

# Minipigs as models for the toxicity testing of new medicines and chemicals: impact assessment

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## Ethics & Welfare

### Ethics

Minipigs cannot be considered as “more acceptable” than dogs or monkeys either on grounds of lesser sentience or on the basis of a popular belief that food animals somehow matter less than pets or primates. The capacity of an animal to experience suffering must be defined in terms of its own sentience, not its status in human society. Similarly, it is not possible to generalise as to the potential of the minipig as a subject for studies designed to improve the implementation of the “three R’s”. However one would encourage any sound proposals for specific developments in this regard.

### Recommendations

- Codes of practice on minipig husbandry, care and procedures
- Mandatory training specific to the species of technical staff
- Recognise early signs of physical or mental suffering, and take appropriate actions
- To communicate any animal welfare concerns to those whose prime responsibility is the successful outcome of the trials to ensure that the welfare of the animals is not unduly compromised.

### Welfare

Minipigs have been selectively bred for small size, light skin pigmentation and docility. All the while it is assumed that the motivations and consequent behavioural needs have remained little changed from their wild ancestors. Overall studies on refinement of minipig housing and procedures are few, and hence any extrapolation from farm pig

studies contains some uncertainty, the same holds true for physiological or behavioural indicators.

Standard handling and dosing program should be adapted in the laboratories, and positive interaction with the minipig is crucial to minimizing stress of both animal caretaker and minipig; hence staff should be well trained in handling this species. It is recommended that all minipigs, with the exception of mature boars, are kept in groups when housed for biomedical research purposes.

Collecting blood samples requires instruction and training as the peripheral blood vessels of the minipig are deeply located in the surrounding tissues. Temporary and chronic vascular catheters can be used for frequent sampling, and are likely to improve welfare.

### Recommendations

- Develop and validate simple welfare assessment tools for minipigs which can be used under practical conditions.
- Define the optimal group size and conditions for group housing
- Investigate whether an individually housed minipig is more stressed than a group-housed pig
- Assess amount and type of bedding/enrichment suitable for minipigs
- Development of neurological tests, together with the definition of humane endpoints.



## Development of chemicals/pharmaceuticals

**Primary Pharmacodynamics.** There are areas of pharmacological research where pigs and minipigs are established research models mainly because of anatomical and/or functional similarities to humans and/or because of availability of disease models.

**Safety Pharmacology.** The minipig is used for invasive and non-invasive cardiovascular safety pharmacology, both anaesthetized and conscious. Regarding the other areas of safety pharmacology little information is available on the minipig.

**Pharmacokinetics.** The minipig is a suitable model for most routes of administration and for evaluation of most ADME endpoints. There are similarities (CYP3A, CYP2E, acetylation activity) between man and pig with regard to biotransformation, but there are also differences (e.g. very low CYP2D and CYP2C19 activity, lack of sulphation for many drugs).

**Toxicology.** The minipig is a long established model for dermal toxicity studies because of the similarities of human and pig skin, but during the last decade an increasing number of studies using other dosing routes are performed. Minipigs are not mentioned as the species of choice, but most guidelines do not specify too much about the choice of species. The use of minipigs can be seen as acceptable for most purposes provided the use of the species is adequately justified.

Drugs where minipigs are of use and with known oversensitivity of the dog for dose-limiting side effects of no or only limited relevance for humans:

- Non-steroidal anti-inflammatory drugs
- Sympathomimetics and anti-hypertensives
- Vitamin D-analogues
- Hormones with oestrogenic activity and antigestagens
- Drugs having an emetic effect in dogs
- Drugs causing histamine liberation in dogs: Some solvents and excipients are known to cause anaphylactic reactions in dogs. Such reactions may cover other side effects and may be dose-limiting and are often not representative for man.

**Food additives.** Pigs have been used extensively for testing food additives, although they are rarely recommended in the guidelines that are applicable.

### Agrochemicals/Biocides /Chemicals/ Cosmetics/Veterinary Pharmaceuticals.

For various reasons there is little or limited opportunity for the minipig to be used to a significant degree within these areas.

### Devices & Human surgical methodology development and medical devices.

Because of its anatomical features, pigs and minipigs have eminent advantages in testing medical devices and surgical methods, especially in the abdominal and thoracic parts of the body.

