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REVIEW ARTICLE

The importance of minipigs in dermal safety assessment: an overview

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Abstract

The use of miniature swine as a non-rodent species in safety assessment has continued to expand for over a decade and their use has become routine, particularly in pharmacology as a model for human integumentary diseases. Translational preclinical swine study data are now favorably compared and contrasted to human data, and miniature swine models provide important information in dermal safety assessment and skin pharmacology. For example, the miniature swine model has been well-accepted for cutaneous absorption and toxicity studies due to swine integument being morphologically and functionally similar to human skin. Subsequently, this model is important to dermal drug development programs, and it is the animal model of choice for assessment of dermal absorption, local tolerance and systemic toxicity following dermal exposures. In conclusion, the miniature swine model has an important role to play in the safety assessment of pharmaceutical products and in multiple aspects of human dermal drug development.

Keywords

Dermal drug safety, dermal pharmacology, dermal toxicology

Introduction

The study of pigs as medical models is recorded from as early as the time of Galen in 2nd century A.D.1 and their study as biomedical models has slowly progressed to modern times. During the past half century, pigs have been used in preclinical dermal toxicology, dermal pharmacokinetics (PK), dermal phototoxicity, dermal wound healing studies and a broad array of other biomedical research applications2,3. Now in modern times, particularly over the past 30 years, the use of swine as biomedical models has grown exponentially.

Swine have been used extensively in dermal research because of the comparability of their integument to that of humans (cf. Figure 1). Reviews of the use of swine in such studies have been previously published4–7. In the field of toxicology, swine skin has been used for acute and repeat dose dermal toxicology, dermal absorption, allergic contact dermatitis, phototoxicity and photosensitization studies. Models have been created both in vivo as well as in vitro with skin membranes and grafts. Both miniature and domestic breeds have been used for these types of studies; however, miniature breeds such as Sinclair, Gottingen, Yucatan and Hanford may be more advantageous because of their smaller size at sexual maturity. Table 1 presents some primary aspects to consider when selecting miniature swine versus domestic swine. Using these miniature breeds allows investigators to conduct experiments in mature (rather than pediatric) animals with a consistent size and health status as compared to farm pigs. Each breed may be utilized in some aspect of dermal toxicology.

Selection of animal species for toxicology

Selection of the most appropriate animal species for preclinical toxicology testing during human drug development can be a challenge; seeking advice from regulatory bodies such as the U.S. Food and Drug Administration (FDA), the European Medicines Agency (EMA), and the Organization for Economic Cooperation and Development (OECD) is encouraged. Similarity to man with regard to anatomy and physiology is certainly important, but highly specific translational predictive value for safety of the drug class under consideration is optimal. Miniswine have increased in rank as an animal model in recent years; in some areas, the swine model is preferred over non-human primates (NHP) or dog models to translate directly into man8–10. Not all miniswine translational research is yet fully realized or openly published as some data remain confidential and are sequestered in private archives. Under most conditions, swine data are evaluated in conjunction with data from other species for making drug development and regulatory decisions.

Selection of the most desirable species for dermal toxicology additionally has many factors requiring consideration, including skin surface area, animal housing and handling, per diem expenses, and predictability. For example, miniswine, and pigs in general, have limited neck and leg mobility and are unable to lick or scratch their backs.
They can, however, scratch dorsal dermal application areas (DAA) on surrounding structures, therefore test sites are generally protected with covers or wraps, though some studies do call for the sites to be left intentionally uncovered.

Beneficially, miniswine are easily trained and socialized, and are able to be placed into slings for short periods of time, not to exceed 3 h, without overt stress. Certain processes and factors also must be closely monitored in order to avoid

Figure 1. Comparative histological (H&E staining) examination of integument of seven species of mammals including two lineages of minipigs. 1: human, 2x; 2: cynomolgus monkey, 10x; 3: Yucatan minipig, 10x; 4: Hanford minipig, 2x; 5: Beagle dog, 10x; 6: rabbit, 2x; 7: guinea pig, 20x; 8: mouse, 20x. Hair follicle (H), Primary hair (PH), Secondary hair (SH), Sebaceous gland (S), Apocrine gland (A), Eccrine gland (E).
Miniswine Smaller, reaches similar to human skin. Macroscopically, the swine is a minor differences, the skin of swine is known to be very differences between swine and humans. Aside from a few As with all animal models there are both similarities and Comparative dermal anatomy and physiology

Ganderup et al. reviewed miniswine safety and efficacy data on 43 marketed drugs with previously reported adverse responses. Fifty-eight percent of the reviewed drugs had a dermal indication, and 27 drugs had both human and miniswine data to enable a comparison. Overall, the predictive value (PV) of miniswine safety and efficacy studies to human outcomes were 89% and 100%, respectively (Table 2). The results of this review support the value of the miniswine model for preclinical safety.

