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Animal models for percutaneous absorption

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ABSTRACT: Animal models are important tools to predict human *in vivo* percutaneous absorption/penetration. Monkey, pig, rat, rabbit, guinea pig, hairless rodents, such as hairless rat, hairless mouse, hairless guinea pig and hairless dog, are among the most used animals for this purpose. Each animal model has its own advantages and weakness or limitation. To better correlate animal data with human skin absorption, we need to be familiar with each animal model's characteristics as well as experimental method and condition. We reviewed the original papers published after 1993 that described permeability of both animal skin and human skin. It showed that monkey, pig and hairless guinea pig are more predictive of human skin absorption/penetration and common laboratory animals, such as rat, rabbit, guinea pig, generally overestimate human skin absorption/penetration. Copyright © 2014 John Wiley & Sons, Ltd.

Keywords: percutaneous absorption/penetration; human; monkey, pig; rat; rabbit; guinea pig; hairless rat; hairless guinea pig

Introduction

A most relevant way to determine the percutaneous penetration rate or absorption rate of chemicals in human is *in vivo* human studies. However, it has become increasingly complex to perform *in vivo* human studies because of regulations such as U.S. EPA's human research rule (U.S. EPA, 2006). An alternative way is *in vitro* human skin absorption study which is not banned by the current human research guidelines. However, it does not have an intact physiologic and metabolic system present in *in vivo* models, and is associated with limited tissue durability, and subject to practical issues of obtaining human tissue. Therefore, animals remain practical models because they are easier to obtain, less subject to regulation, have less intersubject variability owing to inbred animals, and there is a large body of valuable data not only on percutaneous absorption/penetration but also on related toxicokinetic and toxicodynamic parameters (Jakasa and Kezic, 2008). However, animal skin is generally more permeable than human skin. Therefore, the animal model should be phylogenetically as close as possible to humans. Animal model's physiology, biochemistry and anatomy should be similar to humans to develop most predictive data of the human skin penetration or absorption (Simon and Maibach, 2000). Two basic criteria help judge whether an animal is relevant; the animal model should give the same percutaneous absorption as that in a human; if it is not possible, then percutaneous absorption in the animal model should be constantly different from that in a human.

Bartek *et al*. (1972) challenged the world of comparative cutaneous biology to begin to understand relative percutaneous penetration in several species. Subsequently, extensive observations have extended this Bartek's investigation – and here we attempt to evaluate the subsequent three decades – in hopes of aiding dermatopharmacology and dermatotoxicology studies. We describe monkey, pig, rat, rabbit, guinea pig, hairless rodents such as hairless rat, hairless mouse, hairless guinea pig and hairless dog, and then some alternative models such as a human skin grafted onto a nude mouse model (HuSki model).

Monkey: Rhesus Monkey/Squirrel Monkey

It is a most relevant animal model for percutaneous absorption because it is phylogenetically most close to humans, therefore, its skin is also similar to human skin and areas like the inner arm, legs and trunk are relatively hairless like human. Its regional variation in percutaneous absorption resembles human, therefore, the same anatomical site can be used in comparative study. It is also large enough for serial blood sampling. However, the use of monkey in experiments is somewhat limited by cost and restricted availability.

We found three studies for four chemicals, which described the permeability of both monkey skin and human skin and were published after 1993. 2,4-dichlorophenoxyacetic acid penetrated similarly to human skin (Wester *et al*., 1996). Acitretin was found to be 0.3 times permeable of human skin (Surber *et al*., 1993). Water and 7-hydroxycoumarin were 2.3 and 3.8 times more permeable than human skin even if the thickness of full-thickness and stratum corneum (SC) as well as hair density of monkey skin were similar to those of humans (Panchagnula *et al*., 1997). Thus, percutaneous absorption across monkey skin often, but not always resembles human skin.

Pig

Another appropriate animal model for human skin absorption is pig both *in vivo* and *in vitro* (Jakasa and Kezic, 2008). It has several advantages over other animal models; porcine skin is easily obtainable; the pig is large enough for collection of multiple samples (body fluids, biopsies) over extended periods, while at the same time not too large to be conveniently handled in standard laboratory animal facilities. There are similarities between porcine and human skin; the skin of both man and pig is characterized by a spare hair coat, a thick epidermis that has a well-differentiated undersculpture, a dermis that has a well-differentiated papillary body and a large content of elastic tissue (Simon and Maibach, 2000). The follicular structure of pig skin also resembles

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that of humans. The average of 20 hairs per cm^2 of porcine ear skin is similar to 14–32 hairs per cm^2 in humans (Capt *et al*., 2007).

The histological appearance of the epidermis is similar in both man and pig (Simon and Maibach, 2000). Porcine and human epidermises appear similar in tissue turnover time and the characterization of keratinous proteins. Porcine SC contains protein fractions grossly similar to human. It has similar variable filament density and areas of cell overlapping with human skin SC. The epidermal-dermal junction of pig resembles that of man. The number, size, distribution and communications of the dermal blood vessels of the pig were remarkably similar to those of human skin. The architecture of collagen fibers and fiber bundles as wells as the thickness of collagen fibrils in the dermis of the pig is generally similar to those of human skin. In immunohistochemical study with 93 monoclonal or polyclonal antibodies, many antibodies showed similar immunoreactivity on porcine skin with human skin (Debeer *et al*., 2013). Biochemical similarities were found while studying glycosphingolipids and ceramides in human and pig epidermis. The enzyme patterns of the skin of the domestic pig revealed by enzyme histochemical investigations mirror that in man (Simon and Maibach, 2000). Thickness of skin layers in the porcine skin also resembles that of human skin (Tables 1 and 2) (Zendzian, 2000; Boudry *et al*., 2008).

