Journal of Pharmacological and Toxicological Methods 62 (2010) 184–195

Contents lists available at ScienceDirect

Journal of Pharmacological and Toxicological Methods

journal homepage: www.elsevier.com/locate/jpharmtox

Original article

Regulatory acceptability of the minipig in the development of pharmaceuticals, chemicals and other products☆,☆☆

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ARTICLE INFO

Article history:
Received 30 December 2009
Accepted 24 May 2010

Keywords:
Minipigs
Pharmaceuticals
Food additives
Biocides
Agrochemicals
Medical devices

ABSTRACT

As part of the *RETHINK* European FP6 Project an overview of the acceptability and usefulness of minipigs has been carried out in the regulatory arenas of human and veterinary pharmaceuticals, food additives, cosmetics, biocides and agrochemicals, chemicals and medical devices.
The safety of new pharmaceuticals for human use should be tested in non-rodents, but the regulatory world is not too prescriptive regarding the choice of species. The choice is most often dogs through long tradition. When dogs are not appropriate, in many cases non-human primates are chosen as an alternative.
From information in the public domain as well as literature from the EMA and FDA, it is clear that minipigs have already been identified as suitable to take the role of non-rodent species in toxicity testing of pharmaceutical products.
In the field of foodstuffs, the pig is used more extensively because of the apparent similarity in the omnivorous food pattern and digestive tract between humans and pigs. The extensive use of pigs in this field provides historical data.
In the field of medical devices the ISO Guidelines indicate that the pig is regarded as a suitable animal model because of its haematological and cardiovascular similarities to man. The pig is also mentioned as suitable for testing local effects after implantation.
Political and societal support for using nonhuman primates is decreasing, and it is an appropriate time to consider the role of the minipig.
We have reviewed the costs of testing in minipigs, and these are not significantly higher than the costs for a study in dogs. Economical reasons should therefore not be used to argue against the use of minipigs instead of dogs or monkeys.
For most purposes, minipigs may be considered an acceptable choice as non-rodent species, provided adequate justification for this choice is made.

1. Introduction

In this article we review regulatory issues relating to the use of minipigs in regulatory toxicity testing, as a contribution to the *RETHINK* project (Forster et al., 2010a,b-this issue).

The safety testing of medical devices, pharmaceuticals and other chemicals in animals is subject to regulation by national and international bodies. A large number of published guidelines make recommendations regarding the scope of testing and the methods to be used for each specific type of product. Some of these guidelines also recommend which animal species should be used. In certain cases there is a degree of harmonisation between the testing requirements and recommendations of different countries and regions. However, national and regional differences still exist in the testing requirements for certain categories of product.

The recommendations for the use of non-rodent species in animal testing have been reviewed. The major categories of product requiring such testing are dealt with in separate sections. These major categories

☆ The opinions expressed in this paper are those of the authors and not necessarily those of the organizations they represent.
☆☆ This work was supported by the *RETHINK* project, a Specific Support Action funded by the European Community 6th Framework Programme under contract no.: PL01877.
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doi:10.1016/j.vascn.2010.05.005

include pharmaceuticals, food additives, agrochemicals, biocides and medical devices. Categories where testing in non-rodents, apart from rabbits, is unusual or not required are covered in a separate section. These categories include chemicals in general, cosmetics and household products. A short section on development of surgical procedures is also included.

In conducting this review, consideration has been given to the requirements for the scope of testing as described by governmental, regulatory and advisory bodies such as EMA, EU, EPA, FDA, and related organisational frameworks ICH and REACH.[2] The detailed requirements for study design, including animal numbers and species, as published by EPA, FDA, OECD[3] and others, have also been examined and reviewed, particularly for recommendations for the use of non-rodent species.

The primary purpose of this review was to identify instances where the choice of species for non-rodent animal testing has been restricted in some way. The justification, if any, for these restrictions has been examined and evaluated from both a scientific and a practical point of view.

In the final section of this article, some actions are proposed to allow a more scientific and ethical approach to the choice of non-rodent species for use in animal testing.

2. Pharmaceuticals

In the early phase of development, lead identification and optimisation consists of pharmacodynamic characterisation and early safety screening for a large number of compounds. The needs for a high capacity for high throughput testing, fast availability of results as well as minimised compound needs are dominating factors for selection of test systems. This allows a large variety of test systems and experimental designs to be used.

For the later phases of drug development with detailed characterisation of preclinical safety for a small number of drug candidates, standardised test systems have been developed over time. These test systems allow evaluation of specific endpoints of safety pharmacology, pharmacokinetics and toxicology. Regulatory bodies have issued very specific guidelines defining the requirements to be fulfilled in this later phase. In addition, the synthesis and manufacturing process of the drug substance generally includes several up-scaling steps during the course of development because of higher compound needs for the various studies and as part of the preparation for market supply. Thus, larger amounts of drug substance are available and the costs of the drug are reduced over time. This has a major impact on the technical feasibility of the studies and on costs.

2.1. Primary pharmacodynamics

Characterisation of primary pharmacodynamics includes *in vivo* pharmacology studies in healthy animals as well as in disease models. Species selection is mainly driven by the availability of a suitable therapeutic model, in which the drug shows the intended pharmacological activity.

- **Species selection:** Rodents, especially mice and rats, are generally the species of first choice for the development of disease models for a variety of reasons: small size, low costs and simple housing conditions, short reproductive cycle facilitating breeding of strains with spontaneous mutations and spontaneous defects (such as immune-suppressed animals and spontaneously hypertensive rats). More recently, the development of genetically engineered strains, such as transgenic and knock-out strains, have enabled tailor-made pharmacodynamic models to be developed, allowing very specific studies of the modes of action of drugs to be performed. In addition, the short life span of rodents facilitates fast development of chronic changes (e. g. age-related and dietary induced diseases).
- **Non-rodent use:** The purpose of the use of non-rodents in addition to rodents is in most cases for confirmatory experiments to facilitate extrapolation of the experimental results to man. Furthermore, for some indications, where no suitable rodent model is available, the use of non-rodents is important as an alternative animal model.
  When pharmacodynamic activity is expected in a multitude of species and no specific disease model is needed, the non-rodent species is generally selected according to low weight and easy availability (e.g. rabbit).
  For pharmacodynamic targets with highly species-specific expression or function, which is often found for biologics and the new generation of targeted therapies designed for high selectivity for receptor or kinase subtype interaction, non-human primates such as the marmoset or the various macaque species such as rhesus and cynomolgus monkey are commonly used, as there is a high likelihood that they will express the target in a similar way to man.
- **Use of minipigs:** There are some areas of pharmacological research where pigs and minipigs are established research models mainly because of anatomical similarities to humans (e.g. body size, skin, cardiovascular system, and urinary system), because of functional similarities (gastrointestinal system and immune system) or because of availability of disease models (e.g. arteriosclerosis, metabolic syndrome, gastric ulcer, and wound healing) (Earl, Tegeris, Whitmore, Morison, & Fitzhugh, 1964; Swindle & Smith, 1998). Therefore, pigs are commonly used research models for identification of new cardiovascular agents (especially in the field of arteriosclerosis and myocardial infarction), agents for treatment of metabolic syndrome (such as diabetes), dermatology drugs, and diagnostic agents. Here, the minipig can have a significant advantage over the pig because of the reduced size, thus reducing compound needs for the experiments and allowing easier handling, especially if adult animals are required.
- **Gaps in knowledge:** There has been no systematic attempt to compare the utility of minipigs and non-human primates as non-rodent toxicology models for a wide range of different therapeutic approaches. In addition, broader pharmacological characterisation of the minipig might promote selection of the best predictive model for the human situation, when testing drugs with highly species specific target expression or highly species specific pharmacodynamic activity.

