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Original article

The *RETHINK* project on minipigs in the toxicity testing of new medicines and chemicals: Conclusions and recommendations

Roy Forster [a,*], Gerd Bode [b], Lars Ellegaard [c], Jan-Willem van der Laan [d]

[a] *CIT, BP 563, 27000 Evreux, France*
[b] *Herzberger Landstrasse 93, Gottingen D-37085, Germany*
[c] *Ellegaard Göttingen Minipigs A/S, Soroe Landevej 302, DK-4261 Dalmose, Denmark*
[d] *Section on Safety of Medicines and Teratology, Centre for Biological Medicines and Medical Technology, National Institute for Public Health and the Environment (RIVM), P.O.Box 1, 3720 BA, Bilthoven, The Netherlands*

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ABSTRACT

The objective of the *RETHINK* project was to evaluate the potential impact of toxicity testing in the minipig as an alternative approach in regulatory toxicity testing that can contribute to the replacement, refinement and reduction of animal testing (3Rs). Expert study groups (Working Groups) were assembled to review five different areas relating to the use of minipigs in regulatory safety testing: ethical issues, welfare and animal care, development of new medicines and chemicals, safety testing issues and emerging technologies in safety testing. The conclusions and recommendations of the projects are presented in this article. It is concluded that there are no specific areas where restrictions to the use of minipigs in toxicology are required for welfare reasons. The minipig model is generally acceptable to regulatory authorities, provided it is adequately justified. The minipig is an interesting model for safety testing since there are numerous anatomical, physiological, genetic and biochemical similarities to humans. In addition many features of the minipig make it a practical and flexible model for safety testing. The use of the minipig in development of products does not bring any financial penalty in terms of the cost of testing. Benefits in terms of 3Rs can be identified in terms of life-cycle analysis of the use of minipigs compared to dogs and non-human primates. Finally the minipig (unlike the dog) is well positioned to take advantage of genomics and gene manipulation technologies. Specific recommendations for further research are made, which could bring 3Rs benefits. To deploy the minipig to the best advantage, clear information is needed about the predictivity of the minipig for human toxicities, and focussed action to define the potential role of the minipig in testing of biologics.

1. Introduction

The objective of the *RETHINK* project was to evaluate the potential impact of toxicity testing in the minipig as an alternative approach in regulatory toxicity testing that can contribute to the 3Rs, replacement, refinement and reduction of animal testing (Forster, Bode, Ellegaard & van der Laan, 2010-this issue).

Working Groups composed of more than 30 invited experts drawn from around the European Union worked over the period 2006 to 2008 to review the impact of toxicity testing in minipigs and potential 3Rs contribution in five different subject areas. These were namely ethical issues, animal welfare, regulatory issues, safety testing issues and emerging technologies (such as genomics and gene manipulation).

The conclusions of the five Working Groups are presented in the previous articles of this journal special issue (Webster, Bollen, Grimm, & Jennings, 2010-this issue; Ellegaard et al., 2010-this issue; van der Laan et al., 2010-this issue; Bode et al., 2010-this issue; Forster, Ancian, Fredholm, Simianer, & Whitelaw, 2010-this issue; Simianer & Köhn, 2010-this issue, all in this issue). In the present article, these conclusions are brought together in order to provide an overview together with the principal recommendations of the project.

2. Ethical framework

The ethical framework was developed taking into account the viewpoint of all concerned parties. The moral agents are the different groups of human stakeholders including society at large, regulatory bodies, industrialists and animal care staff. The moral patients are the laboratory animals, both breeding stock held by the animal supplier, and experimental animals in laboratories. In

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* Corresponding author. Tel.: +33 23229 2626; fax: +33 23267 8705.
E-mail addresses: roy.forster@citox.com (R. Forster), gerd-bode@t-online.de (G. Bode), ellegaard@minipigs.dk (L. Ellegaard), Jan-Willem.van.der.Laan@rivm.nl (J.-W. van der Laan).

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considering these animals it cannot be assumed that dogs, monkeys and minipigs differ with regard to the pain and suffering that they may experience and undergo when treated in studies designed for safety assessment. This differs from the views generally held by the general public and in publicly available reports.

