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# Ethical implications of using the minipig in regulatory toxicology studies

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and under the auspices of the Steering Group of the *RETHINK* Project

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ABSTRACT

Two key questions are addressed in this article. 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? In considering the answers to these questions, we present an ethical framework which was developed taking into account the viewpoint of all concerned parties. This 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, which includes both the moral agents and the moral patients. 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 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. On this basis we rejected the argument that minipigs are more acceptable experimental animals than dogs or monkeys despite the fact that their use may prove less offensive to some groups within society at large. Species selection must be made on a case-by-case basis where the benefits are assessed by weighing the scientific evidence relating to the predictivity of the animal model, against the harm that may accrue to the animals both from the test procedures and their lifetime experience within the laboratory environment.



## 1. Introduction: remit and aims

Current safety legislation requires that new pharmaceuticals, chemicals and chemical products be assessed for their potential risk to humans and the environment. In many cases this includes the use of animal models for the generation of hazard information. For example, product safety legislation requires that pharmaceuticals are tested in rodents and non-rodents (except in exceptional circumstances) before the clinical phases of drug development programmes to help assure their safe use in humans. The selection of the most appropriate second, non-rodent species has been a topic of discussion for various groups For example, a "points to consider" document has been developed by the ABPI in conjunction with the Home Office, including scientific, ethical, animal welfare and technical criteria that need to be taken into account (Smith and Trennery, 2002).

So far as the industry and the needs of public safety are concerned, several factors influence the choice of a second, non-rodent species for regulatory toxicology and drug testing:

- The predictivity of the process being modelled in the test animal relative to the analogous process in humans.
- The strength of existing scientific evidence concerning the physiology and metabolism of the animal and the organ system under investigation.
- The availability and cost of a healthy, uniform source of experimental animals
- Life history parameters: e.g. growth rate and mature size, reproduction rate and litter size.
- Behaviour and manageability and interaction with humans under laboratory conditions.

The stated aim of the *RETHINK* project is "to provide an assessment of the minipig as an alternative in toxicology testing, and the potential contribution of the minipig to the three R's (replacement, refinement and reduction) in animal testing" (for definition of the 3R's see below).

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The ethics Working Group was invited to address questions such as the following:

- Are there any ethical issues specific to the use of minipigs in toxicology and drug trials?
- Are the ethical issues associated with using the minipig different from those associated with the dog or primates? To what extent should these be influenced by public identification of the pig as a food animal, the dog as a companion animal and primates as species closest to man?
- Can the use of the minipig offer scope for the three R's; i.e. replacement, reduction, and refinement of procedures used in toxicology and drug testing?
- Does the use of the minipig present special issues in respect to European Community policy on respect for animal life?

The group was *not* asked to debate whether or not animals should be used in experiments at all. This has been considered elsewhere (e.g. Animals Procedures Committee (2003a); Nuffield Council on Bioethics 2005). Our remit can be defined by a single, critical question: "Are the potential benefits of using the minipig (relative to other mammalian species) commensurate with any changes, for better or worse, in the harm done to the test animals?" ("Harm" is used here as shorthand for the expression "pain, suffering, distress or lasting harm" as defined within the U.K. Animals (Scientific Procedures) Act 1986). This article first identifies and explains the ethical principles that should govern the use of animals in regulatory toxicology and drug testing. It draws attention to the ethical and legislative principles incorporated into international treaties such as the Amsterdam Treaty Protocol (1997), international legislative provisions including the Council of the European Union Conventions ETS123 (1986) and Council of the European Union (1986) and European Union Directive 86/609 (1986). These endorse the principle of respect for animal life and impose a requirement within science to minimise harm and improve welfare through critical assessment of necessity and application of the 3R's. The article then directs a series of ethical questions to the other RETHINK Working Groups concerning the benefits (to humans), harms (to animals) and potential for application of the three R's arising from use of the minipig, relative to dogs and primates.