Predictability of miniswine data for preclinical safety

Ganderup et al. reviewed miniswine safety and efficacy data on 43 marketed drugs with previously reported adverse responses. Fifty-eight percent of the reviewed drugs had a dermal indication, and 27 drugs had both human and miniswine data to enable a comparison. Overall, the predictive value (PV) of miniswine safety and efficacy studies to human outcomes were 89% and 100%, respectively (Table 2). The results of this review support the value of the miniswine model for preclinical safety.

Comparative dermal anatomy and physiology

As with all animal models there are both similarities and differences between swine and humans. Aside from a few minor differences, the skin of swine is known to be very similar to human skin . Macroscopically, the swine is a relatively hairless animal with a fixed skin that is tightly attached to the subcutaneous tissues. Swine skin surface character, thickness, layers, pigmentation, turnover kinetics, number of hairs and hair follicles, blood flow, and variations by gender, age and body region are generally representative of humans. The skin pH of the pig is slightly higher than human skin, having a less acidic mantle, and the hypodermal adipose can be thicker in older overfed swine. In juvenile swine, hairs and follicles can occasionally be found in triads; the overall percentage of triad formations is low and they tend to outgrow this pattern. Hair follicles contain an intrafollicular muscle which contributes to the erection and rotation of hair shafts in addition to the arrector pili muscles. Seasonal shedding is not generally an issue for miniswine when they are housed indoors under controlled photoperiod and temperature.

topical test article cross contamination between treatment groups, including blood collection, biopsies, pen changing, caging sanitization procedures, dosing procedures, gloves, slings, worker clothing and laundry.

Table 2. Predictive value of swine studies for human drug safety and efficacy.

<table>
<thead>
<tr>
<th>Predictive value</th>
<th>Safety</th>
<th>Efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good</td>
<td>17 (63%)</td>
<td>7 (100%)</td>
</tr>
<tr>
<td>Acceptable</td>
<td>7 (26%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Poor</td>
<td>3 (11%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

Table 3. Histological layers of swine skin and underpinnings.

<table>
<thead>
<tr>
<th>Epidermis</th>
<th>Dermis</th>
<th>Underpinnings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stratum corneum</td>
<td>Papillary layer</td>
<td>Hypodermis (adipose)</td>
</tr>
<tr>
<td>Stratum lucidum</td>
<td>Reticular layer</td>
<td>Fascia and muscle</td>
</tr>
<tr>
<td>Stratum granulosum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stratum spinosum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stratum basale</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basement membrane</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Miniswine breeds can either have pigmented or non-pigmented skin, and the Sinclair and Yucatan breeds may be procured in a spotted variety in which both types of skin are available on the same animal. Additionally, miniswine and humans have similar body surface areas, making miniswine more comparable to humans than smaller animals such as rodents.

While humans, NHPs and swine all have fixed skin that is adherent to underlying structures, rodents – rats, mice and guinea pigs – have loose, poorly adherent skin. This “tightness” of skin is related to the subcutaneous connective tissue structures and the epidermal–dermal connection. Porcine and human skin is similar in appearance on magnified H&E-stained images. Normal pig skin has been described both microscopically and ultrastructurally. The histologically evident layers of miniswine skin and underpinnings are presented in Table 3. The cellular epidermis is similar in thickness, melanin distribution appears the same, and the epidermis undulates with many rete pegs projecting inward and correspondent dermal ridges projecting upward. The collagen of both swine and human dermis appears glassy and eosinophilic and has a compact criss-crossed collagen meshwork. In contrast, the loose-skinned species have much thinner epidermis and fewer rete pegs. The dermis of loose-skinned species is also less eosinophilic and the collagen appears less dense.