The human stakeholders and experimental animals were integrated in an ethical matrix, to assist in better understanding and defining the benefits, freedoms and responsibilities of each group. The ethical matrix provides a framework upon which to identify and explore issues raised by the moral imperative to seek a fair compromise between the differing needs of different interest groups. The notion of fairness is particularly difficult to achieve when all the potential benefits are directed at one interest group (humans) and all the potential harms are directed at the other (the experimental animals). In practice, we can do no more than seek to achieve the most humane solution to the harm/benefit assessment for every class of experimental animal and every procedure.

The *RETHINK* project poses two key questions:

- What are the potential harms to minipigs relative to the harms for dogs and non-human primates and can these harms be reduced more easily in minipigs than in other species?
- Are there potential benefits resulting from the use of minipigs relative to dogs and non-human primates?

These two key issues generate the following relatively straightforward questions.

- How does the minipig compare with the alternative species as a model for humans in regulatory toxicology? What scope is there for increasing benefits?
  - It was concluded that the applicability of the model has to be assessed on a case-by-case basis in terms of the specific physiological or metabolic function under test and not on the basis of a priori assumptions.
- How does the minipig compare with the alternative species in relation to the nature and level of suffering that may be experienced as a direct consequence of scientific procedures? What are the relative advantages and disadvantages of the minipig, compared with the alternative species, in relation to our obligation to provide husbandry appropriate to their physical, behavioural and emotional needs?
  - These are complex questions and any response must take into account different factors such as (i) a comparison of the direct harms (physical and emotional) associated with procedures and the restraint involved with procedures (ii) the adequacy of knowledge and procedures for assessment of pain and distress and identification of humane end points, (iii) the quality of housing and husbandry for test, stock and breeding animals based on assessment of their physiological and behavioural needs and (iv) the quality of animal care based on a competent and compassionate understanding of the human/animal bond.
- What is the potential of the minipig, compared with the alternative species, for the development and application of the principles of the three R's?
  - 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".
- To what extent is, or should the use of the minipig be encouraged or constrained by human values that are unsupported by scientific evidence?
  - The minipig should not be considered a replacement for the dog or primate simply on the grounds that it may prove less offensive to some groups within society at large.

It was concluded that the capacity of an animal to experience suffering must be defined in terms of its own sentience, not its status in human society. The general argument was therefore rejected that minipigs are "more acceptable" experimental animals than dogs or monkeys simply on the basis of public identification of the pig as a food animal, the dog as a companion animal and primates as species closest to man in the species hierarchy. Species selection must be made on a case-by-case basis where the benefits (to humans) are assessed on the basis of scientific evidence relating to the predictivity of the animal model for the specific function (e.g. physiological, immunological) under test, weighed against the harm likely to accrue to the animals both from the test procedures and their lifetime experience within the laboratory environment. 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". It is recognised that there are areas where case-by-case analysis will favour the use of the minipig in toxicology and drug trials. It is also recognised that minipigs show potential in new studies designed to improve the implementation of the "three R's" and any sound proposals for specific developments in this regard are encouraged.

3. Welfare needs of minipigs

The current status and present needs relating to animal welfare indicators for the minipig, animal welfare considerations in minipig husbandry, welfare issues arising from the use of minipigs in biomedical research, and welfare issues (in comparison with other commonly used species) relating to the use of minipigs in regulatory toxicology studies were reviewed.

Animal welfare is concerned with the fulfilment of animals needs. As regards minipigs, what can be said about the definition of those needs? Minipigs have been selectively bred for small size, pale skin and docility in order to make them ideal animal models for laboratory biomedical research. Relatively little scientific data exists addressing the extent to which selective breeding may have modified needs in the minipig, but it is expected that the motivations and consequent behavioural needs of the minipig have remained unchanged or little changed from those of their wild ancestors.

Specific studies on the refinement of minipig housing and procedures are very limited in number. Studies on the welfare needs of production pigs probably represent a useful source of this guidance, and the welfare article makes numerous references to the literature on production pigs) but there is an element of uncertainty in any extrapolation from production (farm) pig studies.

Regardless of the definition of the needs of minipigs, can we assess welfare directly? It emerges from the available data that no single parameter is adequate to assess the level of welfare. The concept of triangulation, in which measures of welfare from different perspectives are integrated, was supported. To date, no physiological or behavioural indicators of welfare have been specifically validated in minipigs, but indicators that have been validated in farm pigs should be appropriate. Since the extent to which behavioural and physiological coping strategies are adopted may differ between breeds, it will be important to characterize the stress responses of minipigs and to assess both types of measure in any welfare study.