### *1.2. Ethical principles*

Ethics, synonymous with moral philosophy, is a structured approach to examining and understanding the moral life. There are two classic approaches to addressing moral issues, conveniently abbreviated as "top-down" and "bottom-up". The classical "top-down" approach asks the question: "Which general moral norms for the evaluation and guidance of conduct should we accept and why?" The principal aim of this approach is to justify moral norms. The drawback to this approach is that practical issues tend to be given little emphasis or ignored. The alternative "bottom-up" approach is first to identify a specific practical issue then construct an analysis of relevant moral issues by a process of induction. The traditional criticism of this approach has been that it lacks the foundation of classical theory and is therefore more susceptible to bias and prejudice. However, Beauchamp and Childress (2001) have developed a powerful and widely adopted "bottom-up" approach to addressing problems in Biomedical Ethics. It builds upon the well-established principles of "common morality"; i.e. those principles and norms identified as relevant and important by reasonable minded people. They know that to violate these norms without having a morally good and sufficient reason is immoral and should lead to feelings of remorse. The three pillars of common morality are defined as "promoting well-being" (the utilitarian principle of beneficence and non-maleficence), "autonomy" (respect for the individual or "do as you would be done by"), and "justice" (which incorporates principles of equality and fairness). Application of these principles to the use of animals in regulatory toxicology is complicated by the fact that the animals cannot contribute to the debate, and no benefit accrues to the individuals used in the process. This applies particularly to the principle of justice. Humans are moral agents and carry moral responsibilities. The animals are 'moral patients.' In this context therefore, the concept of justice demands that we should always seek a fair and humane compromise between the likely benefits to humans and the potential to cause pain, suffering, distress or lasting harm to the test animals.

#### *1.2.1. The ethical matrix*

One of the most basic principles of moral philosophy is that some harms are unacceptable in any circumstances. This report accepts the premise that the use of animals for test procedures that is necessary to protect human health and safety does not fall into this category. Thus the aim must be to seek an ethical compromise between the reasonable expectations of all concerned parties. Mepham (1996, 2000) has developed the principles of Beauchamp and Childress (2001) to create an "ethical matrix", which identifies the concerned parties whose interests command respect in relation to a specific practical issue, then applies the principles of beneficence, autonomy and justice to each of the affected interest groups. The human interest groups are those whose business it is to conduct trials with animals, and market the products, those who regulate such trials and those who benefit by way of improved health and safety from the knowledge gained from such trials. The moral patients are the animals; those used in scientific procedures and those used for breeding purposes. Since all of human society can potentially benefit from the results of regulatory toxicology and drug testing, the principle of justice requires that all of human society should recognise its responsibility to minimise the harms done to the animals.

Table 1 briefly illustrates how the ethical matrix may be applied to this subject. The three columns identify the three ethical principles, well-being, autonomy and justice (as applied to the test animals). The five concerned parties are:

- Human society at large (the users of pharmaceuticals and other substances tested on animals).
- Regulators of the industry, those regulating requirements for products safety and those regulating procedures with animals.
- Producers: controllers of pharmaceutical and testing companies, suppliers of test animals.
- Animal care staff: technicians and veterinarians directly concerned with animal care.
- Experimental and breeding animals.

The first four (human) groups are moral agents with moral rights and responsibilities. The experimental animals, the moral patients, command respect on grounds of both utilitarianism, (i.e. respect for their sentience and capacity for suffering) and autonomy. The concept of autonomy implies that respect must go beyond the need simply to minimise pain and suffering. As Rollin (1993) states: "Not only will welfare mean control of pain and suffering, it will also entail nurturing and fulfilment of the animals' natures, which I call telos." The term "telos" goes back to ancient philosophy. According to Aristotle, every creature possesses a goal in life, which he designated its telos (Mepham 2000). In modern terminology, "telos" can be understood as "the unique, evolutionarily determined, genetically encoded, environmentally shaped set of needs and interests which characterize the animal in question—the 'pigness' of the pig, the 'dogness' of the dog, and so on" (Rollin 1998). The creature will flourish if it lives up to its telos and flounder if this is not allowed.