2.2. Safety pharmacology

Safety pharmacology studies comprise a variety of tests to identify potentially undesirable pharmacodynamic effects of drugs. Requirements for these studies are outlined in the ICH guideline S7A: "Safety Pharmacology studies for human pharmaceuticals" (CPMP/ICH/539/00). With regard to the animal models used, justification for the selection of the model should be given and internationally recognised methods are generally recommended.

Central nervous function**:**

- **Species of choice:** Rats and mice.
- **Non-rodent use:** limited to conditions where no suitable rodent species is available; in such cases, the dog is the most common species for this type of test. Minipigs are not well established for such tests in this regulatory environment, although a recent review draws attention to the potential utility of pigs in neurobehavioural assessments (Lind et al., 2007).

[2] EMA — European Medicines Agency. EU — European Union. EPA — United States Environmental Protection Agency. FDA — United States Food and Drug Administration. ICH — International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. REACH — Registration, Evaluation and Authorisation of Chemicals.

[3] OECD — Organisation for Economic Co-operation and Development.

In vivo cardiovascular function:

Specific requirements in addition to those outlined in ICHS7A were defined to address the potential for delayed ventricular repolarization in ICHS7B: "The non-clinical evaluation of the potential for delayed ventricular repolarization (QT interval prolongation) by human pharmaceuticals" (CPMP/ICH/423/02).

- **Species selection:** Rodents can be used for assessment of effects on blood pressure, but they are not considered appropriate for evaluation of effects on action potential profile, because the ion channels mediating repolarisation in rodents differ from those in humans
- **Non-rodent use:** They are recommended for these tests (Bass, Tomaselli, Bullingham, & Kinter, 2005) with specific mention of dog, monkey, *swine*, rabbit and ferret in guideline ICHS7B. There are species-specific ECG features, which should be taken into account when choosing the species.
- **Use of minipigs:** Pigs are considered suitable for cardiovascular safety studies. Although it has been indicated that minipigs have a long QT interval (circa 250 ms) compared to other species, (Hamlin, 2005), a recent telemetric study with Goettingen minipigs suggests that this is not a constraint for the use of these animals (Stubhan et al., 2008)

Respiratory function (often combined with the cardiovascular function test):

- **Species selection:** Rats and dogs are the most established species, but alternate species may be needed to address specific requirements, e.g. transgenic mice, nonhuman primates or guinea pig (Murphy, 2002).
- **Use of the minipig:** Minipigs are not well-established for this purpose. They have a large number of pulmonary intravascular macrophages typical for species of the order Artiodactyla (Warner, Barry, & Brain, 1986, see also Bode et al., 2010-this issue). With the knowledge of this anatomical specificity a valid histopathological evaluation can be performed.

Gastrointestinal function:

- **Species selection:** Rodents and rabbits are mainly used (Harrison, Erlwanger, Elbrønd, Andersen, & Unmack, 2004). Only rarely, other species are used for these tests because of specific requirements for the individual drug.
- **Use of minipig:** As pigs are omnivorous, they could possibly be well suited for such function tests, but it is not well established.

Renal function:

- **Species selection:** Rats and rabbits are the most commonly used species. However, another possibility is the inclusion of endpoints for renal function into toxicity studies using different biomarkers (Hart, 2005).
- **Use of minipig:** The inclusion of endpoints for renal function can be done with minipigs in systemic toxicity testing.
- **Gaps in knowledge:** Compared to rats and dogs, testing of new laboratory markers is often more difficult in minipigs because of the lack of commercially available routine test kits and the lack of background data.

2.3. Pharmacokinetics

Data on pharmacokinetics (PK) comprising data on absorption, distribution, metabolism and excretion (ADME) are collected in appropriate *in vitro* and *in vivo* models.

- **Species selection:** ICH Guideline S3: "Toxicokinetics: A guidance for assessing systemic exposure in toxicology studies" (note 1) states that confirmation is needed that the metabolic profile in the species used was acceptable. Data to support this will normally be derived generally, the species used in pharmacology and toxicology studies also being used for PK studies, whenever possible, based on their similarity to man. In addition, EU guidance 3BS11a: "Pharmacokinetics and metabolic studies in the safety evaluation of new medicinal products in animals" states that "the animal species in these studies usually should be those normally used in the laboratory for pharmacological and toxicological investigations. The reasons for selection of any other species should be given."
- **Non-rodent use:** PK information is very important, since the metabolite pattern of the drug in the selected species should be similar to man and sufficiently high systemic exposure should be reached in the toxicity studies both for parent compound and for major metabolites. Preliminary pharmacokinetic data such as data on *in vitro* and *in vivo* metabolism and on bioavailability should be collected as early as possible not only in the traditional species of first choice, the dog, but in a broader range of species including the minipig.
- **Use of minipig:** Methods to study ADME in pigs and minipigs are available, and some background data (e.g. on liver metabolism) have been published (Witkamp & Monshouwer, 1998). The pig is a suitable model for most routes of administration and for evaluation of most ADME endpoints. There are some similarities between man and pig with regard to biotransformation, but there are also some significant differences (e.g. very low CYP2D and CYP2C19 activity compared to man). The high biotransformation activity in the intestinal wall of the pig may result in more similarity with man for some orally administered drugs, but may on the other hand cause a low *in-vivo* bioavailability for other drugs. Because of the similarity of skin anatomy, the pig is a well established model for transdermal penetration studies and for other dermal ADME studies.

2.4. Toxicology

Toxicological testing is well standardised and harmonised with international guidelines (ICH) giving recommendations on the extent and timing of toxicological testing during the drug development process as well as more specific recommendations on specific study types. In addition, regional guidelines exist for some study types as summarized in Table 1.

Extended single dose toxicity studies and repeat-dose toxicity studies:

- **Selection of the species:** Basic requirements are that the pharmacological activity of the drug and the pharmacokinetic profile allow sufficient exposure to be achieved and that similarity of metabolism of the drug to humans ensures that the main human metabolites are also formed in the species tested. Traditionally, dog is considered the non-rodent species of first choice, since an extensive historical background knowledge and well standardised methods exist. In addition, the dog is well adapted to cooperate with man and thus easy to train, so that the stress level of the animal is relatively low during the experiments. Furthermore, most dogs interact with humans naturally which facilitates close monitoring of clinical effects. The use of other non-rodent species in toxicological programs have to be specifically justified based on scientific arguments.
- **Use of the minipig:** Dermal products: the minipig is a well-established model to study toxicity of dermal products because of the similarities of human and pig skin, (Mortensen, Brinck, & Lichtenberg, 1998; Mahl et al., 2006) with several marketing applications being accepted by FDA (e.g. Tri-luma™ — fluocinolone acetonide/hydroquinolone/tretinoin cream; Elidel™ — pimecrolimus cream; Anthelios SX™ — cream containing avobenzone, ecamsule and octocrylene) (also Mainigi, 2007) and EMA (Vaniqa® — eflornitihine hydrochloride cream; and others) in recent years. (see European Public Assessment Reports (EPAR's), available at http://www.EMA.europa.eu)

Table 1
Recommendations on species selection in toxicology studies in international and regional guidelines.