In terms of housing, it is recommended that all minipigs, with the exception of mature boars, are kept in groups when housed for biomedical research purposes. The problem of aggressive mounting behaviour of young boars places constraints on possible group housing schemes. How to group minipigs and the optimal group size under specific conditions has not been specifically investigated and should be the subject of further research. More research is needed to identify if single housing with close and positive social contact with other animals and technical staff is better than group housing where a strong hierarchy may bring detrimental consequences.

During the use of minipigs in toxicology studies, handling of the minipigs in different ways is necessary. In order to minimise the stress associated with handling (and consequent impact on experimental variability), positively-reinforced socialisation to human contact and training of the minipig for procedures that will be performed are considered to be essential. It is also essential that animal handling staff have a high standard of training for working with minipigs. Minipig handling and dosing programs should be adopted in all laboratories working with these animals.

During toxicology studies it is necessary to perform different scientific procedures on the experimental animals for the administration of the test item, and for the evaluation of reactions and responses. All commonly used routes of administration of test items in the performance of toxicity testing can be quite readily applied in the minipig. Routine clinical examinations can also be performed readily in minipigs. Further technical development and broader application of telemetry equipment can contribute to refinement and reduction within product safety testing. The peripheral blood vessels of the minipig are deeply located in the surrounding tissues. Therefore the collection of venous blood samples can be demanding, both for the animal and the technician. Temporary and chronic vascular catheters can be used for frequent sampling, and are likely to improve the welfare of the animals. Various schemes and protocols are available for the recording and scoring of clinical condition and clinical signs or symptoms in minipigs. The development of a body condition scoring protocol specifically for minipigs is needed because of their different conformation and adiposity in comparison with farm pigs.

From a welfare point of view it is clearly a "plus" for minipigs that there is an extensive background of veterinary medicine experience (as also for dogs) unlike the situation for non-human primates. Minipigs are also quite tolerant of telemetry implantation procedures. Constraints on the materials that can be used for bedding and enrichment, are limiting for welfare approaches in these areas. The differing needs and behaviour of young and older animals of each sex place constraints on the design of possible group housing schemes.

3.1. Welfare: conclusions and recommendations

In conclusion, from an animal welfare perspective the needs of minipigs as laboratory animals and as experimental models in toxicology appear to be addressed and there are no areas where significant restrictions or limitations to the use of minipigs in toxicity testing are required. The potential (welfare) advantages and disadvantages of selection of the minipig as the toxicology model will be dependent on the specific study type and study requirements. Opportunities exist for refinement and reduction. We consider that the welfare of minipigs before and during use in toxicology studies will be strongly dependent on the skills, knowledge and empathy of the handling staff.

Based on these considerations we have the following recommendations to make for the improvement of minipig welfare:

- Research efforts are needed to understand the breed-specific welfare needs of minipigs (as opposed to production pigs)
- There is a need for development of simple welfare assessment tools that can be applied in a laboratory or animal house setting,
- On the basis of this review of minipig welfare, we consider that it would be an important contribution to the welfare of minipigs to prepare and disseminate a "Code of Practice" for the optimal housing and management of minipigs whilst being used in research.
- It is recommended to further develop detailed protocols for the handling and training of minipigs subjected to procedures used in toxicity testing as well as humane endpoints and training procedures for the technical staff.

4. Role of minipigs in toxicology testing strategies

The role of minipigs in toxicology testing strategies can now be considered taking into account the viewpoints of animal welfare, regulatory acceptance, utility as a toxicology model and emerging technologies.

From examination of the ethical considerations, we have the "nulla obstat" to perform safety studies with minipigs, and similarly from an animal welfare perspective there are no basic restrictions to using minipigs in toxicity testing. From a regulatory point of view, based on our review of guidance documents relating to cosmetics, pharmaceuticals, food additives, chemicals, biocides and medical devices we can conclude that for most purposes, minipigs are and have been considered an appropriate choice as non-rodent species, provided adequate justification of this choice is made. For some non-pharmaceutical product categories it is explicitly indicated that the dog is first choice as a non-rodent species for toxicity testing (for example in the testing of pesticides and biocides); this approach appears to be based on historical experience, more based on tradition than scientific rationale.