Hair follicles and sebaceous glands have been recognized as important pathways for percutaneous penetration of topically applied lipophilic drugs via the pilosebaceous route. Increased hair follicle density (Tables 4 and 5), as is the case with most animal models outside of humans and swine, can to a degree influence skin drug absorption for selected compounds with unique chemistry. In a comparison across species, it was found that NHP skin tends to have relatively many hair follicles while the mouse appears to have the most hair follicles. Hairless does not mean there are no follicles, though; the hairless rat has a follicle density of
The skin of the pig is thicker and somewhat less vascular than human skin. The skin thickness is especially pronounced on the dorsal surface of the neck and back of sexually mature animals and particularly in some breeds such as the Yucatan. The thinnest skin is located on the ventral abdomen and pinnae. There are approximately 60–75 capillary loops/mm² in human and pig skin and approximately 0.7 m of blood vessel length/cm². Blood flow varies by anatomical region and is highest in the ventral abdomen at approximately 18 ml/min/100 g and lowest in the dorsum and buttocks at approximately 3 ml/min/100 g. The capillaries are more involved with body temperature regulation than in humans but otherwise they perform the same essential functions of nutrient transport, waste products removal and blood pressure regulation. The cutaneous blood supply and sequence of events in wound healing are similar to that in humans.

Table 4. Comparative pelage: hair follicle density.

<table>
<thead>
<tr>
<th>Species</th>
<th>Area of skin</th>
<th># hair follicles/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>Abdomen</td>
<td>11 ± 1</td>
</tr>
<tr>
<td>Pig</td>
<td>Back</td>
<td>11 ± 1</td>
</tr>
<tr>
<td>Rat</td>
<td>Back</td>
<td>289 ± 21</td>
</tr>
<tr>
<td>Mouse</td>
<td>Back</td>
<td>658 ± 38</td>
</tr>
<tr>
<td>Hairless mouse</td>
<td>Back</td>
<td>75 ± 6</td>
</tr>
</tbody>
</table>

75 per cm² and the nude guinea pig has many large empty hair follicles evident as well. Humans and miniswine have less hair than most animals, approximately 11 follicles per cm², therefore follicular uptake is less significant. In miniswine, the range of hair counts or follicle density per unit area does not vary much across lineages or from human counts, thus it is not a major contributor to significant differences in percutaneous absorption. Additionally, the interfollicular area predominates over the follicular area by several-fold for both swine and humans. The human follicular area is typically only 0.1% of the total skin surface area, but the follicular area on the face and scalp can be 10% of the total face and scalp surface area. Finally, enhanced follicular uptake of highly lipid soluble drugs is a unique prospect applicable to only a few drug classes. Swine hair follicles are typically larger than those of humans (177 μm versus 70 μm) but this has minimal, if any, effect on uptake. Swine follicles are reported to be no more penetrable than the epidermis in general.

Sebaceous glands as well as apocrine and eccrine sweat glands of swine have differences in function, number and location from those of humans. In pigs, apocrine sweat glands are extensive but do not significantly contribute to sweating or thermoregulatory functions, and eccrine sweat glands are limited to the snout and carpal glands. There is also a mental gland on the ventral chin which is a mass of apocrine and sebaceous glands with tactile hairs. The secretions onto the gland on the ventral chin which is a mass of apocrine and sebaceous glands with tactile hairs. The secretions onto the gland are exocytosis into the intercellular space. Hydrolytic enzymes interact with the lipids resulting in an intercellular lipid matrix. This lipid matrix between keratinized cells is both the epidermis–dermal junction. Six of these cross react with those of humans: laminin, type IV collagen, fibronectin, GB3, BP and EBA. The remaining two, L3d and 19-DEJ-1 do not. This structure is ultrastructurally similar to humans and invaginates into the dermis with epidermal pegs and dermal papilla. In addition to

Table 5. Human versus swine epidermal turnover, pH and skin hair density.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Human</th>
<th>Swine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epidermal turnover (days)</td>
<td>27–28</td>
<td>~30</td>
</tr>
<tr>
<td>Epidermal pH</td>
<td>5</td>
<td>6–7</td>
</tr>
<tr>
<td>Hair density (per cm²)</td>
<td>11</td>
<td>11</td>
</tr>
</tbody>
</table>

