Chapter 2
Animal Models for Percutaneous Absorption

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2.1 Introduction

A most relevant way to determine the percutaneous penetration rate or absorption rate of chemicals in humans is *in vivo* studies. However, it has been increasingly complex to perform *in vivo* human studies because of regulation such as US EPA's human research rule [1]. An alternative way is *in vitro* human skin absorption study 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 due 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 [2]. However, animal skin is generally more permeable than human skin. To develop most predictive data of the human skin penetration or absorption, animal model's physiology, biochemistry, and anatomy of skin should be similar to humans [3]. Thus, animals phylogenetically close to humans would be good models, but it is not absolutely required for an animal to be genetically close to humans to be a good animal because an animal that is not genetically close to human can have skin characteristics similar to humans. Two basic criteria help judge whether an animal is relevant; the animal model should give percutaneous absorption similar to that in humans; if it is not possible, then percutaneous absorption in the animal model should be constantly different from that in humans.

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In 1972, Bartek et al. [4] challenged the world of comparative cutaneous biology to begin to understand relative percutaneous penetration in several species. Subsequently, extensive observations have extended Bartek's investigation—and here we evaluate the subsequent four decades—in hopes of aiding dermatopharmacology and dermatotoxicology studies.

This chapter describes monkeys, pigs, rats, rabbits, guinea pigs, and hairless rodents such as hairless rats, hairless mice, and hairless guinea pigs (HGPs), and then some alternative models such as a human skin grafted onto the nude mouse model (HuSki model).

2.2 Monkeys: Rhesus/Squirrel

The monkey is a most relevant animal model for percutaneous absorption because it is phylogenetically close to humans; therefore, its skin resembles human skin and areas such as the inner arm, legs, and trunk are relatively hairless like human skin. Its regional variation in percutaneous absorption resembles human skin; therefore, the same anatomical site can be used in comparative study. It is sufficiently large for serial blood sampling. However, the use of monkeys in experiments is somewhat limited by cost and restricted availability. Also, they are difficult to handle and require expertise with special facilities. There are some differences in skin anatomy between monkeys and humans; monkeys are covered with a dense coat of pelage not hair; their epidermis has little undersculpture; they have numerous apocrine glands over nearly the entire hairy skin; monkeys have fewer sebaceous glands that directly open to the skin surface [5].

We found three studies for four chemicals, which described the permeability of both monkey and human skin and were published after 1993. 2, 4-dichlorophenoxyacetic acid penetrated similar to human skin [6]. However, acitretin was 0.3 times as permeable as in human skin [7]. In Panchangnula et al.'s study [8], water and 7-hydroxycoumarin were 2.3 and 3.8 times more permeable, respectively, 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. Thus, percutaneous absorption across monkey skin often, but not always, resembles human skin.

2.3 Pigs

Another appropriate animal model for human skin absorption is the pig, both *in vivo* and *in vitro* [2]. 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 inconvenient to handle in standard laboratory animal facilities. There are similarities between porcine and human skin; the skin is characterized by a spare hair coat, a thick epidermis that has a well-differentiated

Table 2.1 Thickness of skin layers of different species. (Modified from [11])

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

Table 2.2 Thickness of human and animal skin. (Modified from [12])

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

undersculpture, a dermis that has a well-differentiated papillary body and a large content of elastic tissue [3]. The follicular structure of pig skin also resembles that of humans. The average of 20 hairs/cm^2 of porcine ear skin is similar to 14–32 hairs/cm^2 in humans [9].

The histological appearance of the epidermis is similar in humans and pigs [3]. Porcine and human epidermis appear similar in tissue turnover time and the characterization of keratinous proteins. Porcine SC contains protein fractions grossly similar to humans. It has similar variable filament density and areas of cell overlapping with human skin SC. The epidermal–dermal junction of pigs resembles that of humans. 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 and human skin [10].

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 [3]. The thickness of skin layers in porcine skin resembles that of human skin (Tables 2.1 and 2.2) [11, 12].