However, dissimilarities also exist; vascularization is rich in man, but poor in the pig and humans have mostly eccrine sweat glands, whereas the pig has mostly apocrine glands (Simon and Maibach, 2000). As there is high fat component in pig, lipid soluble compounds concentrate in the fatty area of pig rather than the central compartment (blood sampling).

Barbero and Frasch (2009) performed an extensive quantitative review on porcine skin as surrogates for human *in vitro* penetration studies and included 18 studies of 41 permeability measurements. The correlation efficient (r) between pig skin and human skin is 0.88 ($P < 0.0001$). In 20 studies of 50 measurements on 40 chemicals that did not report permeability and Factor Of Difference (FOD) calculated from permeability studies, 80% fell within the range of ±1/2 log interval; that is $0.3 < FOD < 3.0$. Average intra species coefficient of variation for pig skin is 21% and for human skin 35%. The smaller variation in pig skin than human skin means that fewer experiments would be required to attain sufficient statistic power to confirm subtle differences. In lag time data (13 measurements from 9 studies on 10 compounds), there is no significant correlation between lag time in pig skin compared with human.

We reviewed the original papers published after 1993 that described permeability of both pig skin and human skin. This included 46 studies, which measured permeability of 77 chemicals. For 38 chemicals in 26 studies, percutaneous permeability of porcine skin is close to that of human skin ($0.66 < FOD$ 1.5). For 25 chemicals in 15 studies, percutaneous permeability of pig skin is higher than that of human. In this group, 9 chemicals absorbed in porcine skin at a much higher rate than that of human skin ($FOD > 3$). For 16 chemicals in 6 studies human skin permeability is higher than that of pig. However, only 3 chemicals showed a higher difference ($FOD > 3$). In conclusion, 86% (65 chemicals of 76) fell within the range of ±1/2 log interval.

As seen above, experiments with many chemicals showed similar permeability through pig skin and human skin. But, the degree of resemblance varies with groups of compounds of different chemical characteristics.

Table 1. Thickness of skin layers of different species

Species, anatomic site	SC (μm)	Epidermis (μm)	Whole skin (mm)
Human forearm	17	36	1.5
Pig, back	26	66	3.4
Pig, ear	10	50	1.3
Mouse, back	5	13	0.8

Modified from Boudry *et al*. (2008).
SC, stratum corneum.

Table 2. Thickness of human and animal skin

Species	SC (μm)	Epidermis (μm)	Whole skin (mm)
Human	16.8	46.9	2.97
Pig	26.4	65.8	3.43
Rat	18	32	2.09
Mouse	9	29	0.70
Hairless mouse	8.9	28.6	0.70

Modified from Zendzian (2000).
SC, stratum corneum.

Rat

Rodents are readily available; they are small and easy to handle, inexpensive, and there are considerable cumulated data about them, so they are most commonly used in permeation studies as well as regulatory toxicity studies. However, rodent skin generally shows higher permeation rates compared with human skin. Among rodents, rat skin has more structural similarities to human skin (Table 2) (Godin and Touitou, 2007). Therefore, permeation kinetic parameters of rat skin are frequently comparable with human skin. However, still differences between rat skin and human skin are significant. In rat skin, the epidermis and SC are thinner, the number of appendages is higher, intercellular lipid composition of the SC is different and the corneocyte surface is lower than in human skin (Capt *et al*., 2007).

We reviewed the original papers published after 1993 that described permeability of both rat skin and human skin. This included 79 studies, which measured absorption of 110 chemicals. For 23 chemicals in 21 studies, permeability of rat skin resembled that of human skin ($0.66 < FOD < 1.5$). For 83 chemicals in 54 studies, rat skin is more permeable than human skin. Only four chemicals are less permeable through rat skin than through human skin. And the permeability difference between rat skin and human skin is much higher than in porcine skin. 28 chemicals show FOD with range of 3 to 10, 24 chemicals show FOD with range of 11 to 99, and 5 chemicals shows FOD with range of 100 to 500. In conclusion, 48% (53 chemicals of 110) fell within the range of ±1/2 log interval and rat skin is generally more permeable than human skin.

van Ravenzwaay and Leibold (2004a, 2004b) compared the *in vivo* rate of penetration of 14 pesticides with a wide range of lipophilicities and molecular weights with an *in vitro* rate of

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penetration in rat as well as an *in vitro* rate of penetration in human. *In vitro* studies, rat skin was always more permeable for all tested substances than human skin. FOD ranged from 2.3 to 36.5, the mean: 13.4 ± 11.1-fold). The *in vivo* rat skin is always less permeable than *in vitro* rat skin, but, in most cases (9/12), higher permeable than *in vitro* human skin. No constant factor of difference was identified. The factor of difference would not appear to be determined by molecular weight, lipophilicity, or aqueous solubility. Because of an inconsistent difference in permeability between rat and human skin, it is not possible to derive a general adjustment factor for estimation of human skin permeability. Thus, the systemic exposure of humans may be significantly overestimated if risk assessment is based only on the results of an *in vitro* or an *in vivo* rat study.

