

# The Use of the Minipig in the Safety Evaluation of Topical Drug Products

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## Introduction

Regulatory safety assessment of pharmaceuticals requires the use of a rodent and non-rodent species. For a variety of reasons that are predominantly ones of availability, familiarity and history, the choice of the non-rodent in the past has been confined to the dog or non-human primate. However, there is now a clear realisation that selection of an appropriate non-rodent species can be one of the most important aspects in the design of the non-clinical development programme. Selection of a test species with close similarity to man in respect of absorption, metabolism and relevant physiological systems and anatomical structures is required to optimise understanding and development of a potential new drug. In the development of a topical dermal product, the close anatomical, physiological and biochemical resemblance of porcine skin to human skin makes the minipig the most suitable non-rodent model in dermal toxicology.

## The Minipig

The minipig strains that are used today in research and safety assessment were developed out of a desire to have a smaller and more manageable version of the domestic pig. Strains of miniature pigs have been developed in the USA, including the widely used Yucatan (mini and micropigs), Sinclair and Hanford breeds. In Europe, the Göttingen minipig is the most popular breed used by pharmaceutical companies and contract research organisations.

## Physiology and Anatomy of Human and Porcine Skin

As with all animal models there are both similarities and differences between pigs and humans.

### Morphological and Functional Similarities of Porcine Skin to Human Skin

- Non-pigmented skin with sparsely haired coat
- Thick epidermis (see Tables 1 and 2)
- General morphology, cellular composition and turnover rate approximately 28-30 days
- Immunological reactivity
- Enzyme pattern of the epidermis
- Skin penetration of compounds

Species	Epidermis (µm)	Stratum corneum (µm)	Overall epidermal thickness (µm)
Mouse	13.32	2.90	
Rat	21.66	5.00	10-20
Rabbit	10.85	6.56	
Dog	21.16	5.56	
Monkey	26.87	12.05	
Minipig	51.89	12.28	70-140
Human	46.90	16.80	70-120

Table 1. Thickness (at the back) of epidermal and stratum corneum layers of commonly used species in safety evaluation studies<sup>1</sup>

Species	Age (months)	Epidermal (µm)	Dermis (mm)
Minipig	1.5	50	1.15
Minipig	3	64	1.47
Minipig	6	63	2.28
Human	Adult	70	1.20

Table 2. Thickness of epidermal and dermis of developing minipig compared with man<sup>2</sup>

### Morphological and Functional Differences between Porcine Skin and Human Skin

- Thickened hyperkeratotic stratum corneum
- Poor vascularisation of the cutaneous glands
- Absence of eccrine cutaneous glands in the common integument
- Apocrine skin glands (not involved in thermoregulation)
- Extensive deposition of fat below the subcutis (see Figure 1)
- Seasonally regulated shedding of hair
- Differences in microenvironmental skin conditions (pH value of 6-7 compared with 5 in man)

## Challenges in Dermal Minipig Studies

- Animal welfare/husbandry/environmental conditions
  - Skin irritation and sensitisation potential investigated in other species
  - Termination of treatment due to painful skin lesions or severe systemic toxicity
  - Thorough prestudy examinations to exclude animals with skin lesions or abnormalities
  - Avoidance of skin injury by individual housing, clipping of hair, rigorous cleaning of skin
  - Variability in the sensitivity to dermal effects (age, skin condition, skin type/secretions, skin thickness, blood circulation)
  - Standard environmental conditions to avoid changes in skin microcirculation in connection with thermoregulation
- Test Item Formulations
  - Dermatitis following treatment with creams or ointments
  - Skin penetration and systemic toxicity affected by formulation
- Administration procedures
  - Selection of the most appropriate method, risks with long-term occlusive application
- Examination procedures
  - Blood and tissue sampling
  - Skin biopsies with risk of inflammation/infection
  - Marking of altered skin areas before euthanasia
- Histopathological examination
  - Non-specific dermatitis as background lesion in about 30% of the minipigs
  - High inter-individual variability in severity of dermal effects