We have also reviewed the potential economic impact of testing in minipigs, considering in particular the costs associated with the quantities of test product required for testing. 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. Questions of cost cannot therefore be used to argue against the use of minipigs as a non-rodent toxicology model.

From a detailed review of the comparative biology of the minipig as it relates to toxicity testing, the minipig presents a very favourable profile as a non-rodent toxicology model, in terms of the similarity to man and also in terms of applicability to different study types. There are numerous examples where the pig and/or minipig is considered to have close similarities with humans or to be an excellent model for human physiology and responses. There are also features of the minipig that make the minipig a particularly convenient and pertinent model for toxicity testing.

- Studies of general toxicology can be performed in the minipig by oral, cutaneous, parenteral and inhalation routes. In some areas (e.g. cutaneous toxicity testing) the minipig is generally accepted as an excellent model
- For reproductive toxicology studies the minipig offers numerous advantages as a non-rodent model. Nevertheless, the lack of placental transfer of antibodies (and probably of macromolecules in general) may limit the role of the minipig in reproductive testing of some categories of biotechnology products.
- For safety pharmacology studies the minipig is an advantageous model, particularly as regards cardiovascular safety pharmacology.
- At the present time when much creativity is going into the development of therapeutic approaches based on manipulation of the immune system. The immune system of the pig is better characterised than that of the dog, making the pig an interesting alternative model to the non-human primate.

Everything that has been reviewed leads us to believe that the minipig might be a better non-rodent toxicology model than the dog. This is based on the numerous similarities to man that make the minipig a relevant model, and also on the characteristics of the minipig that make it a flexible and convenient animal model. At the present time, however, insufficient comparative data is available to permit a rigorous evaluation of the predictivity of the minipig for

human drug-induced toxicities. It is therefore a conclusion of this project that research is needed to provide experimental data that allows evaluation of the hypothesis that minipig studies may better reflect human drug-induced toxicities than studies performed in traditional non-rodent toxicology models. It would be of particular value to gain a better vision of the potential utility of the minipig as a model for the safety testing of new biologics, where the minipig could potentially replace the use of non-human primates in the testing of some new products.

The potential of the minipig as a platform for future developments in genomics, high density biology, transgenic technology, in vitro toxicology and related emerging technologies was reviewed. It was a recurrent theme that commercial interests in the pig as an agricultural production species have driven scientific progress in these areas. There is no equivalent economic driver for progress in the dog or the monkey. As a result the available knowledge-bases are much greater for pigs (than for dogs or monkeys) in many areas (physiology, disease, genetics, immunology etc.). Fundamental genomic knowledge and phenotypic characterization in regard to the pig is well in advance of the dog or the monkey and basic knowledge of the pig is therefore likely to stay ahead of the other two species.

While the emerging technologies are essentially "species neutral" and can in principle be applied to all species, for all the technologies that we examined, basic knowledge and technical capabilities are greater for the pig than the dog or monkey. In concrete terms, in application to safety testing we have seen that:

- The Göttingen minipig is well positioned for the performance of toxicogenomics studies.
- The close sequence homology between pigs and humans suggest that minipigs will be useful for the testing of biotechnology products (and possibly for *in silico* toxicology).
- And finally the minipig is the only non-rodent toxicology model where transgenic animals can be readily generated, and reproductive technologies are well developed in the pig. These properties should also make the minipig an interesting model for the testing of biotechnology products.

These factors all support the idea that the minipig is well placed to meet the challenges of the emerging technologies and the toxicology of the future. It also seems likely that the minipig can be an advantageous model for the testing of biotechnology products.

5. Scope for application of 3Rs

How can all of this be achieved while respecting and contributing to the 3Rs. For the purposes of the *RETHINK* project it was necessary to arrive at a common view on the application and interpretation of the 3Rs. The general principles outlined below provided an agreed framework.

Reduction means using the smallest possible number of living animals to achieve the desired objective. The main, though not only, route to reduction is to ensure that experiments are well-designed and well-conducted, to deliver reliable results and reduce the need for repetition, establishing in advance the numbers of animals necessary to achieve the required level of statistical power. Similar gains may also be achieved by careful dose-level selections in dose–response studies. If minipigs are considered as a non-rodent species early in the development process for new products, permitting a fully informed choice of the non-rodent model, then studies using appropriate models can be avoided, with a corresponding reduction in the expenditure of animal life.