However, dissimilarities also exist: vascularization is rich in man but poor in pigs; humans have mostly eccrine sweat glands, whereas pigs have mostly apocrine glands. As there is high fat component in pigs, lipid soluble compounds concentrate in the fatty area of pigs rather than the central compartment (blood sampling) [3].

Barbero and Frasch [13] extensively reviewed porcine skin as surrogates for human *in vitro* penetration studies. In 18 studies which reported permeability coefficient of 26 chemicals, correlation efficient (r) between pig and human skin is 0.88 ($p < 0.0001$). It supports a strong positive correlation between two skins.

In another 20 studies of 50 measurements on 40 chemicals that did not report permeability and factors of difference (FODs) calculated from permeability studies, 80% fell within the range of ±1/2 log interval; that is $0.3 < FOD < 3.0$. Average intraspecies coefficient of variation for pig skin is 21% and for human skin 35%. 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 the human skin.

As the first edition of this book was published in 1993, we reviewed the original papers published after 1993 that described permeability of both pig skin and human skin. These 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 ($0.625 < FOD < 1.6$). For 25 chemicals in 15 studies, percutaneous permeability of pigs is higher than that of humans. In this group, nine chemicals were absorbed in porcine skin in a much higher rate than human skin ($FOD > 3$). For 16 chemicals in six studies, human skin permeability is higher than that of pigs. However, only three chemicals showed 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.

2.4 Rats

Rodents are readily available, small and easy to handle, inexpensive, have 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 to human skin. Among rodents, rat skin has more structural similarities to human skin (Table 2.2). Therefore, permeation kinetic parameters of rat skin are frequently comparable with human skin [14]. However, differences between rat skin and human skin are large. In rat skin, epidermis and SC are thinner, appendage number is higher, intercellular lipid composition of the SC is different, and corneocyte surface is lower than in human skin [15].

We reviewed the original papers published after 1993 that described permeability of both rat and human skin. These included 79 studies, which measured absorption of 110 chemicals. For 23 chemicals in 21 studies, permeability of rat skin resembled that of human skin ($0.625 < FOD < 1.6$). For 83 chemicals in 54 studies, rat skin is more permeable than human skin. Only four chemicals are less permeable through rat than through human skin. In the group of chemicals ($n = 83$) that were more permeable in the rat than human skin, twenty-eight chemicals show FOD within the range of 3–10, twenty-four chemicals show FOD within the range of 11–99, and five chemicals show FOD within the range of 100–500. 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 Razenzwaay and Leibold [16, 17] compared *in vivo* rate of penetration of 14 pesticides with a wide range of lipophilicities and molecular weights with *in vitro* rate of penetration in rat as well as *in vitro* rate of penetration in humans. In *in vitro* studies, rat skin was always more permeable for all tested substances than human skin (FOD ranged from 2.3 to 36.5, mean: 13.4 ± 11.1-fold). *In vivo* rat skin is always less permeable than *in vitro* rat skin, but, in most cases (9/12), it was more permeable than *in vitro* human skin. No constant factor of difference was identified. Factor of difference would not appear to be determined by molecular weight, lipophilicity, or aqueous solubility. Because of 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 (US EPA 1992; Thongsinthusak et al. 1993; van Ravenzwaay and Leibold 2004; WHO 2005) [17–20] suggested a method, the 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: add reference

$$\%\ \text{human dermal penetration} = \frac{[\%\ \text{dermal penetration in rat in vivo}] \times [\text{rate of dermal penetration in human in vitro}]}{[\text{rate of dermal penetration in rat in vitro}]}$$

Ross et al. [21] examined the predictive worth of this method as outlined in Table 2.3 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 ≤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 2.4 are the values predicted using pig data, which also show a good agreement between estimated and measured values [21]. 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 three study types (*in vitro* human, *in vitro* rat, *in vivo* rat) were conducted concurrently under the same condition by the same laboratory [21].