Study type	Guideline	Species recommendation
Acute toxicity deri	ICH M3: non-clinical safety studies for the conduct of human clinical trials for pharmaceuticals, CPMP/ICH/286/95 (R2), 2009	Historically, two mammalian species, but not longer spelled out.
	EU guideline 3BS1a "Single dose toxicity", 1987	Overruled by ICH M3 (R2) 2009
	FDA guidance for industry: single dose acute toxicity testing for pharmaceuticals, 2006	
	Japanese guidelines for new drug registration: single dose toxicity, 1999	
Repeat dose toxicity	ICH M3 (R2): non-clinical safety studies for the conduct of human clinical trials for pharmaceuticals, CPMP/ICH/286/95	Two mammalian species, including one non-rodent species.
	CPMP note for guidance on repeat dose toxicity CPMP/SWP/488313/07	Two mammalian species, including one non-rodent species, selection based on similarity to human PK, exposure to main human metabolite should be ensured.
	Japan's and ICH guidelines for new drug registration: repeat dose toxicity, 1999	Two mammalian species including one non-rodent species other than rabbit.
Toxicity in juvenile animals	CHMP guideline on the need for non-clinical testing in juvenile animals on human pharmaceuticals for paediatric indications, EMA/CHMP/SWP/169215/2005, final 2008	One appropriate species for evaluating toxicity in endpoints relevant for the intended paediatric population; for repeat dose toxicity studies, rats and dogs are traditionally the species of first choice. However, other species might be more appropriate in some instances. Factors to be considered include pharmacodynamic, pharmacokinetic and toxicological properties and the feasibility of conducting the study.
	FDA guidance for industry: nonclinical safety evaluation of pediatric drug products, 2006	Rats and dogs have been the rodent and nonrodent species of choice. In some circumstances, however, other species may be more appropriate, e.g. when drug metabolism in a particular species differs significantly from humans, an alternative species (e.g., minipigs, pigs, and monkeys) may be better suited.
Reproductive and developmental toxicity	ICH S5(R2): note for guidance on the detection of toxicity to reproduction for medicinal products and toxicity to male fertility (CPMP/ICH/386/95), 1994	Mammalian species, generally desirable to use same species and strain as in other toxicological studies; rat as predominant rodent species: practicality, comparability with other results obtained in this species and large amount of background knowledge accumulated. In embryotoxicity studies only, a second mammalian species traditionally required, the rabbit being the preferred choice as a "non-rodent". Reasons for using rabbits: extensive background knowledge, availability and practicality. Where rabbit is unsuitable, an alternative non-rodent or a second rodent species acceptable, considered on a case by case basis. Comment on domestic and mini pigs: malformation clusters with variable background rate, large amounts of compound required, large housing necessary, insufficient historical background data (Note 5).
Local tolerance	CPMP note for guidance on non-clinical local tolerance testing of medicinal products, CPMP/SWP/2145/00, 2001	One appropriate species per type of test.
Photosafety	CPMP note for guidance on photosafety testing, CPMP/SWP/398/01	Photoallergy: guinea pig.
	FDA guidance for industry: photosafety testing, 2003	Photosensitivity: appropriate animal models (generally mice or guinea pigs, but also rabbits or swine).

Further opportunities for the use of minipigs as species of first choice or as alternative to the dog:

- Drugs with known oversensitivity of the dog for dose-limiting side effects of no or only limited relevance for humans:
- Non-steroidal anti-inflammatory drugs: cause severe gastro-intestinal lesions in the dog (Lehmann, 1998). The minipig has been used as the non-rodent species in marketing applications accepted by FDA (e.g. Mobic™ — tablets containing meloxicam). In the EU this product is accepted as Novem®, a veterinary drug for cattle and pigs (EPAR, http://www.ema.europa.eu).
- Sympathicomimetics and anti-hypertensives: cause cardiotoxicity at very low doses in the dog, so that other adverse effects are difficult to identify in the dog (Lehmann, 1998; Stephan-Gueldner, & Inomata, 2000).
- Sex hormones with oestrogenic activity and antigestagens: female dogs are very sensitive to oestrogens and develop severe panmyelophthisis after subacute administration of moderate doses of oestrogens (Crafts, 1948; Hart, 1990).
- Drugs causing an emetic effect in dogs: the dog has a propensity to vomiting which can result in insufficient or erratic systemic exposure to the drug after oral administration and can provoke cardiovascular disturbances.
- 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, resulting in low systemic exposure. Since such effects are often not representative for man, the use of another non-rodent species is necessary.
- Cytotoxic and cytostatic anticancer drugs: dogs are most commonly used as non-rodent species for testing of such drugs (Schein, 1977). However, it is well known that the dog often shows dose-limiting gastro-intestinal effects at relatively low exposure, so that other side effects more relevant for safety assessment may not be identified. In addition, dogs are sometimes very poor predictors for man, as they are more sensitive. In addition to the dog, monkeys are also well established for this type of drug (Schwartsmann, Wingrad, & Pinedo, 1988). Since regulatory recommendations only ask for use of a relevant non-rodent species (ICH S9 CHMP/ICH/646107/2008: Nonclinical Evaluation for Anticancer Pharmaceuticals, 2009), use of other non-rodent species such as the minipig is in concordance with current state of the art.
- With regard to regulatory acceptance, only a few marketing applications can be found on the websites of FDA and EMA where the minipig has been used as the non-rodent model in addition to the ones listed above (e.g. Mirapexin® — tablets containing pramipexole, a dopamine receptor agonist for the treatment of Parkinson's disease; Visudyne™ — an injectate containing verteporfin, a photosensitizer for photodynamic therapy).
- Gaps in knowledge: today, the Cynomolgus monkey is most often used as an alternative species to the dog because of the long experience with this species in toxicity testing. However, the minipig may also be a valid alternative preferable from an animal welfare point of view.

- Disadvantages/issues in the use the minipig: the minipig is susceptible to liver enzyme induction similar to rodents (Lehmann, 1998), which is not found to the same extent in man, dog and primate, so that the minipig may be less suitable than other non-rodent species for drugs found to be enzyme inducers in rodent liver.

2.5. Reproduction and developmental toxicity

Studies on fertility and early developmental toxicity (segment 1 studies):

- Species selection: generally performed in a rodent species, ie the rat.
- Non-rodent use: in those cases where the rat is not suitable, an alternative species is needed for these tests, which can be a rodent or a non-rodent species. It is generally a case by case decision based on the characteristics of the drug, but mouse and monkey are commonly used alternative species.
- Opportunities for the use of minipigs: the minipig represents a well suited alternative because of its short estrus cycle length of about 3 weeks and the high number of piglets in a litter.
- Gaps in knowledge: methods for such examinations in the minipig are only established in a few CROs. Because of the need of background data, data sharing of control animal data is recommended or quite extensive control experiments are required.