Replacement refers to the use of non-sentient organisms, or direct studies with humans, as an alternative to the use of higher animals for experiments. Microorganisms, metazoan parasites, and higher plants were originally suggested as possible alternatives and such experiments were labelled "absolute replacement", since no higher animals were required at any stage. In vitro techniques with cell cultures from animal tissues were defined as "relative replacement" procedures, since the experiments themselves were conducted on non-sentient material, but still depended upon animal material. In recent years, new developments, for example in culture of tissues and stem cells, in molecular biology and in robotics, have enormously improved the number and range of procedures that can be performed relative to the number of animals used, and the harm done to the animals. The option to replace animals by in vitro studies is growing when focused and well-defined endpoints are investigated. For the evaluation of unexpected adverse effects (for example, in preclinical safety assessments of candidate drugs) it is expected that regulatory requirements will continue to recommend combining in vitro and in vivo assays. The concept of replacement does not embrace the notion of replacing one class of sentient mammal (e.g. primates) by another (minipigs).

Refinement refers to any changes in protocol that can reduce the incidence or severity of distress experienced by living vertebrate animals and/or that can improve their welfare. It is now recognised as applying to the lifetime experience of the animal. Thus, in the context of this enquiry, it includes improving husbandry, handling and general care, together with the setting of less severe end points in toxicity tests and the development of less invasive or non-invasive techniques, which may involve biochemical and physiological markers such as blood parameters and telemetry of vital functions (e.g. heart rate, body temperature).

The general conclusions in the individual *RETHINK* articles, together with the detailed tabular presentation in Ellegaard et al. (2010-this issue), allow us to draw a relatively favourable 3Rs profile for the use of minipigs.

- It is easier to keep a population of pigs to a good standard of welfare than it is for dogs or non-human primates. Minipigs are relatively "sedentary" and are not arboreal (like non-human primates) or athletic (like dogs).
- The numbers of animals and the overall period of laboratory housing required to provide animals for a regulatory toxicity test are smaller for minipigs than for dogs and non-human primates, as a consequence of the large litter size and short period of development to sexual maturity of minipigs.
- For laboratories that are located within EU member states, Göttingen minipigs can be obtained within the EU, from breeders meeting EU welfare standards, and require transport within the EU only. Minipigs are in any case more tolerant of travel than non-human primates.
- The greater base of experience with the pig (than the dog or non-human primate) in various scientific domains will bring greater interpretative value to experiments performed in pigs and minipigs.
- Minipigs promise refinement in the future based on a greater knowledge-base for genomics, genetic manipulation, and immunology. This provides opportunities for 3Rs benefits through technologies that can provide a greater volume of more relevant data from a smaller number of animals, or from the development of animal models that are more pertinent for the evaluation of safety.

In addition to these identified 3Rs features of the minipig, further 3Rs benefits may be achieved through the research opportunities and recommendations made in the following section. Many of the research opportunities have a significant Refinement element, and these items are marked in the table in this way: ☑ 3Rs refinement.