At this stage the ethical matrix as set out in Table 1 merely presents a structure for discussion of the ethical issues. However a few examples may be given at this stage to illustrate how the matrix can be made to work. It is (for example) self-evident that improved health and product safety contribute to the well-being of society and we accept that the proper practice of regulatory toxicology is essential to this aim. Equally

**Table 1**
Application of the ethical matrix to the use of animals in regulatory toxicology and drug testing.

| | Well-being | Autonomy | Justice (to the animals) |
|---|---|---|---|
| Human society<br>(patients and consumers) | Improved health<br>Product safety | Freedom of choice among available therapies and products | Compassionate and informed recognition of the harms to the test animals |
| Regulators of products<br>Regulators of animal experiments | Responsibility to society (health and safety)<br>Responsibility to animals (minimise harms) | Open-minded approach to new developments | Respect for animal welfare enshrined in legislation and codes of practice |
| Producers<br>Chemical and pharmaceutical industry<br>Animal breeders | Financial reward<br>Informed and sympathetic regulation of procedures<br>Responsibility to animals (minimise harms) | Free competition among producers. | Compassionate interpretation of legislation. Apply three R's |
| Animal care staff | Pride and security in work<br>Responsibility to animals (minimise harms) | Control over decisions concerning e.g. animal husbandry and "end points" | Input into animal welfare policy |
| Experimental animals | Physical and emotional well-being through good husbandry<br>Minimal harm from procedures | Environmental enrichment to permit freedom of choice | Receipt of justice in context of harm: benefit analysis |

we recognise that financial success and pride in work are proper elements of well-being. This applies both to the leaders of the pharmaceutical industry and to the staff with day-to-day responsibility for animal care. These "rights" bring responsibilities. The utilitarian principle of respect for animal well-being relates, of course, to the principle of minimising harm directly associated with toxicity testing. This applies not only to the physical effects of the substances under test, but also to any emotional effects of the testing procedures and other aspects of the animals' lifetime experience. It therefore requires that proper attention should be given to the physical and emotional welfare of all laboratory animals from their birth to death. Utilitarian principles require regulators of product testing and trials with animals to ensure public safety through the imposition of proper controls. The principle of justice requires that they also ensure proper respect for the test animals. This respect should be enshrined within legislation and codes of practice and implemented in practice.

Two practical expressions of the principle of autonomy, as it applies to humans, are competition and freedom of choice, both of which are encouraged through the development of new, desirable drugs and chemicals. Equally, all the animals should be offered some freedom of choice, for example through provision of an enriched environment wherein they can exert some degree of control over their own welfare. This may be difficult to incorporate into toxicology trials but it can be done. Finally, the demands of justice require all who work with experimental animals, who commission work with experimental animals, or who benefit from the outcome of such work, to promote their welfare and minimise harms through policies based upon the principle of respect for all life.

*1.2.2. Harms to the animals: animal suffering*

The regulation of scientific procedures with living vertebrates is based on the prima facie moral principle of respect for the animals and the need to ensure that any negative impact on the well-being of any animal should be minimised and avoided if possible. The EU Directive 86/609 regulates any scientific procedure with a living vertebrate animal that "may have the effect of causing that animal pain, suffering, distress or lasting harm". Acceptable purposes for which procedures may be conducted include the following, which are relevant to this enquiry.

- The prevention (whether by the testing of any product or otherwise) or the diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants.
- The protection of the natural environment in the interests of the health or welfare of man or animals.

It is necessary to consider in more detail what is meant by the notion of "pain, suffering, distress or lasting harm". It is now beyond cavil that, for all mammals, pain is a physical and emotional experience. It is much more than just an unpleasant sensation; it also induces changes in behaviour and mood broadly similar to those seen in humans (Morton and Griffiths, 1985; Webster, 2005). It is also formally recognised by the Amsterdam Treaty Protocol (1997) that mammals are sentient beings. A sentient animal may be defined as one that has "feelings that matter" (Webster 2005). This definition may be briefly explained thus: sentient animals interpret sensations and experiences primarily in emotional terms and are motivated to behaviour designed to make them feel good and avoid feeling bad. This emotional basis to motivation may, or may not, be modified by cognition (or reason). Having behaved in a way designed to achieve a favourable physical and emotional state, the animal reviews the consequences of its actions. If these are successful, it will achieve a sense of well-being and if it fails it is liable to suffer distress. It follows from this that all sentient mammals can suffer distress not only from direct consequences of scientific procedures, such as pain, fear and malaise, but also from the emotional consequences of failure to achieve their physiological and behavioural needs within the constraints of their environment. Such long-term consequences cover a spectrum of distress that ranges from chronic anxiety to learned helplessness. It also follows that the capacity of a sentient animal to suffer is defined by its emotional potential, and not necessarily its cognitive ability. Thus it *cannot be assumed* that a pig is less (or more) capable of suffering than a primate or a dog.