Studies on embryo-fetal toxicity (segment 2 studies):

- Non-rodent use: a non-rodent species is required. Traditionally, the rabbit is used for this purpose. Advantages of this species are the high litter size, the relatively low body weight and the relatively simple housing conditions. It has a high regulatory acceptance, although there are also some disadvantages in this species.
- Opportunities for the use of minipig: if the rabbit is not suitable, other non-rodent species have to be used. The ICH guideline lists some species including the domestic and minipig including the shortcomings of this species (see Table 1). The advantages of the minipig include early sexual maturity, good availability of mature animals, relatively large litter size and short pregnancy period.
- It should be taken into account that the placenta appears to be a real barrier in case of biopharmaceutical proteins and protein hormones, except for very specific uptake mechanisms (e.g. in humans for insulin in combination with antibodies [Bauman & Yalow, 1981]). In this respect the (mini)pig together with species such as horses, cows, sheep and goat is different from non-human primates and humans (Faber & Thornburg, 1983). In these species the newborn animal receive the maternal antibodies by ingestion of the immunoglobulin-rich first milk (so-called colostrum). Nevertheless the minipig may be of value in the testing of conventional ("small molecule") pharmaceuticals for embryofetal toxicity (Wiest, Swindle, Garner, Smith, & Gillette, 1996). The available data present do not confirm the disadvantages mentioned in the ICH S5a Guideline (Berggren et al., 2008).
- Gaps in knowledge: methods for embryo-fetal examinations in minipigs are only established in a few CROs. Because of the need of background data, data sharing of control animal data or quite extensive control experiments are required. For some of these reproduction and developmental toxicity study types, background data for some parameters were published for the minipig (Damm Jørgensen, 1998a,b; Damm Jørgensen, Ellegaard, et al., 1998; Damm Jørgensen, Kledal, et al., 1998).

Peri-postnatal studies (segment 3 studies):

- Species selection: generally performed in a rodent species, ie the rat
- Non-rodent use: in those cases where the rat is not suitable, an alternative species is needed for these tests, which can be a rodent or a non-rodent species. It is generally a case by case decision based on the characteristics of the drug, but mouse and monkey are the commonly used alternative species.
- Opportunities for the use of minipigs: the minipig can represent a useful alternative species in cases where the above listed species are not suitable in view of the relatively short pregnancy duration and high number of piglets in the litter.
- Gaps in knowledge: experience in the performance of such studies in the minipig is limited to a few specialised CROs. Because of the need of background data, data sharing of control animal data is recommended or quite extensive control experiments are required to establish the tests.

Studies in juvenile animals:

- Species selection: generally one species is considered sufficient for this purpose, and rats are commonly used. In case of the need for a second species, the dog is also quite commonly used because of the broad experience in repeat-dose toxicity studies (Guideline on the Need for Nonclinical Testing in Juvenile Animals of Pharmaceuticals for Paediatric Indications EMEA/CHMP/SWP/169215/2005, 2005).
- Use of the minipig: if these species are not suitable, the minipig might be a valuable species because of the litter size and the length of the oestrous cycle, facilitating synchronising of pregnancies (Svendsen, 2006). For some of these reproduction and developmental toxicity study types, background data for some parameters were published for the minipig (Damm Jørgensen, 1998a,b; Damm Jørgensen, Ellegaard, et al., 1998; Damm Jørgensen, Kledal, et al., 1998).

Local tolerance:

- Species selection: a variety of animal models for the various possible clinical administration routes and possible sites of misadministration exist. Most testing is done in albino rabbits or albino rats, but for some routes, other species may be more appropriate (Jochims, Kemkowski, Nolte, Bartels, & Heusener, 2003).
- Non-rodent use: CHMP has given some specific recommendations with regard to choice of species for different types of local tolerance tests for pharmaceuticals in the note for guidance on non-clinical local tolerance testing of medicinal products (CPMP/SWP/2145/00). For single dose dermal tolerance testing, they recommend the rabbit. Repeated dose dermal tolerance can be tested in rabbits or any other species if adequately validated. For parenteral administration routes, they require that the selection of species is justified, but do not recommend any species. For rectal tolerance testing, selection of species should be justified, but rabbit and dog are mentioned as most commonly used species. For vaginal tolerance testing, rat, rabbit and dog are recommended as most commonly used species.
- Use of the minipig: the minipig is never mentioned in the guideline. However, the minipig may be a very valid model, if a larger species than rat and rabbit are needed e.g. because of large administration volume or better similarity of the skin region to human anatomical situation.

Epidermal, intracutaneous, subcutaneous, intravascular and perivascular injection: one advantage of the minipig over dog and monkey are the unpigmented skin with little hair growth, facilitating visual in-life control of injection sites. Some data on local irritation testing in the minipig are published. Use of the subcutaneous injection route is documented in some marketing applications for insulin preparations (Novomix 30®, Levemir™) and for an antiviral drug (Fuzeon®).

Other published use of the minipig for local tolerance testing:

- Vaginal irritation (D'Cruz, Erbeck, & Uckun, 2005)
- Submucosal injection (Fujishiro et al., 2005)
- Local intranasal injection (Ranklove, Kaae Thorhuage, Eriksen, & Glerup, 2006).

General discussion of the use of minipigs in toxicity studies

- Handling. Handling of the minipig may be technically demanding and careful training of staff is required. For a discussion of handling issue, see Ellegaard et al., 2010-this issue. It is questionable, however, whether this training eventually is more labour intensive as compared with working with dogs and monkeys
- Costs. Since adult minipigs have a higher body weight than other common non-rodent species, compound need might be higher, and will be especially for subchronic and chronic studies, which may be a critical issue, when compound synthesis is time-consuming and expensive (see Table 2 on comparisons of costs of the toxicological testing programme with dog or minipig as the non-rodent species (study costs, compound need, and compound cost scenarios)). Between companies there are differences in strategies in choosing the starting weight of animals, which can be responsible for a different outcome of the costs of a study. In evaluating the scenarios described in Table 2 the following considerations should be taken into account:
  - Experiments with minipigs can be started at a lower age, and a lower body weight than at full adult age (12 vs. 25 kg). This results in a smaller difference with dog experiments with respect to compound need. This difference in compound need might be higher in the studies usually performed parallel to phase 1–phase 2 of a new active pharmaceutical ingredients.
  - Longer duration (9–12 months) studies are needed in a phase where upscaling has reduced the cost of compound. In the same time frame also carcinogenicity studies are carried out in rodents, with high costs, and high need for compound.
- With respect to the costs it can be concluded that although in general the choice of the approach using minipig in the studies (as compared with dogs) this might be slightly more expensive (10 to 15%), the impact on the total development costs is but the differences are small, and the slightly higher costs that are outweighed might outweigh the benefits of the choice of the most appropriate animals (e.g. using sexually mature animals, most relevant species with regard to metabolite profile), if applicable.