To overcome this problem, several research groups (U.S. EPA, 1992; Thongsinthusak *et al*., 1993; van Ravenzwaay and Leibold, 2004a, 2004b; WHO, 2005) suggested a method, so-called 'parallelogram', to estimate dermal penetration through human skin from the combined use of *in vivo* and *in vitro* rat data and *in vitro* human data, using the following equation:

$$\%\,\text{Human dermal penetration} = \frac{[\%\,\text{dermal penetration Rat } \textit{in vivo}] \times [\text{rate dermal penetration Human } \textit{in vitro}]}{[\text{rate dermal penetration Rat } \textit{in vitro}]}$$

Ross *et al*. (2011) examined the predictive worth of this method as outlined in Table 3 for five other compounds with widely varying log K_{ow} (log *P* varies from –0.1 for caffeine to 6.1 for permethrin). Agreement between estimated and measured values is remarkable. More importantly, the predicted dermal absorption estimate is ≤ 1.7-fold of the actual human *in vivo* measured value for each compound except fluazifop-butyl and *o*-phenylphenol.

The parallelogram method to estimate human dermal absorption can also be utilized with other test animal data besides rat. Shown in Table 4 are the values predicted using pig data, which also show a good agreement between estimated and measured values (Ross *et al*., 2011). While the ratio of animal to human absorption varies with the compound, this approach is only valid if the ratio of *in vivo* to *in vitro* absorption for a given compound remains the same in both human and animal species. It is also desirable if the three study types (*in vitro* human, *in vitro* rat and *in vivo* rat) were conducted concurrently under the same condition by the same laboratory.

Rabbit

Similar to rat skin, rabbit skin is generally more permeable than human skin and the difference in percutaneous absorption between rabbit and human is not consistent. Nicoli *et al*. (2008) performed an experiment to compare rabbit ear skin with pig ear skin on histology, lipid composition and permeability of

Table 3. Comparison of measured human absorptions and new predictions of human dermal absorption using the Parallelogram method

Compound	Rat $_{in\ vivo}$ / Rat $_{in\ vivo}$	Human $_{in\ vivo}$ (%)	Human $_{in\ vivo}$ P (predicted %)	Human $_{in\ vivo}$ M (measured %)	Human $_{in\ vivo}$ p / Human $_{in\ vivo}$ M
Benzoic acid	1.3	46.5	60.5	60.6	1.0
Caffeine	1.0	40.6	40.6	40.6	1.0
Fluazifop-butyl	0.9	2.2	2.0	8.0	0.25
o-Phenyl phenol	3.5	16.3	56.7	24.2	2.4
Permethrin	1.3	1.3	1.7	1.2	1.4
Piperonyl butoxide	1.2	7.4	8.9	5.3	1.7
Propoxur	0.6	25.9	14.5	14.5	1.0

Modified from Ross *et al*. (2011).

Table 4. Estimated human dermal absorption using the Parallelogram method with pig data

Compound	Pig $_{in\ vivo}$ / Pig $_{in\ vivo}$	Human $_{in\ vivo}$ (%)	Human $_{in\ vivo}$ P (predicted %)	Human $_{in\ vivo}$ M (measured %)	Human $_{in\ vivo}$ p / Human $_{in\ vivo}$ M
Benzoic acid	1.9	46.5	88.4	60.6	1.5
Caffeine	1.2	40.6	40.6	48.7	1.2
Lindane	1.3	7.5	9.8	9.0	1.1
Malathion	0.4	17.0	6.8	8.0	0.9
Testosterone	0.5	39.4	19.7	49.5	0.4

Modified from Ross *et al*. (2011).

skin (Tables 5 and 6). Rabbit ear skin is characterized by a density of hair follicles (80 per cm^2) much lower than that of the skin of the rabbit back and of other rodents (rat 8000 per cm^2). Rabbit ear skin also showed comparable permeability in some molecules (lidocaine, triptorelin and thiocolchicoside). One of these works demonstrated that rabbit ear skin is a reasonable model for studying the iontophoretic transport of drugs *in vitro* as the relative electro-osmotic and electrorepulsive contributions were almost the same for human and rabbit skin (Nicoli *et al*., 2003).

As seen in Tables 5 and 6, rabbit ear skin has a similar SC thickness with pig ear skin and human skin. However, the lipid composition of rabbit SC was substantially different from that of the pig, which showed a higher content of nonpolar lipids. The viable epidermis of rabbit ear skin was also much thinner than that of pig ear skin. Hair follicle density is also still higher than pig and human (human back and abdominal skin are 29–93 per cm^2 and 6 per cm^2) although it is much lower than other hairy rodents. In permeation studies, hydrophilic chemicals (caffeine and nicotinamide) were four to seven times less permeable through rabbit ear skin than through pig skin, probably because of the higher lipophilicity of its SC whereas lipophilic chemical, progesterone showed similar permeability with pig ear skin (Nicoli *et al*., 2008).

We reviewed the original papers published after 1993 that described permeability of both rabbit skin and human skin. It included 16 studies, which measured absorption of 19 chemicals. Only two chemicals showed very similar permeability in both skin. Sixteen chemicals showed higher permeability through rabbit skin than through human skin. Among 14 chemicals, di-n-butylphthalate is 24 times and terbutaline is 14 times more permeable through rabbit skin than through human skin. In conclusion, rabbit skin is generally more permeable than human skin and 10 out of 19 chemicals (53%) fell within the range of ±1/2 log interval.

