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Original article

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

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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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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.