pharmaceutical Press, London.

Williams, A.C., Cornwell, P.A., Barry, B.W., 1992. On the non-Gaussian distribution of human skin permeabilities. International Journal of Pharmaceutics 86, 69–77.