Scenarios evaluated for setting up Table 2:

Compound 1: expensive drug substance: 5000 Euro/g up to Ph 1, 1000 Euro Ph 1–Ph 3, 200 Euro/g after Ph 3, highly potent resulting in relatively low human dose
Compound 2: low costs for drug substance: 100 Euro/g up to Ph 1, 20 Euro/g Ph 1–Ph 3, 4 Euro/g after Ph 3, mediocre potency resulting in relatively high human dose
Further assumption: costs of goods are reduced in the course of CMC development process and up-scaling of production

Study programme included in the calculations:
Prior to Phase 1: Acute toxicity rat i.g. and dermal; acute toxicity mouse i.g.; exploratory repeat-dose toxicity study in rat and non-rodent over 14 days, i.g.; 4-week repeat-dose toxicity studies i.g. in rat and non-rodent; in vitro mutagenicity testing; single dose non-rodent i.v.; local tolerance i.v.; skin sensitisation assay in guinea pig
Prior to Phase 2: repeat-dose toxicity studies i.g. in rat over 13 and 26 weeks, in non-rodent over 13 and 52 weeks; dose-range finding studies of embryofoetal development, i.g. in rat and rabbit, male and female fertility study i.g. in rats, micronucleus test in vivo
Prior to Phase 3: dose-range finding studies of carcinogenicity (13 weeks mouse and rats, oral); pivotal studies on embryofetal development in rats and rabbits.
Prior to submission: 2-year carcinogenicity studies rat and mouse; peri-post natal toxicity study i.g. rat

Dose selection:
Low dose: HD in mg/kg for a 70 kg person scaled for body surface area (BSA) (scaling factors according to recommendations of FDA)
Top dose: limit dose of 2000 mg/kg for acute, 1000 mg/kg for repeat-dose/repro and 25x HD for carcinogenicity; alternative: subacute 100x HD scaled for BSA, subchronic/chronic: 50x HD scaled for BSA
Mid dose: geometric mean of low and high dose
Body weight: mouse: 30 or 40 g, rat: 150, 300 or 450 g, dog: 10 kg; minipig: 12, 15 or 25 kg, dependent on duration of the study

Table 2
Comparative listing of compound needs and study costs for different development scenarios.

	Compound 1		Compound 2	
	HD[a] 10 mg/day	HD 10 mg/day	HD 2000 mg/day	HD 2000 mg/day
	NR[a]: dog	NR: minipig	NR: dog	NR: minipig
Prior to Ph I				
Compound need	255 g	250 g	7259 g	8392 g
Compound costs	€ 1275 K	€ 1250 K	€ 726 K	€ 839 K
Study costs	€ 553 K	€ 597 K	€ 553 K	€ 597 K
Ph I–Phase II				
Compound need	1582 g	2092 g	91707 g	189602 g
Compound costs	€ 1582 K	€ 2092 K	€ 1834 K	€ 3792 K
Study costs	€ 1922 K	€ 2107 K	€ 1922 K	€ 2107 K
Ph II–Phase III				
Compound need	217 g	217 g	6683 g	6683 g
Compound costs	€ 217 K	€ 217 K	€ 134 K	€ 134 K
Study costs	€ 605 K	€ 605 K	€ 605 K	€ 605 K
After Phase III				
Compound need	2180 g	2180 g	109077 g	109077 g
Compound costs	€ 436 K	€ 436 K	€ 437 K	€ 437 K
Study costs	€ 3110 K	€ 3110 K	€ 3110 K	€ 3110 K
Total				
Compound need	4234 g	4739 g	214724 g	313754 g
Compound costs	€ 3510 K	€ 3995 K	€ 3131 K	€ 5202 K
Study costs	€ 6190 K	€ 6419 K	€ 6190 K	€ 6419 K
Total costs	€ 9700 K	€ 10,414 K	€ 9321 K	€ 11,621 K

[a] HD: human dose and NR: non-rodent species.

3. Veterinary pharmaceuticals

A survey of the literature concerning the minipig as a model species revealed that these animals have been rarely used for pharmacological studies of veterinary products. In such trials always the target species were used to evaluate the effects of drugs, which in the case of pigs would be the large domestic swine.

Pharmaceuticals developed to cure animals do have the advantage that they can be relatively easily studied in the target animals. Apart from testing the efficacy in the target animal population its safety for this population is also important.

The description for veterinary pharmaceuticals is laid down in Directive 2001/82/EC as amended. In fact the requirements are not recommending a specific except for developmental toxicity (teratogenicity) studies. Single dose studies should be carried out in two mammalian species one of which may be replaced by the target animal species. For repeated dose toxicity testing a single species (which might be the target species) is sufficient in case of products intended to be used in non food-producing animals. In case of medicines to be used in food-producing animals the product should be tested in two species, one of which should be a non-rodent. No further requirements are given other than that the investigator shall give his reasons for the choice of species.

As indicated above only for embryo/fetal toxicity (teratogenicity) studies there is a specification of the species. In case of products intended for use in food producing animals studies shall be carried out in at least two mammalian species, usually a rodent and the rabbit. The target species is not even mentioned.

The EPAR on Novem containing meloxicam (see http://www.ema.europa.eu) as a veterinary drug indicates that minipigs were used for single dose toxicity as well as for 13 and 52 week chronic toxicity. The reason for the choice of the minipig was not described in this report.

- Use of the minipig
  Pigs are important food-producing animals. The use of minipigs may be considered in order to reduce the costs of the compound. However, it is highly probable that the minipigs are more expensive that the target animal even in case the target animal species is the farm pig. The smaller size of minipigs is an important advantage in this respect.

4. Food additives

Food additives are among the most intensively tested products. The testing of new additives almost invariably includes use of non-rodents over long treatment periods.

The pig and minipig are good choices for testing food additives for various reasons:

- Pigs, like humans, are omnivorous.
- Gut transit time is similar to humans, and much slower than in dogs.
- There are many similarities in the stomach and small intestine compared with the same organs in humans.
- Enzyme activity in the gut and absorption show many similarities.
- Pigs are among the group of species providing meat for humans that are tested for the effects of various growth promoters, e.g. antibiotics.

The published literature confirms that pigs have been used extensively for testing food additives, e.g. many food colours, flavourings, artificial sweeteners, pectins, fat substitutes, gums, microalgae, monosodium glutamate, and antioxidants (Gaunt, Hall, Grasso, & Goldberg, 1968; Gaunt, Grasso, Kiss, & Gangolli, 1969; Gaunt, Kiss, Grasso, & Gangolli, 1969a,b; Gaunt, Colley, Creasey, & Grasso, 1969; Gaunt, Butterworth, Grasso, & Hooson, 1975; Olsen & Hansen, 1973; Butterworth, Gaunt, Grasso, & Gangolli, 1976; Sondergaard, Hansen, & Wurtzen, 1977; Hansen, Wurtzen, Sondergaard, & Skydsgaard, 1978; Hansen, Meyer, & Olsen, 1982; Hendy, Butterworth, Gaunt, Hooson, & Grasso, 1978; Grundschober, 1977; Bertelsen, Jensen, & Buemann, 1999; Nofre, Glaser, Tinti, & Wanner, 2002; Ahrens, Hagemeister, Pfeuffer, & Barth, 1986;Ahrens, Pfeuffer, Hagemeister, & Barth, 1991; Baekey et al., 1988; Cerda et al., 1994; Drochner, Kerler, & Zacharias, 2004; Liu, Fishman, Hicks, & Kende, 2005; Greenwood-van Meerveld, Neeley, Tyler, Peters, & McRorie, 1999; McRorie et al., 2000; Poulsen, 1973; Abril, Garrett, Zeller, Sander, & Mast, 2003; Stegink, Brummel, Boaz, & Filer, 1973; Stegink, Filer, & Baker, 1973; Olsen, 1983; Wurtzen & Olsen, 1986).