6. Research opportunities and proposals

Item	Topic area	Proposal
1	Identification of welfare needs of minipigs ☑ 3Rs refinement	*Issue*: the extent to which selective breeding may have modified motivations and consequent behavioural needs in the minipig is unknown. *Proposal*: Research is needed to identify the extent to which selective breeding may have modified needs in the minipig, in comparison with other porcine breeds and strains, and how these basic needs translate into welfare needs.
2	Assessment of welfare ☑ 3Rs refinement	*Issue*: There are no welfare assessment schemes for minipigs. Monitoring schemes for pork production pigs exist such as the Animal Needs Index used in Austria and Germany (Bartussek, 1999) and the decision-support system in the Netherlands (Bracke, Metz, & Dijkhuizen, 2001; Bracke, Metz, & Spruijt, 2001). These assessment schemes focus on housing parameters and on selected animal observations and are not oriented to the specifics of laboratory housing and biomedical experimentation. *Proposal*: Research is needed to develop and validate simple welfare assessment tools for minipigs which can be used under practical (laboratory and animal house) conditions. The triangulation approach described by Webster (1994), provides a useful principle on which an overall assessment tool could be based.
3	Saliva based assays for non-invasive sampling ☑ 3Rs refinement	*Issue*: Collection of saliva (or swabs) rather than blood would place less physiological and interventional stress on experimental animals. In the minipig this issue is of special relevance, in view of the difficulties (real or imagined) in obtaining blood samples. *Proposal*: Development of blood biochemistry assays (for welfare indicators such as hormone levels) based on saliva samples rather than blood samples would be of general interest for animal welfare. Saliva sampling could also be achieved in settings where trained laboratory technicians may not be available for blood sample collection (eg during transport), permitting a broader vision of welfare during the animal's life-cycle. The saliva technology could subsequently contribute to non-invasive sampling during toxicology studies or other experimental work (for example in drug level determinations).
4	Minipig husbandry: group housing schemes ☑ 3Rs refinement	*Issue*: How to group minipigs and the optimal group size under specific conditions has not been the subject of focussed research, although some work has been performed on the impact of stocking density on the behaviour of group housed minipigs (Krohn, Ellegaard, & Hansen, 2000). The housing should take account of the different social needs of immature and mature male and female minipigs. *Proposal*: Research is urgently needed to define the optimal group size for minipigs housed in groups. Appropriate methods should be developed to ensure socialisation to human contact for individual minipigs within these groups.
5	Minipig husbandry: benefits of peer and human social contact ☑ 3Rs refinement	*Issue*: There are occasions when single housing of minipigs is necessary as a constraint of experimental designs or techniques. Single (individual) housing has traditionally meant social isolation for minipigs since little provision was made in housing units for tactile or visual contact between animals. *Proposal*: Research is needed to establish the benefits of providing singly-housed minipigs with visual, olfactory and tactile contact with other minipigs. This research should be extended to evaluate whether singly-housed minipigs, with a strong and positive socialisation with animal handlers and with visual, tactile and olfactory contact to other pigs, enjoy a greater (or lower) level of welfare (or reduced stress) in comparison with minipigs kept in different configurations of group housing. These approaches must nevertheless not permit cross contamination between experimental animals.
6	Minipig husbandry: benefits of bedding materials and enrichment ☑ 3Rs refinement	*Issue*: There has been no systematic study of the bedding and environmental enrichment needs and benefits for minipigs. *Proposal*: Research is needed to assess the amount and type of bedding and/or enrichment which is optimal for minipigs, particularly under the constraints of barrier rearing, to reduce stress and enhance welfare.
7	Minipig husbandry: reducing transport stress ☑ 3Rs refinement	*Issue*: Loading and unloading are probably the most stressful stages of transport. In farm pigs physiological values of stress indicators have been known to return to baseline very rapidly (within 2 days). Relevant data is not available to evaluate the stress involved in minipig transport and to evaluate the measures required to minimise any impact on the quality and reliability of toxicology and biomedical research subsequently undertaken with these animals. *Proposal*: Systematic study of the impact of the various phases of transport on minipigs is needed. The period required for full recovery should be evaluated.
8	Use of Minipigs in toxicology: toxicology data sharing schemes in reproductive toxicology	*Issue*: 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. For some of these reproduction and developmental toxicity study types, minipig background data for some parameters have been published by Damm Jørgensen, Kledal, Svendsen and Skakkeboek (1998) and more recently by Berggren, Jensen and Boegh (2008). *Proposal*: 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. Further characterisation of the different stages of spermatogenesis in the minipig boar is also required.
9	Minipig reproductive biology: IVM and IVF ☑ 3Rs refinement	*Issue*: Further understanding of the reproductive biology of the pig focused on refinement of IVM/IVF techniques would bring animal welfare benefits. In particular it could permit the use of slaughterhouse-derived oocytes for embryo manipulation and genetic modification work, with a corresponding positive impact on the reduction of animal numbers used for experimental work. The same principles can apply to necropsy derived minipig oocytes. *Proposal*: in view of the potential 3Rs benefits, as well as the benefits in terms of facilitation of research methods, further effort should be devoted to these areas.
10	Use of minipigs in toxicology: cognitive testing	*Issue*: To date cognitive testing in minipig is an area not well-known in the field of pharmaceutical industry. *Proposal*: Neuroscience research in pigs is extensive (Lind, Moustgaard, Jelsing, Vajta, Cumming, & Hansen, 2007). In this respect advantage can be taken from modern approaches in camera-monitoring of experimental animals, in combination with complex techniques to learn