There is a separate ethical issue that relates to the number of animals bred, and subsequently killed, in laboratories irrespective of whether or not they have suffered during life. Death is, by any definition, a lasting harm. One of the central principles of the three R's is the reduction of animals used in experiments and it is one that we embrace.

*1.2.3. "The three R's": replacement, reduction and refinement*

Perhaps the most effective application of the principles of practical ethics to the conduct of experiments with animals is the concept of the "Three R's": reduction, replacement and refinement, introduced by Russell and Burch (1959)

*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, so deliver reliable results and reduce the need for repetition. This requires close cooperation with statisticians to establish in advance the numbers of animals necessary to achieve the required level of statistical significance. Similar gains may also be achieved by careful dose-level selections in dose–response studies.

*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 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).

Legislation on the protection of vertebrate animals used for experimental and other scientific purposes has been formulated in line with the principle of the three R's. It was first presented in EU Directive 86/609/ECC (EU Directive European Union Directive 86/609, 1986) and with regard to other decisions in 2003 in the Council of the European Union (2003). These directives require the use of alternatives to living animals if these are available. In addition, national legislation such as the Animals (Scientific Procedures) Act, (ASPA 1986) incorporates the principles of the three R's as follows: "the regulated procedures to be used are those which use the minimum number of animals, involve animals with the lowest degree of neurophysiological sensitivity, cause the least pain, suffering, distress or lasting harm, and are most likely to produce satisfactory results".

### 1.3. Ethical issues and practical questions

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. In the specific context of this investigation, we need to consider 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.

1. How does the minipig compare with the alternative species (dogs and primates) as a model for humans in regulatory toxicology? What scope is there for increasing benefits to patients and consumers?
2. 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, including cloning and transgenic techniques?
3. What are the relative advantages and disadvantages of the minipig, compared with the alternative species, in relation to our obligation to provide conditions of housing, handling and husbandry appropriate to their physical, behavioural and emotional needs?
4. 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?
5. To what extent is, or should the use of the minipig be encouraged or constrained by human values that are unsupported by scientific evidence?

Question 1, the validity of the pig model, is strictly a matter of science and is addressed in detail by van der Laan et al., Bode et al. and Forster et al. (all this issue). Questions 2 and 3 arise from the moral need to minimise harms. These issues and practical solutions to these issues are reviewed in detail in Ellegaard et al. (2010-this issue). Question 4, implementation of the three R's, presents questions of science, in particular the development and application of new technologies, and these have been addressed in Forster et al. (2010-this issue). It also poses questions of ethics, in particular, what constitutes replacement and refinement? Question 5 is clearly a matter of ethics.

### 1.4. Species selection

In this section we review very briefly the issues of science, animal welfare and human concern that govern the choice of species for toxicology studies. The potential benefits (to humans) and harms (to the animals) are described in detail in subsequent articles. The aim of this section is to place these issues within a proper ethical context.

#### 1.4.1. Predictivity and existing knowledge

The scientific case for the use of minipigs in regulatory toxicology is based on their many similarities to human anatomy, physiology and pathophysiology (Bode et al., 2010-this issue). However, it is not possible to come to a general statement regarding the comparative aspects of minipigs, dogs and primates. In a scientific sense, the species of choice can sometimes be based on apparent similarities between organs and tissues; e.g. skin, where a valid case can be made for the use of the minipig. Similarly, dogs may be more appropriate in certain studies because of their anatomical features. For this reason the dog was chosen as the preferred species in the development of anti-ulcer drugs, since dogs have a similar gastric mucosal membrane as in humans, whereas pigs have a squamous mucosal region in the stomach. Some expert groups have suggested that the use of primates should be restricted to those experiments for which there is, at this time, no known alternative (Boyd Group 2002, Royal Society (Weatherall Report) (2006)). This could restrict the choice of primates to certain studies in neuroscience and brain function and communicable diseases common to man and other primates (e.g. HIV/AIDS and tuberculosis). For some important questions, e.g. studies of the immune system, none of these species may prove a suitably predictive model for humans [ICH S6, 1997].