Guinea Pig

Guinea pig skin is also generally more permeable than human skin, similar to other rodents. Barbero and Frasch (2009) performed an extensive quantitative review on guinea pig skin, including hairless guinea pig skin as well as porcine skin as surrogates for human *in vitro* penetration studies. This included data from 14 *in vitro* studies consisting of 15 measurements of 13 different chemicals on permeability through both human and guinea pig skin. Their review showed that an excellent correlation exists between guinea pig skin and human skin; the linear correlation of the log transformed data gave an r^2 of 0.90 with a slope very close to 1.0 (0.96 ± 0.10), and an intercept not distinguishable from 1 (0.11 ± 0.3). But, for those where FOD only is measured (17 studies, 25 measurements, 21 different chemicals), 65% fell within the range $0.3 < \text{FOD} < 3.0$. These FOD studies generally exhibit less agreement between guinea pig and human permeation.

The average intra species coefficient of variation for guinea pig skin is 19%, which is less than for human skin (24%). Twelve lag time measurements of 12 chemicals taken from 11 studies comparing human and guinea pig skins have a Pearson's coefficient of correlation of 0.90 ($P < 0.0001$). The linear correlation slope was 1.07 with an intercept of –0.22 h, and r^2 of 0.82. Thus, time lag correlations between guinea pig and human skin were significant. From these results they concluded that, in general, guinea pig is a good model for human skin *in vitro* permeability measurements. For chemicals with substantial disagreement they suggest that higher hair density in guinea pig may contribute to high permeability of guinea pig for those chemicals, particularly hydrophilic ones (e.g. paraquat dichloride and sodium chloride).

We reviewed the original papers published after 1993 that described permeability of both guinea pig skin and human skin. This included 10 studies, which measured absorption of 10

Table 6. Mean thickness of different layers of rabbit, pig, human and mouse skin

Species	SC (μm)	Epidermis (μm)	Whole skin (mm)
Human	12.5	53.5	-
Pig, out ear	9.1 ± 0.8	61.7 ± 3.0	1.1771 ± 0.0097
Rabbit, inner ear	11.7 ± 0.5	17.0 ± 1.2	0.276 ± 0.01
Mouse	6.7	9.6	

Modified from Nicoli *et al*. (2008).
SC, stratum corneum.

Table 5. Rabbit ear skin as a skin model for *in vitro* transdermal permeation experiments

	Rabbit ear skin	Pig ear skin (control)
SC thickness	11.7 μm	9.1 μm
Lipid amount in SC	6%	5%
Lipid composition in SC	more lipophilic	less lipophilic
Ceramide *(polar)*	35%	43%
Cholesterol *(polar)*	11%	32%
Cholesterol esters *(non-polar)*	32%	1%
Triglycerides *(non-polar)*	5%	1%
Epidermis thickness	17 μm	62 μm
Hair density	80 per cm^2	11–30 per cm^2
Permeation		
hydrophilic (caffeine, nicotinamide): 4–7 times less permeable than pig skin		
lipophilic (progesterone): comparable with isolated pig epidermis		

Summarized from Nicoli *et al*. (2008).
SC, stratum corneum

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chemicals. Six chemicals showed higher permeability through guinea pig skin than through human skin. Three chemicals are less permeable through guinea pig skin than human skin. In conclusion, 5 chemicals out of 10 fell within the range of ±1/2 log interval and there is no consistent pattern of permeation rate in guinea pig skin such as always higher permeable or always less permeable than human skin. This result differs from Barbero and Frasch's result. It may be from small number of studies reviewed, and that they also included hairless guinea pig that showed much more comparable results to human skin as well as haired guinea pig in their review.

Hairless Rodents (Hairless Rat/Hairless Mouse/Hairless Guinea pig) and Hairless Dog

Hairy rodents have a disadvantage of an extremely high density of hair follicles and require hair removal before permeation experiment. As both issues can affect percutaneous absorption of chemicals, hairless rodents have been gaining more ground in permeation studies.

Hairless Rat

In the past there were some *in vivo* studies in which chemicals showed very similar permeability through hairless rat skin to human skin. Therefore, Shah *et al*. (1991) stated in 1991 that, together with pigs and rhesus monkeys, hairless rats are the only animals in which permeation data are consistently qualitatively and quantitatively similar to human permeation data.

We reviewed the original papers published after 1993 that described permeability of both hairless rat skin and human skin. This included 13 studies, which measured absorption of 21 chemicals. For 4 chemicals from 3 studies, absorption was very similar in both hairless rat and human skin. For 14 chemicals from 7 studies absorption through hairless rat skin is higher than through human skin. Most (12 of 14) are more than 3-times permeable than human skin and 7 chemicals showed more than 10-times permeability than human skin. Three chemicals from three studies are less permeable through hairless rat skin than through human skin. In conclusion, 33% (7 chemicals of 21) fell within the range of ±1/2 log interval. Thus, hairless rat skin seems to be generally more permeable than human skin.