Despite the popularity of using pigs and minipigs for non-rodent testing of food additives, they are rarely recommended in the testing guidelines, even though available evidence indicates that studies utilising swine have full regulatory acceptance. An example of this can be found in the WHO "Guidelines for the Preparation of Toxicological Working Papers" (World Health Organisation (WHO), 2000), which specifies where in the document pig data should be included, and refers to pigs in Appendix F which provides information on how to calculate dietary parts/million of additive for various species.

The recommendation for non-rodent species in the FDA Food Ingredient Guidelines (U.S. Food and Drug Administration, 2003) does not encourage the use of swine. In the guidelines for short-term, subchronic, and one-year studies in non-rodents it is stated "These guidelines are for studies with non-rodents (usually dogs); if other species are used, modifications of these guidelines may be necessary". The guidelines also recommend discussion with the FDA in selecting suitable species. However, despite the above, in the section of the guidelines relating to the basal diet to be fed to the non-rodents, reference is made to the nutritional requirements of swine (U.S. National Research Council, 1998).

- Use of the minipig
  Pigs have been used extensively for testing food additives, although they are rarely recommended in the guidelines that are applicable. The use of minipigs should be justified (when compared to the default species dog).

5. Cosmetics

A cosmetic is defined in the EU in Article 1 of Council Directive 76/768/EEC and its amendments as "any substance or preparation intended to be placed in contact with the various parts of the human body (epidermis, hair system, nails, lips and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance and/or correcting body odours and/or protecting them or keeping them in good condition" (Council Directive, 1976). Exposure is therefore mainly external, and thus the pig can be considered to be a suitable species for safety testing purposes, because of the similarities of the skin compared with human skin. As well as direct effects upon the skin, it is also important with cosmetic ingredients to determine penetration through the stratum corneum, resulting in systemic exposure to both the chemical and its metabolites.

In Europe, the safety evaluation of cosmetic ingredients is carried out by the Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers (SCCNFP, 2003a). Their evaluations rely largely on standard toxicology screening of ingredients, and the Committee relies heavily on the OECD Guidelines for this purpose. However, the Committee has a strong commitment to tests *in vitro*, and has therefore issued an Opinion on the basic criteria required for assessment of dermal absorption of cosmetic ingredients *in vitro* (SCCNFP, 2003b). The Opinion specifies that OECD Guideline 428 (OECD 428, 2004) should be followed, but some specific species requirements are stipulated, i.e. "only human or pig skin should be used, skin of rodents is not representative for human skin". It is also specified that the pig skin should be obtained from the abdomen, breast, back, flanks or ears. Use of pig skin has the further benefit of not requiring testing in live animals, and the skin samples can be obtained from abattoirs, as long as transport to the testing facility at or below 4 °C is possible.

The regulatory situation in the USA for cosmetics and cosmetic ingredients is very different to Europe (U.S. Food and Drug Administration, 1992, 2005, 2006). The FDA has no specific requirement for use of animals in testing cosmetics for safety, and there is no premarket approval. It is the responsibility of manufacturers to substantiate the safety of both ingredients and finished products. The FDA does require, however, demonstration of safety of new colour additives. There is no advice available as to whether use of pigs is appropriate for testing cosmetics and cosmetic ingredients.

The published literature confirms that pigs, whole animals or skin, have been used extensively for cosmetic testing, e.g. sun-screens, emollients (moisturisers), skin care products, fragrance ingredients, hair dyes and anti-oxidants (Gupta, Zatz, & Rerek, 1999; Hori et al., 1999; O'Goshi, Tabata, Sato, & Tagami, 2000, Wang et al., 2004; SCCNFP, 1997a;1997b;2002;2004a;2004b;2004c; Steiling, Kreutz, & Hofer, 2001; Dressler & Appelqvist, 2006; Rangarajan & Zatz, 2001a,b, 2003). The literature also indicates that, when used, pigs and/or pig skin are acceptable to regulatory authorities, and in the case of dermal absorption, their use is mandatory in Europe if human skin is not available (SCCNFP, 2003a).

- Use of the minipig
  Although the pig can be considered to be a suitable species for safety testing purposes because of the similarities of the skin compared to human skin, the use of live animals is not recommended for cosmetics. For testing of local tolerance *in vitro* studies are preferred using human or pig skin. Pig skin samples can be obtained from abattoirs, and no specific use of minipigs is foreseen in Europe in this respect.

6. Chemicals

Annexes to Directive 92/32/EEC, the 7th amendment to Directive 67/548/EEC concerning the classification, packaging and labelling of dangerous substances, specify data requirements. Annex VIII — additional information and test required under Article 7 (2) states:

- "Sub-chronic and/or chronic toxicity study, including special studies (one species, male and female, most appropriate route of administration) shall be required if the results of the repeated-dose study in Annex VII or other relevant information demonstrate the need for further appropriate investigation."

While not specifically stated, the rodent (rat) is regarded as the default species.

The Directive requires that all tests should be conducted in accordance with Annex V methods. Exceptions to this should mainly be as a consequence of changes to OECD test guidelines or developments in the field of animal welfare.

Annexes to the REACH regulation that will replace the above legislation specify the data requirements under the new regulations. Annex IX — Additional standard information requirements for substances manufactured or imported in quantities of 100 tonnes or more states:

- "Sub-chronic toxicity study (90-day), one species, rodent, male and female, most appropriate route of administration, comparable to the likely route of human exposure."
- Use of the minipig
  As testing in nonrodents is not foreseen, the use of minipigs to test the safety of chemicals may be negligible.

7. Biocides

Annexes to Directive 98/8/EC define data requirements under the Directive. Annex IIA — common core data set for active [chemical] substances states:

- "Subchronic toxicity — 90-day study, two species, one rodent and one non-rodent
- Chronic toxicity — one rodent and one other mammalian species
- Carcinogenicity study — one rodent and one other mammalian species."

Similarly, Annex IVA — common core data set for active substances [fungi, micro-organisms and viruses] states:

- "Sub-chronic toxicity — 40-day study, two species, one rodent, one non-rodent
- Chronic toxicity — two species, rodent and one other mammal
- Carcinogenicity — one rodent and one other mammal"

The Directive states that, as a general principle, tests must be conducted according to the methods described in Annex V to Directive 67/548/EEC.