In swine, the SC contains approximately 15 layers of keratinized stratified squamous epithelium which is continuously desquamated. Beneath it is the thin translucent stratum lucidum with keratinized cells devoid of nuclei. This layer contains protein-bound phospholipids and eleidin, which is especially abundant in thick, hairless skin. The stratum granulosum is the next layer and consists of cells containing keratohyalin and lamellar granules that release lipid by exocytosis into the intercellular space. Hydrolytic enzymes interact with the lipids resulting in an intercellular lipid matrix. This lipid matrix between keratinized cells is both the primary barrier against and the pathway for penetration of topical drugs. The physical properties of this matrix contribute to the similarities of transdermal penetration in swine to humans. The stratum spinosum is immediately below this layer, then the stratum basale which consists of columnar or cuboidal cells that have the dual function of attaching to the dermis and producing new epidermal cells. There are approximately four viable cell layers in the stratum basale of swine. Non-keratinocytes present in the epidermis are melanocytes, Merkel cells and Langerhans cells. The epidermal–dermal junction provides the basement membrane to the epidermal cells and the connection to the dermis. There are eight separate antigenic epitopes in this structure. Six of these cross react with those of humans: laminin, type IV collagen, fibronectin, GB3, BP and EBA. The remaining two, L3d and 19-DEJ-1 do not. This structure is ultrastructurally similar to humans and invaginates into the dermis with epidermal pegs and dermal papilla. In addition to
its adherence and maintenance functions, this structure is also a selective barrier for molecular restriction and transport, wound healing, and immunological function\textsuperscript{5,13,14}.

The dermis, or corium, is composed of dense irregular connective tissue containing elastic, collagen and reticular fibers in amorphous ground substance. This layer contains numerous cellular and adnexal structures. Commonly found cells are fibroblasts, mast cells, plasma cells, macrophages, chromatophores and fat cells. Blood vessels, lymphatics and nerves are predominantly located in this layer, as well as sweat glands, sebaceous glands, and hair follicles with their associated intrafollicular and arrector pili muscles\textsuperscript{5,13,14}.

The hypodermis, or subcutis, is a layer of loose connective tissue which produces fascia and elastic fibers connecting the skin to muscle. The subcutaneous fat, or panniculus adiposus, is located in this region. In boars, this layer becomes traversed with collagen fibers which provide a protective shield on the dorsolateral aspects of the body\textsuperscript{5,13,14}.

In summary, the dermal anatomic and physiologic similarities between pigs and humans include a sparse hair coat, a relatively thick epidermis, epidermal turnover kinetics, lipid composition, lipid biophysical properties, and arrangement of dermal collagen and elastic fibers. The differences are the interfollicular muscle, the distribution and function of apocrine versus eccrine sweat glands, thickness of the SC, the basement membrane epipithelium, and Cytochrome P-450 bio-transformation isoenzymes\textsuperscript{3,5,6,24}. Also, miniature swine offer advantages over domestic pigs for preclinical applications, considerations of which are presented in Table 1\textsuperscript{2}.

Distribution of skin thickness

Full-thickness skin is composed of the SC, cellular epidermis and dermis. It is well recognized that skin thickness varies from region to region of the body and the density of hair follicles varies greatly by region as well\textsuperscript{18}. The Yucatan miniswine is no exception, as demonstrated in a study evaluating body surface sites for 18 animals including neck, back, flank and abdomen. The dermis made up the majority of the thickness of Yucatan skin (92–99%). The Yucatan epidermis (stratum corneum plus cellular epidermis) made up 1.0–8.0% of full-thickness skin. The full-thickness epidermis ranged from 62.36 μm on a 10.3-month-old castrated male flank to 134.15 μm on the neck of this same castrated male. Group mean full-thickness skin ranged from 955.83 μm (0.95 mm) on the back of 3.5-week-old females to 5666.32 μm (5.6 mm) on the neck of a 10.3-month-old castrated male. Cellular epidermis thickness ranged from 32.80 μm on the abdomen to 140.93 μm on the flank. SC thickness ranged from 6.23 μm on the abdomen to 88.13 μm on the neck. Dermis thickness ranged from 587.29 μm on the abdomen to 6741.67 μm on the neck.

The thickness of the SC and epidermis plays a significant role in affecting absorption of percutaneously applied drugs as the near surface vascular supply underlies the epidermis between the epidermal rete pegs in the uppermost dermis. The thickness of the SC influences the resistance of the skin to physical and chemical trauma and potentially to transdermal drug delivery. The thickness of the dermis may also have an impact on dermal absorption as the dermis depths contain hair follicles, apocrine sweat glands, and the subcutaneous layer of adipose, blood vessels, nerves, and the beginnings of skeletal muscle.