Hairless Mouse

Chantasart *et al*. (2004) described the advantage of hairless mouse skin: hairless mouse skin SC has relatively constant lipid content whereas human skin lipid content varies considerably, thus making the interpretation of the partition experiment data difficult; hairless mouse skin SC lipid composition resembles that of human skin; the large body of hairless mouse skin data available in the literature allows the direct comparisons of the present results with those in previous studies; and hairless mouse skin has been found to be an adequate, quantitative model for human skin in the investigation of chemical permeation enhancers when defined protocols are employed.

Simon and Maibach (1998) reviewed the relevance of hairless mouse as an experimental model for human skin penetration. Regarding histology, SC of the hairless mouse is less than half as thick as that of the human tissue and accordingly with lower barrier properties. It is more susceptible to chemical perturbations than human skin. Their conclusion was that statistically significant correlations were not obtained between the hairless mouse skin and human skin and the *in vivo* hairless mouse data is not a useful predictive for human skin *in vitro* permeability. For *in vitro* studies, hairless mouse skin needs to be hydrated thoroughly to be a model for human skin penetration. Some compounds penetrated in an almost similar manner, but many differed in at least one logarithmic order, human skin being the less permeable. The relative effect of each enhancer formulation on the two skins was not consistent and therefore the hairless mouse model should not be used to predict the effects of penetration enhances in human skin.

We reviewed the original papers published after 1993 that described permeability of both hairless rat skin and human skin. This included 16 studies, which measured absorption of 17 chemicals. Five chemical penetrated through hairless mouse skin with a similar rate as human skin. Twelve chemicals penetrated through hairless mouse skin more than through human skin, 7 of these chemicals being more than a three-fold difference between hairless mouse skin and human skin. These results support that hairless mouse is not good model to predict human skin absorption.

Hairless Guinea Pig (HGP)

Hairless guinea pig (HGP) has some structural similarities to human skin that haired guinea pig does not have (Sueki *et al*., 2000). The epidermis of HGP is thick and has distinct layers (5–10 layers) similar to human epidermis. The thickness of the SC and the amount of blood vessels in the dermis are similar as well.

Skin permeability values in HGP were similar to those of human. Frasch and Barbero (2009) performed an experiment to compare HGP skin permeability and lag time measurements for six chemicals with a wide range of lipophilicity (log K_{ow} 0.90–3.40) with those of human skin. They found an excellent correlation between HGP skin and human skin in terms of permeability (Kp) and lag time. The data of permeability (Kp) for six chemicals through HGP skin are mostly slightly more permeable, but close to those of human. Thus, they concluded that HGP is a good substitute for human skin.

We also reviewed the original papers published after 1993 that described permeability of both HGP skin and human skin. This included 20 studies, which measured absorption of 28 chemicals. Seventeen chemicals from 10 studies showed a very close absorption rate through HGP to human skin. Only one chemical found less permeable through HGP than human skin and 12 chemicals from 9 studies showed higher permeability through HGP skin than human skin. Overall, 89% (25 of 28) of chemicals are within range of $0.3 < \text{FOD} < 3$. These results support that HGP skin is a good model for human skin absorption.

Hairless Dog

Percutaneous absorption in hairless dog has been compared to man. Absorption of benzoic acid, progesterone and testosterone was significantly slower and longer in hairless dogs than human (Hunziker *et al*., 1978). The percentage of penetration of *N*, *N*-diethyl-*m*-toluamide (12.8 ± 4.6%) in hairless dog was comparable to that of human reported by Feldmann and Maibach (16.7 ± 5.1%) (Reifenrath *et al*., 1981). However, when percutaneous absorption of nine compounds (caffeine, benzoic acid, *m*-deet, 3 steroids and 3 insecticides) were compared with

human, no significant correlation existed between hairless dog values and human values ($r = 0.58$) (Reifenrath *et al*., 1984). Thus, additional comparative studies are needed to determine its usefulness as an animal model for man.

In vitro Species Comparison and *in vitro*/*in vivo* Correlation

Compared with the *in vivo* study, *in vitro* animal models are more easily available, easy to perform and can provide results in a shorter period. They provide important tools for screening a series of drug formulations, evaluation of skin permeation enhancing properties and mechanism of action of the carrier systems and estimation of rank of skin transport for a series of drug molecules (Godin and Touitou, 2007).

In vitro test guidelines have recently been adopted (OECD, 2004; SCCNFP, 2006; U.S. EPA, 2004). They have brought a significant improvement in the standardization of *in vitro* tests and comparison of data between studies. However, still there are no strict recommendations regarding the type and preparation of the skin sample, type of diffusion cell and receptor fluid, for example some use fresh skin and others use dead skin. Skin membranes prepared in various ways have been used: full-thickness skin, dermatomed skin (split thickness skin) or epidermal membranes. Regarding diffusion cells, different kinds of system are accepted for use: Franz-type diffusion cells; flow-through diffusion cells, side-by-side diffusion cells, Keshary–Chien diffusion cell, horizontal static diffusion cells or vertical diffusion cells. Receptor fluid may also vary. All these factors influence the outcome. There is a debate on the way in which *in vitro* tests should be performed and how the resulting data should be interpreted in the risk assessment of dermal exposure. There are a number of ways in which the data from *in vitro* percutaneous absorption experiments are calculated (Jakasa and Kezic, 2008).