The technical guidance document in support of Directive 98/8/EC concerning the placing of biocidal products on the market (Guidance on data requirements for active substances and biocidal products) states:

- "Subchronic toxicity
  Should usually be studied in two species, one rodent and one non-rodent.
  Usually rat is the preferred rodent species and dog as the non-rodent species. If there is evidence from the 90-day studies that the dog is significantly more sensitive and where such data is likely to be useful in extrapolating results to man, in addition to the 90-day study a 12 month toxicity study in dogs may need to be conducted and reported. It is possible to replace a 90-day study in dog by a one-year study in a dog. An expert judgement is required to determine whether the one-year test is needed.

EC methods B.26 (90-day repeated oral dose study using rodent species) and B.27 (90-day repeated oral dose study using non-rodent species) or the corresponding OECD 681 guidelines 408 or 409."

- "Chronic toxicity
  The test is required for one rodent and one other mammalian species. It is recommended to study the rat first, and based on this result more testing in another mammalian species may be necessary A test should be performed in a rodent, the rat being the preferred species.

  EC methods B.30 or the corresponding OECD guidelines 451, 453."
- "Carcinogenicity study
  One rodent and one other mammalian species should be tested. The rat and the mouse are usually the species used for testing carcinogenic potential, while the rat is used for a combined chronic toxicity/carcinogenicity testing."

The use of the dog as the preferred non-rodent species is confirmed in the technical guidance document on risk assessment in support of:

- Commission Directive 93/67/EEC on risk assessment for new notified substances
- Commission Regulation (EC) No 1488/94 on risk assessment for existing substances
- Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market: "For biocides, at least the common core data requirements have to be met for notification purposes. …. In summary, the primary required data for biocidal active substances encompass oral 90-day studies in a rodent (rat preferred) and in a non-rodent species (dog preferred) performed according to EU Annex V methods or the corresponding OECD guidelines."
- Use of the minipig
  Minipigs might have a place in the testing strategy of biocides,

instead of dogs when required. Thus far, this is not very common, and the dog is the default species.

8. Agrochemicals

Annexes to Directive 91/414/EEC define data requirements under the Directive. Annex II — requirements for the dossier to be submitted for the inclusion of an active substance in Annex I states:

- "Oral administration — two species, one rodent (preferably rat) and one non-rodent, usually 90-day study"
- "Chronic toxicity — oral long-term toxicity and carcinogenicity (rat and other mammalian species)"

Commission Directive 94/79/EC, updating toxicological data requirements, states: "The short-term oral toxicity (90 day) of the active substance to both rat and dog, must always be reported. Where there is evidence that the dog is significantly more sensitive and where such data are likely to be of value in extrapolating results obtained to man, a 12-month toxicity study in dogs must be conducted and reported."

- "A long-term oral toxicity and carcinogenicity study (two years) of the active substance must be conducted using the rat as test species; these studies can be combined. A carcinogenicity study of the active substance must be conducted using the mouse as test species."

Tests must be conducted according to the methods described in Annex V to Directive 79/831/EEC.

It is noted that all regulations refer to Annex V testing methods. The repeated dose 90-day oral toxicity study in non-rodents (Annex V, B 27) states that the method described is a replicate of the OECD Test Guideline 409 (1998). The method states:

- Selection of animal species
  The commonly used non-rodent species is the dog, which should be of a defined breed; the beagle is frequently used. Other species, e.g. swine, mini-pigs, may also be used. Primates are not recommended and their use should be justified. Young, healthy animals should be employed, and in the case of the dog, dosing should begin preferably at 4–6 months and not later than nine months of age. Where the study is conducted as a preliminary to a long-term chronic toxicity study, the same species/breed should be used in both studies.
- Use of the minipig
  This implies that the use of species other than the dog, such as the mini-pig, is acceptable. However, data requirements for both agrochemicals and biocidal products state that the dog is the preferred non-rodent species. Use of a species other than the dog will require justification.

9. Human surgical methodology development and medical devices

A literature search has been carried out regarding the use of minipigs in methodology development in human surgery. Most of the literature refers to the use of domestic pigs in testing medical devices, and hardly any data on the use of minipigs in human or veterinarian surgical methodology development was found. Where swine were used for methodological improvement, the animals involved were usually large domestic pigs of different ages. For these purposes there was no good reason to use far more expensive and less available minipigs.

On the other hand, many physiological and pharmacological studies have been published where the methods included surgical procedures. In some cases these procedures are rather remarkable and demonstrate a high level of technique from an instrumental and manual point of view. In most cases, anatomy similar to humans played an important role, as in spleen and kidney transplantation, radiologic and pathologic studies, new solutions in tracheal stenosis and arterial remodelling after balloon angioplasty (see below). For such examinations, minipigs may be of special value, because they are available in a very good and well defined quality and have a body size easy to handle even when sexually mature. This can be of special importance, when the studies require longer follow-up periods.

9.1 Spleen transplantation between some strains of rodents can lead to donor-specific tolerance either spontaneously or after a short course of immunosuppression. A surgical technique for spleen transplantation in miniature swine has been developed to investigate its immunologic impact in a large animal model (Gollackner et al., 2003).Transplantation tolerance is still a challenge in the case of xenotransplantation. In this study, minipigs were donors as well as recipients of kidneys, the recipients providing data during 60 days after the day of transplantation. Fuchimoto et al. (1999) also used minipigs as a close anatomical model for man for kidney transplantation.

9.2 Another application is the study of pathophysiological processes. Kieschlichova et al. (2005) has described the pathophysiology of fulminant hepatic failure in a minipig model. Livers were devascularised to stimulate the process of failure. In this study the minipig was considered to be a good model due to the close similarities abdominal and liver anatomy in humans and pigs.

The anatomical benefits of the pig and minipig have already been described. These features are also of value for the assessment of medical devices, a particularly heterogeneous group of products involving biological, physical and chemical testing to ascertain safety. Pigs have been found in many circumstances to be suitable for screening new devices.

The general guiding principles for testing medical devices are found in the ISO guidelines issued by the International Organisation for Standardisation.

The similarity of the human and porcine cardiovascular systems makes the pig an exceptionally good model for evaluation of cardiovascular devices and prostheses. This is true for both the assessment of anatomical aspects influenced by the medical device, as well as determining haemocompatibilty of the device at the site of its use. The value of the pig is emphasised in the ISO Guidelines: "The pig is generally regarded as a suitable animal model because of its haematological and cardiovascular similarities to the human" (Biological evaluation of medical devices — Part 4: Selection of tests for interactions with blood, ISO 10993-4: 2002 (October 2002)).

9.3 Coronary restenosis after balloon angioplasty is an important issue in the use of stents. In atherosclerotic iliac arteries of Yucatan micropigs, PTA or stenting was performed. Serial intravascular ultrasound (IVUS) and quantitative angiography were applied before and after surgery and at 2 or 42 days of follow-up, followed by histomorphometric analysis (Post et al., 1997). All animals were fed an atherogenic diet from 3 to 5 months of age. Two weeks and 6 weeks thereafter, they underwent Fogarty denudation of the internal iliac, external iliac, and femoral arteries and were continued on the atherogenic diet. Five to 8 months later, selected stenosed arterial segments underwent either balloon dilation or stenting.