Background histopathology

As shown in Table 6, inflammation and mononuclear infiltrates were the most common incidental background findings across the four most common miniswine breeds in the US: Göttingen, Yucatan, Hanford and Sinclair. Yucatan miniswine exhibited mononuclear infiltrates in 5.6% of the animals. The Hanford strain exhibited a variety of types of inflammation, ranging from acute to chronic dermatitis in 13.5% of the animals to chronic perifollicular inflammation and multifocal lymphohistiocytic inflammation, each in just under 2% of the tested population. The Göttingen breed had a broader range of findings, including crusts (9.1%), hyperkeratosis and parakeratosis (4.9%), and epidermal and subepidermal edema (6.3%). They also had mononuclear and inflammatory cells present in 13.3% of the population.

Dermal toxicity models

The landscape of dermal study models and applications is expanding, and Hanford, Sinclair, and Yucatan miniswine are each utilized in some aspect of dermal toxicity testing or dermal research\textsuperscript{3,3,6,24}. Below are selected model descriptions.

Dermal toxicity

In light of the morphological and physiological similarities between human and porcine skin that exceeds other laboratory animal species, the miniature pig is a preferred model for evaluating the safety profile of dermally applied xenobiotics. Swine as a model in toxicity testing of pharmaceuticals and other chemicals is now being well-accepted by Japan, EU, Canada and USA regulatory agencies. Miniswine are also an accepted second species for GLP toxicity/safety assessment\textsuperscript{25}; the OECD 409 Guideline even lists swine and minipigs as options for the second non-rodent species in toxicology testing. In most cases, swine should be selected as a primary species over dogs and rabbits as a dermal toxicity model\textsuperscript{16}. Young adult, 3–6-month-old Hanford miniswine are most commonly used. Dermal studies in miniswine allow the evaluation of both local and systemic toxicity, and miniswine normal reference data is readily available.

Transdermal absorption

In general, the pig is accepted as an appropriate model for topical agent testing and skin penetrance is second only to macaques in its similarity to humans for both lipophilic and hydrophilic drugs.

Swine permeability is higher than pigs for most compounds tested\textsuperscript{27}, but miniature swine are still a recognized predictive model for human drug candidate dermato-pharmacology studies\textsuperscript{28}.

Penetration of the skin by topical agents may be due to intercellular, pore, interfollicular and/or skin breaks pathways.
The lipid matrix within the stratum granulosum also affects penetrability. Absorption may be variable depending upon temperature, humidity, the skin condition, the surface area of the application, the location on the skin and whether the area is covered or uncovered. The properties of the agent and its vehicle are also important. Sometimes transdermal drug delivery also includes the technique of iontophoresis, which involves the use of electrical current to enhance penetration of drugs that ordinarily would not be permeable. With this technique, charged drugs are transported after applying an opposing electrical field. The pig ear has been used as a predictive model because it is relatively thin and highly vascularized, but transdermal absorption has been performed on the ventral abdomen and dorsum as well. In the caudal ventral abdomen, there is opportunity to study absorption in areas of direct cutaneous blood supply in the region of the last nipples versus the musculocutaneous blood supply cranial to that region.

Body surface area

Dermal maximum tolerated dose (MTD or dose escalation) studies are common in miniswine. Their dermal surface area is adequate for these studies, and changes during growth have been investigated to determine the potential effect upon the dose per unit surface area and dose per unit body weight. Several methods for calculating body surface area of domestic swine have been published, including those by Spector and Wachtel et al. The Spector method is highly respected, thus it was selected and applied to miniswine in a study where the correlation of the three TBSA calculation methods was evaluated. The correlation of the Spector and Wachtel methods was 0.99; all three methods were comparable with 10% difference. No actual TBSA reference value or skin area measurements were available to ascertain accuracy and precision of the formulas.