Item	Topic area	Proposal
		animals technical skills for cognitive experiments. (Smulders, Verbeke, Mormede, & Geers, 2006). Development of such tests would contribute also to juvenile toxicity testing in the minipig, CNS function safety pharmacology in the minipig and the definition of humane endpoints in the minipig.
11	Use of minipigs in toxicology: blood pressure monitoring ☑ 3Rs refinement	*Issue*: The unsatisfactory use of cuff approaches in measuring blood pressure leads to the use of invasive approaches for the determination of this parameter. *Proposal*: The development of non-invasive or minimally invasive approaches for blood pressure measurement in the minipig.
12	Use of minipigs in toxicology: inhalation toxicity and respiratory administration	*Issue*: Inhalation toxicology and respiratory administration is currently an area of weakness for the minipig model. There are few publications and methods are not well established for inhalation toxicology or respiratory function studies. Until inhalation approaches are better established, the minipig will not be seen as an alternative model for products administered by this route. *Proposal*: A program of work designed to area to provide basic information in this area (RMV values, deposition efficiency) and to consolidate methods (inhalation administration, respiratory function measurements) would open the way to the use of the minipig for such products.
13	Use of minipigs in toxicology: collaborative program on cross-species metabolism	*Issue*: There is a paucity of data on cross-species comparative data for drug metabolism. Information on the proportion of drugs for which the minipig is a relevant (metabolic) model, and the families of chemical structures where minipig metabolism resembles metabolism in man. *Proposal*: Some issues are best addressed through collaborative programs rather than focussed research actions. Data that could be collected in this way on cross-species comparative data on metabolism providing a database and case-histories where the minipig does or does not provide a close model of drug metabolism and disposition in humans. This information would better define the role of the minipig in regulatory toxicology testing.
14	Use of minipigs in toxicology: collaborative program on cutaneous tolerance testing	*Issue*: It is generally believed that the minipig provides a better model of human cutaneous reactions than other animal models such as the rabbit or the rat. For percutaneous absorption data is available to demonstrate the value of the mini(pig) model, but for skin tolerance we do not have quantitative data. It is known that the correlation between rabbit and human irritant responses is poor for weak irritants (Steinberg, Akers, Weeks, McCreesh, & Maibach, 1975), but the corresponding data is not available for the pig. *Proposal*: this issue can be addressed through collaborative programs rather than focussed research actions. This is an important point to establish, both in the interest of drug safety and also since it could open the way for highly predictive porcine skin-based *ex vivo* models.
15	Safety testing of new biologics: prospective data collection	*Issue*: In order to gain a better vision of the potential utility of the minipig as a model for the safety testing of new biologics, it is crucial to generate data on the responsiveness of porcine molecular targets to human biologics. *Proposals*: In a prospective initiative, companies should be encouraged (by guidance documents or by research initiatives) to include minipigs in the early screening for selection of species responsive to the pharmacodynamic actions of new biologics. The resultant data must be collated and made public.
16	Safety testing of new biologics: retrospective validation	*Issue*: In order to gain a better vision of the potential utility of the minipig as a model for the safety testing of new biologics, it is crucial to generate data on the responsiveness of porcine molecular targets to human biologics. *Proposals*: In a retrospective initiative, a survey of the responsiveness of porcine molecular targets of existing biologics would be a valuable and informative exercise which could make a useful contribution to our understanding of the future role of the minipig in safety assessment.
17	Selective breeding of minipigs for temperament ☑ 3Rs refinement	*Issue*: Animals are not always easy to handle and this can also be true for Göttingen minipigs. The opportunity exists to selectively breed minipigs for smoother temperament and greater tolerance of human contact. *Proposal*: Much in the same way that reduced body size and "dominant white" skin colour of the Göttingen minipig were intended to adapt the minipig to laboratory use, breeding for smoother temperament and greater tolerance of human contact would bring further benefits in the laboratory environment. Better tolerance of human contact and greater amenability to handling would at the same time reduce the stress to animal handlers and scientific staff and also reduce the stress for the animals themselves. This would be a highly ethical development reducing anxiety during laboratory manipulation for both animal handling staff and for the minipigs and certainly contributing to enhancing the scientific quality of the work performed.
18	Minipigs and toxicogenomics: database of drug-induced gene expression signatures	*Issue*: Minipigs are poised for use in toxicogenomic studies. Established experience in toxicogenomics studies would clearly favour the use of the minipig in routine regulatory toxicology studies, because of the additional interpretative data and mechanistic insight that it can bring. *Proposal*: Data on the gene expression changes and signatures after treatment with reference toxicants would be very valuable in this respect, establishing the minipig as a toxicogenomics model and stimulating its further use. Predictive toxicology studies particularly need the comparison of transcription profiles with drug signature databases. Up to now, no such database for the pig and/or minipig has been generated and this leaves a very important gap in the development of genomics tools for predictive toxicity and/or pharmacology studies. The value of the minipig as a model in toxicogenomics would be greatly enhanced by availability of toxicity databases and validated toxicity signatures.
19	Consideration of minipig as a candidate non-rodent species	*Issue*: For each product that must be tested, selection of the most appropriate species as the non-rodent toxicology model will bring benefits to all concerned (whether this is the minipig or not). It would therefore be appropriate to ensure that the minipig is systematically taken into account in the selection of the non-rodent species. *Proposal*: A working group should be established to ensure that all European pharmaceutical and biotechnology companies and CRO's have ready and easy access to any tissues, tissue banks, preparations or reagents that are necessary for the purpose of informed selection of the non-rodent species.
20	Use of minipigs in toxicology: predictivity of the minipig model for human toxicities	*Issue*: Comparative data does not exist to permit comment on the relative utility and predictivity of the minipig, dog and non-human primate as models for toxic actions in man. This fundamental information is required in order to permit the informed and appropriate use of these non-rodent models. There has been no systematic attempt to compare the utility of