**Regulatory acceptance.** Despite not being mentioned in guidelines the European Public Assessment reports reflect clearly the application of minipigs in testing pharmaceuticals, in human as well as in veterinary pharmaceuticals. Recent publications of the FDA (two papers and one poster) confirm the acceptability of the minipigs in the pharmaceutical regulatory field.

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## Safety Assessment

For the selection of non-rodent animals for safety evaluations, dogs and primates are the most frequent used models, but the minipig is becoming a more established research model and seems suitable to be used as “most human like” model for the identification and prediction of undesirable effects and risks for humans due to a number of anatomical similarities like structure and function of the skin, cardiovascular system, gastrointestinal tract, renal and urinary and possibly immune system. All routes of administration (oral gavage, dermal, injections, continuous infusion, inhalation, intravitreal) are available and sufficiently described in the literature. There are a number of advantages of the minipigs: Minipigs reach full sexual maturity when used in 13-week studies and approach maturity when used in 4-week studies. Minipig microsomes and hepatocytes for in vitro assays are commercially available and should be routinely included into the screening for metabolic profiling and stability very early on to improve the selection of the most appropriate predictive species for humans.

The minipig is often not chosen as the primary non-rodent species since researchers feel a lack of available background data and technicians are not sufficiently trained to feel “comfortable” with this species. Furthermore there is the fear that regulatory authorities will not accept the data from minipigs with the same ease as they would accept data from dogs.

In order to reflect today’s situation more adequately, an industry questionnaire was circulated by the Rethink experts with the following results.

- 13 of 22 had experience of using the minipig in safety assessment. The experience within each company was variable
- 6 of 22 of companies includes the minipig in the screen for pharmacological activity / metabolic clearance / profiles
- 6 of 22 companies used minipigs as the only non-rodent species
- 5 of 22 companies used minipigs as the second non-rodent species
- 14 of 22 companies perceived hurdles to the use of minipigs as a more general non-rodent species in safety assessment. The principal concerns were due to weight of minipigs; lack of experience with this species, lack of background and historical data, animal husbandry, supply and logistics were also mentioned as concerns. Finally, the lack of reagents and diagnostic kits specific for minipigs was mentioned.
- Reasons for choosing the minipig as the non-rodent of choice included: lack of tolerance of administered compounds to dogs, hereby vomiting was a jeopardizing reaction, also high counter reaction of the cardiovascular system by dogs with compounds of blood pressure lowering potential; there were major differences in metabolism in those species selected first for testing and accordingly search for higher similarity to humans, further there were special applications like dermal administrations.

In Summary: all animal models have their pros and cons. Most important is the search for the most human like model to improve the precision of predictivity of adverse events in humans. The minipig offers such an option for many substances adequately.

## Genomics and emerging technologies

The Göttingen minipig is a genetically managed model unlike the dog and monkey toxicology models. The basis of the small size of the Göttingen minipig does not involve defective genes.

Commercial interests in the pig as an agricultural production species have driven the area of pig genomics. There is no equivalent economic driver for progress in the dog or the monkey. Fundamental genomic knowledge about the pig is well in advance of the dog or the monkey. Whole genome studies show a closer sequence homology with humans than rodents.

In the area of genetic manipulation, current technical possibilities are well in advance of dogs and monkeys. There is a lack of in vitro reproductive technology in the dog.

In the area of immunology, current technical possibilities are well in advance of dogs and monkeys. Nevertheless, it is unlikely that the minipig can offer a more relevant model than the monkey for the evaluation of new immune based therapies

**Application of 3Rs.** Careful genetic management and opportunities for improvement of minipig as a model, and better models give more pertinent testing and less wasteful use of animal resources

- Gaps and opportunities.** Breed minipigs for smoother temperament
- Development of drug induced gene expression signatures in pig
  - Areas ripe for rapid progress in understanding are functional genomics and isolation of minipig ES cells
  - Development of humanised minipig models
  - Evaluate cross reactivity of minipig to panel of commercialised biologics

**Overall.** Emerging technologies are essentially “species neutral” but in all the technologies that we examined, basic knowledge and technical capabilities are greater for the pig than the dog or monkey. We expect this situation to continue in the future.

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