Skin stripping technique for dermal penetration

Tape stripping is a simple and effective method for removing the SC and is commonly employed during in vivo studies investigating the percutaneous penetration and disposition of topically applied candidate drugs. Skin can be prepared by washing it with gentle detergent; it can also be prepared by adhesive tape stripping to reduce the thickness of the SC for dermal penetration studies. One study was performed with the objective to assess the remaining thickness of the SC following 0, 10, 20, 30, 40 and 50 repetitions of tape stripping of skin on three young adult, male Yucatan miniature swine weighing 33–36 kg. Following clipping of the pelage over the dorsal lumbar and thoracic areas, six 5 cm by 5 cm sites were demarcated and skin was stripped using 1.8 mm clear acrylic adhesive tape applied with uniform, firm pressure. The results of analysis by light microscopy showed an inverse pattern of SC thickness to the number of tape stripping repetitions. After 20 stripplings, the number of layers was reduced from 11–15 down to 2–6 and 50 passes were required to remove nearly all SC. No immediately
detectable underlying changes of the epidermis or dermis were observed. These data demonstrate that skin can be stripped of SC in a linear fashion based upon repetition of the technique.

Clinical evaluation of topical reactions

Draize scoring, developed by Draize\textsuperscript{33}, provides a method of assessing the degree of inflammation based on quantifying the values that are at risk of interpretation bias or being considered insignificant. Originally developed for use in rabbits, it has been modified for use in both swine and humans, thus is often referred to as the Modified Draize Score. In dermal studies, the values that need quantified analysis are erythema and edema. Erythema and edema are each graded on a scale ranging from 0 to 4. For edema this ranges from “no edema” at a score of 0 to “severe edema raised >1 mm and extending beyond the area of exposure” at a grade of 4 and for erythema this ranges from “no erythema” to “severe erythema or slight eschar formation”. The grades 1, 2 and 3 for each value are slight, well-defined and moderate or severe, respectively.

Table 7. Dermal vehicles and their tolerability when applied to miniswine\textsuperscript{a}.

<table>
<thead>
<tr>
<th>Vehicle</th>
<th>Duration (days)</th>
<th>Tolerability</th>
<th>Percent affected</th>
<th>Pathology</th>
<th>Number of animals</th>
<th>Breed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dehydrated ethanol 200 Proof; Hexylene glycol; Dimethiconol blend 20; Hydroxypropylcellulose; Anhydrous citric acid Purified water; NaCl, KCl, L-arginine HCl, NaOH; Emollients, emulsifiers and thickening agents</td>
<td>28</td>
<td>Well-tolerated</td>
<td>20%</td>
<td>Normal</td>
<td>10</td>
<td>Sinclair</td>
</tr>
<tr>
<td>Purified water; Denatured alcohol 190-proof; Propylene glycol; NaOH solution; Phenoxethanol; Emollients, emulsifiers, and thickening agents</td>
<td>7</td>
<td>Severe persistent dose-site erythema beginning on day 2</td>
<td>33%</td>
<td>Three with chronic-active superficial dermis inflammation, one with chronic subcutis inflammation as well; two had bacterial infection, grade 1–3 hyperkeratosis and surface exudate</td>
<td>6</td>
<td>Sinclair</td>
</tr>
<tr>
<td>Methyl- and Propylparaben 50:50 Ethanol:Propylene glycol; BHA and BHT</td>
<td>91</td>
<td>Well-tolerated</td>
<td>0%</td>
<td>Normal</td>
<td>12</td>
<td>Hanford</td>
</tr>
<tr>
<td>Gelatin phosphate buffer</td>
<td>28</td>
<td>Slight erythema, resolved</td>
<td>10%</td>
<td>Skin biopsies: mononuclear infiltrates</td>
<td>10</td>
<td>Hanford</td>
</tr>
<tr>
<td>Purified water; Denatured alcohol 190-proof; Propylene glycol; NaOH solution; Phenoxethanol; Emollients, emulsifiers, and thickening agents</td>
<td>210</td>
<td>Well-tolerated</td>
<td>0%</td>
<td>Not performed</td>
<td>10</td>
<td>Hanford</td>
</tr>
<tr>
<td>PEG 400</td>
<td>90</td>
<td>Mild erythema and edema beginning after 40 days, slight to severe papules and pustules beginning after day 30</td>
<td>50% and 83%</td>
<td>Normal</td>
<td>10</td>
<td>Hanford</td>
</tr>
<tr>
<td>Soybean, coconut, and mineral oil; Beeswax; Stearic acid; Emollients, emulsifiers, and thickening agents</td>
<td>21</td>
<td>Mild edema pre- and post-dose 2 separate occasions</td>
<td>10%</td>
<td>Mild dose-site granulomatous infiltrate</td>
<td>10</td>
<td>Hanford</td>
</tr>
<tr>
<td>Purified water; Olive oil; Shea butter; Emollients, emulsifiers, and thickening agents; Methyl- and propylparaben</td>
<td>14</td>
<td>One male with persistent erythema/edema, then miliary erythema and exudate, one female with slight to moderate miliary erythema beginning day 7</td>
<td>20%</td>
<td>Chronic dermis inflammation at dose site</td>
<td>10</td>
<td>Hanford</td>
</tr>
<tr>
<td>Ethanol 190 proof; Deionized water; Glycerin; Propylene glycol; Salicylic acid; EDTA; Emollients, emulsifiers, and thickening agents Water; Propylene glycol; Ethanol 200 proof; NaOH solution; Emollients, emulsifiers, and thickening agents Phenoxethanol</td>
<td>92</td>
<td>Mild persistent irritation</td>
<td>10%</td>
<td>Normal</td>
<td>10</td>
<td>Hanford</td>
</tr>
<tr>
<td>Water; Propylene glycol; Ethanol 200 proof; NaOH solution; Emollients, emulsifiers, and thickening agents</td>
<td>91</td>
<td>Intermittent dose-site erythema, varied occurrence and severity</td>
<td>50%</td>
<td>Mild superficial dermis inflammation</td>
<td>10</td>
<td>Hanford</td>
</tr>
</tbody>
</table>