There are numerous *in vitro* studies and *in vivo* studies as well, but fewer *in vitro-in vivo* comparative studies. This made it difficult to interpret *in vitro* data. When *in vitro* absorption of the pesticide propoxur (log *P* 1.56) and the fungicide *o*-phenylphenol (log *P* 3.28) were compared with *in vivo* absorption in human and in rat skin, it was found that *in vitro* PA in most cases overestimated the *in vivo* situation (van de Sandt *et al*., 2000; Cnubben *et al*., 2002). Most close agreement between *in vitro* and *in vivo* results could be obtained on the basis of the potentially absorbed dose for both rat and human.

In an *in vivo* and *in vitro* comparative study in rats, van Ravenzwaay and Leibold (2004a, 2004b) determined rates of skin penetration for 14 chemicals. The result showed that *in vitro* results were always higher, irrespective of the compound tested and the duration of exposure, as compared with *in vivo* values. *In vitro* methods provided a more accurate prediction of *in vivo* dermal absorption for water-soluble molecules than lipophilic molecules with a log *P* greater than 3. However, a considerable difference between *in vitro* and *in vivo* values for highly lipophilic compounds was reported for lindane (log *P* 3.5) showing the 40-fold overprediction. Ross *et al*. (2011) reviewed a comparison of *in vivo* and *in vitro* dermal absorption values measured in pigs (Table 7), humans (Table 8) and rats (Table 9). This showed that *in vitro* studies are generally in a good agreement with *in vivo* studies for most compounds tested. This result suggests that a properly conducted *in vitro* study is generally accurate in predicting *in vivo* skin absorption and may avoid *in vivo* studies.

Table 7. Comparison of *in vivo* and *in vitro* dermal absorption values measured in pigs

Compound	Pig *in vitro*	Pig *in vivo*	Ratio, *in vivo*/*in vitro*
Benzoic acid	15	28	1.9
Caffeine	20	23	1.2
DEET	6	9	1.5
Fluocinolone acetonide	4	6	1.5
Lindane	6	8	1.3
Malathion	10	4.4	0.4
Mean			1.2

Modified from Ross *et al*. (2011).

Table 8. Comparison of *in vivo* and *in vitro* dermal absorption values measured in humans

Compound	*In vitro* human	*In vivo* human	Ratio, *in vivo*/*in vitro*
Benzoic acid	46.5	60.6	1.3
Caffeine	40.6	40.6	1.0
Fluazifopbutyl	2.2	8.0	3.6
Lindane	7.5	9	1.2
Malathion	17	8	0.47
Ortho phenyl phenol	16.3	24.2	1.5
Permethrinh	1.3	1.2	0.95
Piperonyl butoxide	7.4	5.3	0.72
Propoxur	25.9	14.5	0.56
Testosterone	39.4	49.5	1.3
Mean			1.0

Modified from Ross *et al*. (2011).

Table 9. Comparison of *in vivo* and *in vitro* dermal absorption values measured in rats

Compound	*In vitro* rat	*In vivo* rat	Ratio, *in vivo*/*in vitro*
Acetyl salicylic acid	29.0	24.8	0.86
Benzoic acid	49.1	37.0	0.75
Caffeine	48	57	1.2
DEET	34	38	1.1
Fluazifop-butyl	80	74.3	0.93
Ortho phenyl phenol	10.3	35.8	3.5
Permethrine	20.7	35	1.7
Piperonyl butoxide	35	42	1.2
Propoxur	31	20.8	0.67
Urea	7.2	8.1	1.1
Mean			1.1

Modified from Ross *et al*. (2011).

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However, the majority of studies reported that *in vitro* percutaneous absorption overestimated *in vivo* percutaneous absorption. *In vitro* studies showed considerable variation depending on the experiment ways or conditions that are mentioned above. The predictive value *in vitro* assays was shown to be influenced by factors such as type and thickness of the skin and choice of the receptor fluid. Agreement between *in vitro* and *in vivo* is better for hydrophilic than for lipophilic compounds. *In vivo*, the capillary bed acts a sink, removing the chemicals as it diffuses into the epidermis and dermis. *In vitro* methods rely on the receptor fluid (RF) as a sink, but the thermodynamics of the partitioning from the skin into the RF is highly dependent on the lipophilicity of the chemicals, the physiochemical attributes of the RF and the solubility of the chemicals into the RF (Capt *et al*., 2007). Most studies showed that the use of full-thickness skin resulted in a lower absorption of lipophilic chemicals into the receptor fluid when compared with the results obtained with split thickness skin, indicating a reservoir effect of these compounds.

There is also a point of debate regarding the amount of a chemical retained in the skin at the end of exposure (Jakasa and Kezic, 2008):

> **OECD**: The test substance remaining in the skin should be considered as absorbed.
> **COLPIA** (European Cosmetic, Toiletry, and Perfumery Association) & **SCCNFP** (Scientific Committee on Cosmetic Products and Non-food Products Intended for Consumers)

i. The amount of a chemical present in the **SC** at the end of the exposure should **not** be considered as systemic available.
ii. The amount of a chemical in the **epidermis and dermis** and in the RF as systemically available.

In conclusion, more comparative studies are needed to determine the factors that influence the predictive value of the *in vitro* and animal models.

Alternative *in vitro* Test Methods

New regulatory guidelines increasingly demand the reduction of tests using laboratory animals in research as well as in drug, chemical and cosmetic screening. Thus, alternative *in vitro* test methods are gaining in importance to avoid excessive animal use.