Table 2.3 Comparison of measured human absorptions and new predictions of human dermal absorption using the parallelogram method. (Modified from Table 4 in [21])

Compound	$\frac{\text{Rat}_{in\ vivo}}{\text{Rat}_{in\ vivo}}$	$\text{Human}_{in\ vivo}$ (%)	$\text{Human}_{in\ vivo}M$ (predicted %)	$\text{Human}_{in\ vivo}M$ (measured %)	$\frac{\text{Human}_{in\ vivo}p}{\text{Human}_{in\ vivo}M}$
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
PBO	1.2	7.4	8.9	5.3	1.7
Propoxur	0.6	25.9	14.5	14.5	1.0

Table 2.4 Estimated human dermal absorption using parallelogram method with pig data. (Modified from Table 9 in [21])

Compound	$\frac{\text{Pig}_{in\ vivo}}{\text{Pig}_{in\ vivo}}$	$\text{Human}_{in\ vivo}$ (%)	$\text{Human}_{in\ vivo}P$ (predicted %)	$\text{Human}_{in\ vivo}M$ (measured %)	$\frac{\text{Human}_{in\ vivo}p}{\text{Human}_{in\ vivo}M}$
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

2.5 Rabbits

Similar to rat, rabbit skin is generally more permeable than human skin and the difference in percutaneous absorption between rabbit skin and human skin is not consistent. In 2008, Nicoli et al. [22] performed an experiment to compare rabbit ear skin with pig ear skin on histology, lipid composition, and permeability of skin (Tables 2.5 and 2.6). Rabbit ear skin is characterized by the density of hair follicles (80/cm^2) much lower than that of the skin of the rabbit back and of other rodents (rat 8000/cm^2). Rabbit ear skin also showed comparable permeability in some molecules (lidocaine, triptorelin, thiocolchicoside). One study demonstrated that rabbit ear skin is a reasonable model for studying the iontophoretic transport of drugs *in vitro* since the relative electro-osmotic and electrorepulsive contributions were almost similar for human skin and rabbit skin [23].

As seen in Tables 2.5 and 2.6, rabbit ear skin has SC thickness similar to pig ear 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. And viable epidermis of rabbit ear was much thinner than that of pig ear skin. Hair

Table 2.5 Rabbit ear skin as a skin model for *in vitro* transdermal permeation experiments. (Summarized from [22])

	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 *(nonpolar)*	32%	1%
Triglycerides *(nonpolar)*	5%	1%
Epidermis thickness	17 μm	62 μm
Hair density	80/cm^2	11–30/cm^2
Permeation		
Hydrophilic (caffeine, nicotinamide)	4–7 times less permeable than pig skin	
Lipophilic (progesterone)	Comparable with isolated pig epidermis	

Table 2.6 Mean thickness of different layers of rabbit, pig, human, and mouse skins. (Modified from Table 2 in [22])

Species	SC (μm)	Epidermis (μm)	Whole skin (mm)
Human	12.5	53.5	–
Pig, outer ear	9.1	61.7	1.1771
Rabbit, inner ear	11.7	17.0	0.276
Mouse	6.7	9.6	–

follicle density is also still higher than pigs and humans (human back and abdominal skin are 29–93/cm^2 and 6/cm^2, respectively) though it is much lower than other hairy rodents. In permeation studies, hydrophilic chemicals (caffeine, nicotinamide) were 4–7 times less permeable through rabbit ear than through pig skin, probably because of the higher lipophilicity of its SC while lipophilic chemical, progesterone showed permeability similar to pig ear skin [22].

We reviewed the original papers published after 1993 that described permeability of rabbit skin and human skin, including 16 studies, which measured 19 chemicals. Only 2 chemicals showed similar permeability in both and 16 chemicals 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 that through human skin. In conclusion, rabbit skin is generally more permeable than human skin and 10 chemicals of 19 (53%) fell within the range of ±1/2 log interval.