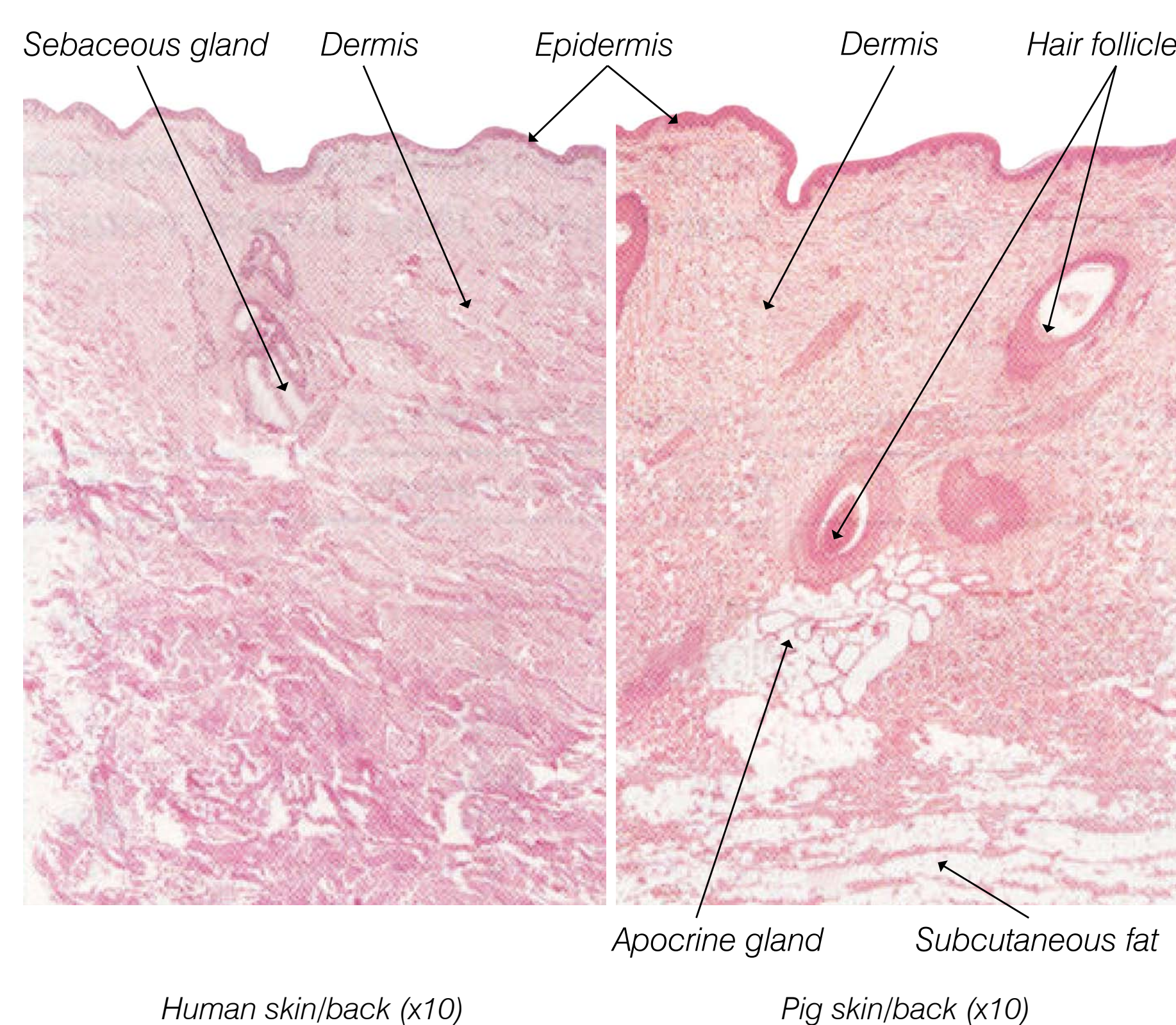


Figure 1. Comparison of porcine and human skin<sup>3</sup>

The skin of minipigs has a similar anatomical structure to human skin, although differences exist. Pigs have apocrine glands associated with hair follicles, whereas humans have sebaceous glands. Pigs have more subcutaneous fat, but minipigs between 3 and 6 months of age have a similar skin thickness and approximately a similar amount of subcutaneous fat to man. Older pigs have significantly more fat than the average human.

## Minipig Dermal Dosing Studies

- Administration of the test substance as a cream, gel, ointment or transdermal patch to the minipig is straightforward and there is no problem in relation to repeat administration up to 12 months. The test substance is readily applied to a designated dosing site of a fixed area, usually on the flank, for a pre-determined number of hours each day.
- The volume of material applied to the dosing area is varied in order to establish a dosage-relationship so that findings can be used to provide human dose selection.
- The dosing area is usually a maximum of approximately 10% of the animal's surface area determined by various mathematical formulae for calculating the animal's surface area based on its body weight.

- The nature of a formulation can influence the quantities applied. Gels are normally rapidly absorbed and are easy to apply. Ointments on the other hand tend to be thick and poorly absorbed and, while they can be applied in large amounts as thick layers, most applied material fails to come into contact with the animal. Liquids are difficult to apply as they tend to run-off and care is required to evenly distribute the material over the dosing area.
- The skin test site can be left open, semi-occluded using gauze or fully occluded with plastic dressing. The local skin reaction at the site of application can be visually assessed and graded according to the common parameters - erythema, oedema, scaling, pigmentation changes, skin erosion or ulceration, etc.
- Clinical signs, body weight and food consumption assessments, physiological examinations (ophthalmoscopy and electrocardiography), blood and urine samples for clinical pathology as well as gross and histopathological procedures, follow the same routines as in other laboratory animals.

## Regulatory Acceptance

- The minipig is fully accepted as an alternative non-rodent species in Europe, Japan and the USA
- Specifically mentioned as potential non-rodent species in guidelines from Japan and Canada and would be generally considered superior to dogs and rabbits as a dermal model
- OECD Test Guideline 409 lists swine and minipig as optional species
- Increases in the amount of minipig background information will continue to demonstrate their usefulness in toxicology and specifically as a dermal model
- Evidence should be provided that the minipig is a suitable species – favourable metabolic or pharmacokinetic profiles for the test substance or close resemblance between a main target organ in the minipig and man e.g. the skin

## Conclusion

The pig is a well recognised animal model for dermal toxicology. The close anatomical, biochemical and physiological similarities of porcine skin to human skin make the minipig a favourable non-rodent species in the efficacy and safety assessment of dermal topical products. However, there are specific differences in the anatomy and physiology of the skin of the two species that should be considered, as these may affect the penetration and/or absorption of the test substance. Absorption may be variable depending on the location of application, skin condition, temperature and humidity, surface area and drug concentration, occlusion and vehicle. The minipig is becoming widespread in its use by pharmaceutical companies and contract research organisations for routine and non-routine studies when scientifically appropriate.

## References

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