9.4 A coronary artery balloon-injury model has been developed in minipigs. A catheter was inserted through the femoral artery to the coronaries and, following the artificial injuries, the process was observed over a 4 week period. After sacrificing the animals histological analysis was carried out. (Matsumoto et al., 2001). ACE-inhibitors were assessed for their ability to inhibit the formation of neointima in this coronary artery balloon-injury model.

9.5 Fibromalacic tracheal stenosis was surgically created in 12 minipigs and afterwards a silicone stent was inserted in the stenotic area. Although most pigs were successfully treated by the stent, some unexpected mortalities occurred, underlining the need for long term experiments perfecting this procedure (Bolliger et al., 1999). The pig model was undoubtedly suitable in this experiment.

Pigs and minipigs are also very useful for testing implanted devices, e.g. in subcutaneous tissue, muscle or bone. Pigs are included in the list of suitable species recommended in the relevant ISO Guideline for long-term studies, especially up to 104 weeks (Biological evaluation of medical devices — Part 6: Tests for local effects after implantation, ISO 10993-6: 1994 (1994)).

The skin of pigs is very similar to that of humans and is therefore useful for testing all types of externally applied devices for potential irritative effects (Biological evaluation of medical devices — Part 10: Tests for irritation and delayed-type hypersensitivity, ISO 10993-10: 2002 (September 2002)).

- Use of the minipig
  The anatomical similarities of pigs compared to humans are clear. Therefore, pigs and minipigs have advantages in the testing of medical devices and surgical methods, especially in the abdominal and thoracic areas.

10. Regulatory acceptance: an overview

Whether or not studies using minipigs to support safety of products (pharmaceuticals, biocides, cosmetics, other chemicals as well as medical devices) will be acceptable to authorities is not clearly spelled out in regulatory guidance documents.

In the field of cosmetics the species pig is mentioned explicitly because of its applicability for testing skin products. However, in the EU *in vivo* studies supporting safety of cosmetics will most probably no longer be allowed after 2009, and *in vitro* studies using pig skin might be carried out using abattoir material.

The regulatory world is not too prescriptive regarding the choice of species and just indicates that the two species should be used, one rodent and one other non-rodent mammalian species.

The regulatory field of the pharmaceuticals is most explicit in all the guidelines that exist, in FDA and EMA/CHMP documents, but even there the minipig is not mentioned as one of the species of choice. Rats and mice are the rodents of first choice, whereas rabbits and dogs are the common first choice as the non-rodent (mammalian) species.

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 (although not formal position papers) confirm the acceptability of the minipigs in the pharmaceutical regulatory field (Jacobs, 2006; Peters, 2007), mainly for dermally applied medicinal products.

From the public literature it is clear that pigs and minipigs may serve and have served as the species of choice to support the safety of products. The choice should be justified scientifically. For conventional pharmaceuticals it is important to know the pathways of metabolism in the various species, and to use the species that is most similar to humans.

In the field of foodstuffs the pig is used more extensively because of the apparent similarity in the omnivorous food pattern and digestive tract between humans and pigs. Although this similarity should be taken with caution, the extensive use of pigs in this field provides historical data, which might be very useful in this area.

In the field of medical devices the policy is written in ISO Guidelines, especially in the ISO10993 series on Biological evaluation of medical devices. The pig is generally regarded as a suitable animal model because of its haematological and cardiovascular similarities to man. The pig is also mentioned as a species suitable for testing local effects after implantation. Surgical methods are being applied especially in pigs because of its size.

11. Gaps and opportunities

In this review of the regulatory acceptability of minipigs in toxicity testing the following gaps and research opportunities have been identified:

General utility and predictivity of the minipig model

- There has been no systematic attempt to compare the utility of minipigs and non-human primates as non-rodent toxicology models for a wide range of different pharmaco-therapeutic classes. In addition, broader pharmacological characterisation of the minipig might promote selection of the best predictive model for the human situation, when testing drugs with highly species specific target expression or highly species specific pharmacodynamic activity.

Since there is great interest in the pig as an animal providing meat in human nutrition, there is broad knowledge of the physiology of this species. This is also reflected in the broad spectrum of commercially available kits to test clinical biochemistry and other laboratory markers such as hormones. In this respect the minipig might be a good candidate as non-rodent species. There is a need to reduce the use of nonhuman primates especially in the development of biopharmaceuticals (e.g. immune system-directed monoclonal antibodies). However, in the screening for the most appropriate animal species (a pharmacologically responsive species is needed) the minipig is often not included. The high level of knowledge of the pig immune system should imply that there is good opportunity for this species to be studied as a possible candidate as the standard animal for toxicity studies in this respect.

Specific needs in toxicology testing

- The minipig appears to offer a promising alternative for reproductive toxicity testing, with several advantages (onset of sexual maturity, litter size etc.). Nevertheless, methods and background data for minipig use in reproductive toxicology are not yet well developed. For example, methods for embryo-fetal examinations in minipigs are only established in a few Contract Research Organizations. Since the interpretation of such studies can depend strongly on background data, the adoption of schemes for data sharing of control animal data is recommended in order to avoid repetition of extensive control experiments or data collection exercises. For some of these reproduction and developmental toxicity study types, minipig background data for some parameters have been published by Damm Jørgensen (1998a,b); Damm Jørgensen, Ellegaard, et al. (1998); Damm Jørgensen, Kledal, et al. (1998) and more recently by Berggren et al. (2008).
- Thus far cognitive testing in minipig is an area not well-known in the field of pharmaceutical industry. However, neuroscience research in pigs is extensive (Lind et al., 2007). In this respect advantage can be taken from modern approaches in camera-monitoring of experimental animals, in combination with complex techniques to learn animals technical skills for cognitive experiments. (Smulders, Verbeke, Mormède, & Geers, 2006).

12. Conclusions

Guidance documents relating to cosmetics, pharmaceuticals, food additives, chemicals, biocides and medical devices were reviewed. In these guidance documents, some reference is made to minipigs, but

most guidelines provide little detail regarding the choice of (non-rodent) species.

Some documents explicitly indicate that the dog is first choice as a non-rodent species for toxicity testing. At the present time, the Cynomolgus monkey is most often used as an alternative to the dog. This is in part because of the long experience with this species, and in part because of the close genetic and physiological similarity to human. Political and societal support for using nonhuman primates is decreasing (SCHER, 2009), as reflected in recent discussions on the revision of the European Animal Welfare Directive 86/609 (EC, 2009) and it is an appropriate time to consider the role of the minipig.

With respect to impact of testing in minipigs, we have reviewed the costs of testing in minipigs, including the cost associated with the quantities of test product, for a pharmaceutical product. We estimate that the total costs of a study in minipigs (dependent in part on the stage of development of a new active pharmaceutical ingredient) are not significantly higher than the costs for a study in dogs. Economical reasons should therefore not be used to argue against the use of minipigs instead of dogs or monkeys.

This review indicates that for most purposes, minipigs will be considered an acceptable choice as non-rodent species, provided adequate justification of this choice is made.

Acknowledgements

This work was supported by the *RETHINK* project, a Specific Support Action funded by the European Community 6th Framework Programme under contract no.: PL01877.

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