Isolated Perfused Porcine Skin Flap (IPPSF)

Among 45 studies done in pig in our review only 4 are *in vivo*. To overcome some of the limitations of *in vitro* study while still taking advantages of the *in vitro* study, Riviere *et al*. (1986) developed the IPPSE (isolated perfused porcine skin flap) model. It provided an anatomically intact, viable, isolated, perfused tube-like preparation in which epidermis and dermis are viable with functional microcirculation which can be used for collecting blood-containing chemicals absorbed through the skin. Wester *et al*. (1998) compared percutaneous absorption of five chemicals through human skin and the IPPSF model. Percutaneous absorption values of the IPPSF model were comparable to those of human skin (correlation coefficient = 0.78; $P < 0.04$). However, they concluded that though their results and other promising studies, this model needs more studies with a broader group of diverse chemicals.

These *ex vivo* models system allow cutaneous toxicology and pharmacology studies to be conducted in viable skin that has a normal structure and intact microvasculature. However, surgical procedures and perfusion techniques for these models are complex and time consuming.

Isolated Blood-Perfused Pig Ear

There are two major advantages of the perfused pig ear model over *in vitro* models. In the pig ear model, blood is used as the recipient medium instead of a buffer containing organic solvents, and the model does not ignore the effect of chemicals on the dermal vascular system (dose-dependent increase in perfusion pressure after noradrenaline and reversal of the same by isoxsuprine) (Simon and Maibach, 2000).

In comparative *in vitro-in vivo* percutaneous absorption studies for propoxur and *ortho*-phenylphenol, the data generated in the perfused pig ear model were generally intermediate between full thickness skin and epidermal membrane of human *in vitro* studies and 5–10 times overestimated the human *in vivo* data for both chemicals (van de Sandt *et al*., 2000; Cnubben *et al*., 2002). And the practical perfusion period is limited to about 6 h (de Lange *et al*., 1994).

Isolated Normothermic Hemoperfused Porcine Forelimb

Wagner *et al*. (2003) developed this model to replace animal testing with maintaining the characteristics of the porcine skin as close to physiologic conditions as possible. The perfusion of the isolated porcine forelimb in several respects meets the requirements of an *in vitro* assay model. However, it was not predictive of *in vivo* absorption in human. The maximal nitroglycerin concentration as determined in their study was considerably higher than that in humans from studies described in the literature (Table 10) (Muller *et al*., 1982; Heidemann *et al*., 1985;).

They explained that the difference from the perfusion of the porcine is run in a recirculating mode, which leads to continuous accumulation of nitroglycerin in the perfusion medium and the isolated porcine limb is deprived of the metabolizing and eliminating mechanisms of the liver and kidney, which are present *in vivo*. They concluded that comparing the penetration rates of nitroglycerin in the porcine limb model with *in vivo* in humans is very difficult. So, further improvement of the perfusion setup is necessary. Like the isolated blood-perfused pig ear model, its vitality of model was maintained for 5–6 h.

Table 10. Comparison of maximum blood nitroglycerin concentration in isolated normothermic hemoperfused porcine forelimb and Human

	Maximal blood nitroglycerin concentration	
Patch	Wagner *et al*. (2003)	Human *in vivo* (published data)
TTS 5[a]	up to 3.86 ng ml^{-1}	0.27 ng ml^{-1}
TTS 10[b]	4.54 ng ml^{-1}	1.1 ng ml^{-1}

[a]Patches containing nitroglycerin 25 mg.
[b]Patches containing nitroglycerin 50 mg.

Mouse Dorsal Skin Fold Chamber Model

Eros *et al*. (2012) used the mouse dorsal skin fold chamber model which permits precise determination of the quantity of drug penetrating living full-thickness skin with a functioning microcirculation. A skin fold in the dorsal region of a nude mouse was fixed with two fenestrated titanium plates. A circular wound was made on one side of the skin fold. A metal cylinder with phosphate buffer was fixed into the window of the titanium plate. The concentration of penetrated drug was measured in the buffer. It is an *in vitro* study under *in vivo* condition, so repeated measurements can be performed in the same animal to determine the kinetics of penetration, which can reduce the number of animal required for study. However, continuous presence of an investigator is required for assessment of the animals and for the maintenance of anesthesia. And observation period was only 6 h.

Eros *et al*. (2012) measured ibuprofen permeation through the dorsal skin fold chamber model. The flux of 11.57 $\mu g\,cm^{-2}\,h^{-1}$ was not comparable with the data of human skin in published data; 20–30 $\mu g\,cm^{-2}\,h^{-1}$ or higher in the human skin *in vitro* study (Iervolino *et al*., 2001; Swart *et al*., 2005). Therefore, it may replace the *in vivo* mouse study, but is not comparable with human studies.

Isolated Bovine Udder

Netzlaff *et al*. (2006) compared bovine udder skin with human and porcine skin in percutaneous permeation experiments. Bovine udder skin seemed to exhibit a weaker, but less variable, barrier against caffeine, benzoic acid, testosterone and flufenamic acid whereas pig and human skin were found to be equally permeable.

Human Skin Grafted Onto Nude Mouse Model (HuSki Model)

This new model has the advantage of allowing the evaluation of chemicals using a system consisting of a viable human skin and SC with a physiological capillary circulation of nude mouse. Reifenrath *et al*. (1984) investigated skin absorption in several *in vivo* models including HuSki model. He demonstrated a significant correlation between the skin penetration values obtained for nine chemicals with the HuSki model and the human volunteer values ($r = 0.74$; $P = 0.05$).