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\textsuperscript{a} Hanford miniswine, \(7\) studies selected for this report, nine were performed on Hanford miniswine, two were performed on Sinclair miniswine and no specific vehicle was used more than once. As shown in Table 7, vehicles that resulted in no adverse
response during treatment or histologic pathology include a combination of dehydrated ethanol, hexylene glycol, dimethicone blend 20, hydroxypropylcellulose and anhydrous citric acid. Methyparaben and propylparaben were also well-tolerated. Water, polawax, denatured alcohol, propylene glycol, isopropyl myristate, sodium hydroxide solution, phenoxyethanol and carbomer 974P was a combination that was well-tolerated for 210 days, but adequate comparison to the other vehicles is not possible since histopathology was not performed. Other vehicles resulted in mild, transient responses. For example, a 50:50 composition of ethanol and propylene glycol with very small amounts of BHA and BHT produced very mild erythema in eight of the 12 animals on the first day of treatment, but this was resolved by the next observation seven days later in all but one animal. A vehicle of soybean oil, coconut oil, mineral oil, cyclomethicone, cetostearyl alcohol, stearic acid, myristyl alcohol, beeswax, stearyl alcohol, and docosanol resulted in mild pre- and post-dose edema in one animal on two separate occasions, and mild granulomatous infiltrate in one of 10 animals on histopathology. A vehicle made of gelatin phosphate buffer resulted in slight erythema in one of 10 animals on day 2 that was resolved by day 3. This particular study analyzed periodic dose-site punch biopsies; three of the animals had mononuclear infiltrates on histopathology from various days throughout the study.

Other vehicles were observed to cause decidedly negative side effects. A vehicle of purified water, sodium chloride, potassium chloride, L-arginine, glyceryl stearate, cetyl alcohol, propylene glycol, squalene, polysorbate 20, sodium hydroxide, and stearyl alcohol, resulted in mild erythema in eight of the 12 animals on first day of treatment, but this was resolved by the next day. PEG 400 caused adverse effects after 30 days of treatment in over half of the animals; these effects consisted of erythema and edema and slight to moderate, occasionally severe, papules and pustules. Interestingly enough, histopathology revealed no abnormal findings.

Summary
Swine as a model in toxicity testing of pharmaceuticals and other chemicals is now being well-accepted by Japan, EU and USA regulatory agencies. Swine are specifically mentioned as a potential non-rodent species in the guidelines of Japan and Canada and would generally be considered superior to dogs and rabbits as a dermal model. The OECD 409 guideline lists swine and minipigs as additional species. However, evidence should be provided that it is a suitable species in order to overcome residual regulatory resistance. Increases in the amount of background information on this species will continue to demonstrate their usefulness in toxicology in general and specifically as a dermal model.\(^{2,3,6,11,24,34}\)

Declaration of interest
The authors report no conflicts of interest.

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