(continued on next page)

Item	Topic area	Proposal
		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. *Proposal*: A predictivity testing program is urgently needed in which an adequate number of compounds or drugs (for example ten) of well characterised human toxicity would be tested in both the minipig, the dog and the monkey in order to demonstrate that predictivity in the minipig is at least as good as the monkey and better than the dog. Such a result would be a significant finding and should provoke rethinking about the role of monkeys in the testing of new drugs.

7. Overall conclusions and recommendations

7.1. Recommendations

In the previous section of this article 20 different proposals are made for research or actions. The majority of these proposals will contribute to 3Rs objectives in refining or reducing minipig use. In addition to these proposals, we give particular importance to three recommendations:

- The preparation and dissemination of a "Code of Practice" for the optimal housing and management of minipigs whilst being used in research (including detailed protocols for the handling of minipigs and training procedures for the technical staff).
- A testing program to provide predictivity data: a number of drugs with well characterised toxicity should be tested in both the minipig, the dog and the monkey in order to evaluate the predictivity of the minipig relative to the other two species.
- A survey of the responsiveness of porcine molecular targets of existing biologics would make a useful contribution to our understanding of the future role of the pig in safety assessment.

These proposals can make important contributions to animal welfare, to gaining a better vision of the predictivity of the minipig in toxicity testing, and to gaining a better vision of the utility of the minipig in testing of new biologics.

7.2. And closing comment|s

This survey of the impact of the minipig in toxicology testing has shown us that there are no welfare restrictions to the use of minipigs in toxicology, and that the minipig model is generally acceptable to regulatory authorities, provided it is adequately justified. The minipig is an interesting model for safety testing since there are numerous anatomical, physiological, genetic and biochemical similarities to humans. In addition many features of the minipig make it a practical and flexible model for safety testing. The use of the minipig in development of products does not bring any financial penalty in terms of the cost of testing. Benefits in terms of 3Rs can be identified in terms of life-cycle analysis of the use of minipigs compared to dogs and non-human primates. Finally the minipig (unlike the dog) is well positioned to take advantage of genomics and gene manipulation technologies. To deploy the minipig to best advantage, clear information is needed about the predictivity of the minipig for human toxicities, and focussed action to define the potential role of the minipig in testing of biologics. This is a moment in time when there is public debate and concern about the use of non-human primates in safety testing, and publicly funded research programs are underway that aim to replace animal testing. At the same time professional toxicologists are seeking new paradigms in toxicology testing, exploiting advances in systems biology and toxicogenomics. In this moment of ferment and re-evaluation, it is an appropriate time to give full reconsideration to the potential benefits that the minipig can bring to the safety evaluation of new products.

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