Capt *et al*. (2007) compared the skin penetration for three reference insecticides (malathion, lindane and cypermethrin) using two *in vivo* (Rat and HuSki), and two *in vitro* (Rat and Human) models. Then they compared the data obtained from these models with human volunteer data (Maibach *et al*., 1971; Feldmann and Maibach, 1974; Woollen *et al*., 1992) for their ability to predict the human skin absorption (Table 11). The human *in vitro* model was most predictive of human *in vivo* absorption, but it could not be used for more than 24 h as the skin samples in the diffusion cells could lose their vitality after 24 h. The HuSki model was similar to the human *in vitro* model in predicting human *in vivo* absorption for the three compounds. The rat *in vivo* model overestimated human *in vivo* skin absorption much more than the human *in vitro* and HuSki model. As HuSki is an *in vivo* model and allows the absorption experiment for longer period (at least 11 days), it was also suitable to study the fate of chemicals in the skin and SC over prolonged periods of time. The evolution of the distribution of cypermethrin between 6 and 120 h was different in the rat model compared with the HuSki model. In the rat model, about 1/3 of cypermethrin present in the SC at 6 h was further absorbed at 120 h. In contrast, in the HuSki model, only a small fraction of the cypermethrin present in the SC at 6 h was further absorbed at 120 h and 3/4 of cypermethrin present in the SC at 6 h was eliminated in the wash-off compartment at 120 h.

Table 11. Absorption (% of applied dose) of malathion, lindane and cypermethrin in five models at 24 h

	Human *in vivo*	Human *in vitro*	HuSki	Rat *in vivo*	Rat *in vivo*
Malathion	9.4%	7%	12.4%	31%	56%
Lindane	9.3%	7.4%	18.4%	31.6%	10%
Cypermethrin	1.1%	4.3%	5%	12.3%	33.65%

Modified from Capt *et al*. (2007).

Animal Skin Physical and Chemical Parameters

There are many physical and chemical differences between skin of a variety of animals and between animal skins and human skin: skin (especially SC) thickness; composition of SC lipids; hair density and thickness of hair follicles; epidermal-dermal junction; architecture of vasculature, collagen fibers and fiber bundles in the dermis; and distribution of fat. In addition to these, dosing variables (concentration, surface area, formulation, time, etc.) affect percutaneous absorption in animal models.

The lack of a correlation in transdermal permeation of molecules across species or from different application sites in the same animal model is due mainly to variations in skin (or SC) thickness, in the composition of intercellular SC lipids and in the number of hair shafts. Netzlaff *et al*. (2006) have shown that the amount of free fatty acids and triglycerides and the density of hair follicles are important factors causing differences between the skin barriers among species.

Dose Response

In vivo percutaneous absorption can vary depending upon skin concentration. Therefore, a topical dose response can give additional information about the relevance of an animal model. The rhesus monkey showed the same dose response with human *in vivo* (Wester and Maibach, 1976). The hairless rat also showed the same dose response to human *in vivo*, which showed a linear increase in absorption with increased dose (Dupuis *et al*., 1984). However, it was different in the absolute amount absorbed between two species. In the hairless rat, 80–90% of the applied dose was absorbed; however, much less was absorbed in rhesus monkey.

Regional Variation in Animals

Regional variation in animal models may affect prediction of human skin absorption. In the Rhesus monkey, regional variation is similar to human, thus it is a most relevant animal model for human regional variation (Wester *et al*., 1980). In rat and hairless rat, skin thickness is much less than human skin, which causes percutaneous absorption to be higher.

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Summary

For critical studies, percutaneous absorption in human remains the best option. However, it is very difficult or impossible to perform human study. Thus, animal models have been introduced to predict percutaneous absorption in human. However, it is very complex to correlate absorption data from animal studies with human because there are differences in percutaneous absorption between human and animal species, which comes from either species themselves or methods or technologies used in the study. Animal models should be selected to be best appropriate for aim of the study. And experimental method and conditions that affect percutaneous absorption should be controlled by the investigator. Some are easy to control, i.e. the site of application, occlusion, dose concentration, surface area and vehicle. However, others may be difficult to control, i.e. skin metabolism, skin age and skin condition.

We also need familiarity with limitation of various animal models and experimental methods. Absorption in common laboratory animals (i.e. rat or rabbit) is generally higher than human but absorption in pig, monkey (squirrel, rhesus) and hairless guinea pig is more predictive of human *in vivo*. In *in vivo-in vitro* comparative studies, absorption data appeared to be comparable in many chemicals. For those chemicals, the *in vitro* study may replace the *in vivo* study. And to overcome limitations of *in vitro* or animal models, new alternative models such as isolated perfused porcine skin flap or HuSki models were introduced.

In conclusion, as noted in several sections here, we are fully conversant with the limitations of these decades of important observations – namely that many variables were not held constant – and that all too few benchmarked to the complexities of the 15 steps to percutaneous penetration *in vivo* in man (Ngo *et al*., 2010). Nevertheless, the themes outlined here provide the bases for refinement and extension of our knowledge as to how to perform studies relevant for man.

Conflict of Interest

The Authors did not report any conflict of interest.

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