2.6 Guinea Pigs

Guinea pig skin is also generally more permeable than human skin like other rodents. Barbero and Frasch [13] performed an extensive quantitative review on guinea pig skin, including HGP skin as well as porcine skin as surrogates for human *in vitro* penetration studies. These included data from 14 *in vitro* studies consisting 15 measurements of 13 chemicals on permeability through both human and guinea pig skin. Their review showed 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 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.

Average intraspecies 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 correlation coefficient of 0.90 ($p < 0.0001$). 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 skins were significant. From these results they concluded that, in general, the 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 pigs may contribute to the high permeability of guinea pig skin for those chemicals, particularly hydrophilic ones (e.g., paraquat dichloride, sodium chloride).

We reviewed the original papers published after 1993 that described permeability of both guinea pig and human skins. These included 10 studies, which measured absorption of 10 chemicals. Six chemicals showed higher permeability through guinea pig skin than through human skin. Three chemicals were less permeable through guinea pig skin than human skin. In conclusion, five chemicals of ten fell within the range of $\pm 1/2$ log interval. This result differs from Barbero and Frasch's result. This may be due to the small number of studies reviewed and that they also included HGP that showed much more comparable results to human skin as well as the haired guinea pig skin in their review.

2.7 Hairless Rats/Hairless Mice/Hairless Guinea Pigs

Hairy rodents have the 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.

2.7.1 Hairless Rats

Earlier there were *in vivo* studies in which chemicals showed permeability through hairless rat skin similar to human skin. Therefore, Shah et al. [24] 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 original papers published after 1993 that described permeability of hairless rat skin and human skin. These included 13 studies, which measured absorption of 21 chemicals. For four chemicals from three studies, absorption was similar in hairless rat and human skin. For 14 chemicals from seven studies absorption through hairless rat skin is higher than human skin. Most (12 of 14) were more than three times permeable than human skin and seven chemicals showed more than ten 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.

2.7.2 Hairless Mice

Chantasart et al. [25] 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 SC lipid composition resembles that of human skin. The large body of hairless mouse skin data available allows direct comparisons of the present results with those in previous studies. 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 [26] reviewed the relevance of the 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 usefully 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. 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 hairless rat skin and human skin. These included 16 studies, which measured ab-

sorption of 17 chemicals. Five chemicals penetrated through the hairless mouse skin at a rate similar to human skin. Twelve chemicals penetrated through the hairless mouse more than through human skin, seven of them showing more than a threefold difference between hairless mouse skin and human skin. These results support that the hairless mouse is not a good model to predict human skin absorption.

2.7.3 Hairless Guinea Pigs (HGPs)

The skin of HGP has some structural similarities with human skin that the skin of the haired guinea pig does not have [27]. The HGP epidermis is as thick as human skin and has distinct layers (5–10 layers) similar to human epidermis and SC thickness and the number of blood vessels in the dermis is similar as well.

Skin permeability values in HGP were similar to those of humans.Frasch and Barbero [28] 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 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 humans. Thus, they concluded that HGP is a good substitute for human skin.

We reviewed the original papers published after 1993 that described permeability of both HGP and human skin. These included 20 studies, which measured absorption of 28 chemicals. Eighteen chemicals from 11 studies showed a close absorption rate through HGP to human skin. Only one chemical was less permeable through HGP than human skin and 11 chemicals from eight studies showed higher permeability through HGP skin than human skin. Overall, 89 % (25 of 28) chemicals are within the range of $0.3 < \text{FOD} < 3$. These results support that HGP skin is a good model for human skin absorption.

2.8 *In Vitro* Species Comparison and *In Vitro*/*In Vivo* Correlation

Compared to *in vivo* animal 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 [14].

There are numerous *in vitro* and *in vivo* animal studies, but fewer *in vitro–in vivo* comparative studies. This makes it difficult to interpret *in vitro* animal data. van de Sandt et al. [29, 30] compared *in vitro* absorption of the pesticide propoxur (log *P* 1.56) and the fungicide *o*-phenylphenol (log *P* 3.28) with *in vivo* absorption in

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