All the arguments outlined above can and should be incorporated into the decision as to the selection of the most suitable species for a specific procedure. However choice of species should never be made on the basis of conservatism. The continued use of dogs and primates as non-rodent species in regulatory toxicology is often justified by the preamble "the species has been used in the past, there is a substantial library of knowledge and it is acceptable to the regulators". The Animals Procedures Committee (2003b) reported that 72% of non-human primates were used for regulatory toxicology and safety testing of pharmaceuticals. The Boyd Group (2002) stated "non-human primates may be selected out of caution of the risk that choosing other species may prove unacceptable to the regulators and thus result in costly delays in bringing a new medicine to market". Nevertheless there is a considerable body of information relating to the use of pigs and other farm animals as models for studies relating to human health (e.g. Kues and Nieman

2004). EU statistics on the use of animals in scientific procedures revealed that approximately 10% (9.9% in 2002) of animals were used for toxicological and safety evaluations, numbering 1,066,047, of which 12,826 (1.20%) were dogs, 6102 (0.57%) old-world monkeys and 2271 (0.21%) pigs. We do not have statistics specifically relating to the minipig. Since we do not draw a distinction between the capacity of primates, dogs and pigs to suffer harm, we can only assume that the physiology and psychology of the minipig is essentially the same as that of its agricultural cousins.

The central point that emerges from detailed consideration of the scientific basis for species selection is that it is facile to conclude that the pig is simply better or worse than the dog as a model for the effects of drugs and toxic substances on humans. It is also dangerous to generalise at the level of organ systems (with the possible exception of the central nervous system). We would conclude therefore 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.

*1.4.2. Minimising harms*

The responsibility of those who work with animals used for scientific procedures, toxicology and drug testing relates not only to the scientific procedures but should also embrace a proper professional concern for the lifetime welfare of all the animals. This ethical principle generates a number of issues concerning species differences in the potential to cause, or minimise, harm that generate questions for the other RETHINK Working Groups, in particular as regards Animal Welfare. In all cases these require a comparison between minipigs, dogs and the different species of primate used in regulatory toxicology. We list the major issues below.

- Comparison of direct harms, both physical and emotional, associated with procedures such as blood sampling, gavage etc. and the restraint involved with such procedures.
- Adequacy of knowledge and procedures for assessment of pain and distress and identification of humane end points.
- Quality of housing and husbandry for test, stock and breeding animals interpreted in terms of a professional assessment of their physiological and behavioural needs.
- Quality of animal care based on a competent and compassionate understanding of the human/animal bond as it applies to the different species.

*1.4.3. Application of the three R's*

Decisions about choice of species are complex and evolve with new knowledge. It is therefore important to review regularly the factors that have to be taken into account and to make use of all of the relevant information currently available. In an ethical context, this means a comprehensive review of the potential scientific, technical and economic benefits, all set against the need to minimise harm.

The quality of the science is defined by:

- The predictivity of the process being modelled in the test animal relative to the analogous process in humans.
- The strength of existing scientific evidence concerning the physiology and metabolism of the animal and the organ system under investigation.

Issues of practical expediency include:

- Regulatory requirements—in some instances, specific test requirements allow little discretion in the species to be used; in other circumstances, regulatory systems allow judgement to be exercised when selecting appropriate species
- Technical issues, e.g. availability of the species in the numbers needed, health and genetic status of the species, animal size and volume of blood or tissues required
- Animal husbandry issues, e.g. availability of suitable housing, ease of handling of the animals, training and level of competence of personnel
- Public concern about use of certain species
- Financial considerations.

Practical concerns for animal welfare in relation to husbandry and procedures for the three species, minipigs, dogs and non-human primates, are considered in Ellegaard et al. (2010-this issue). This section considers ethical issues in relation to the 3R's. The aim must always be to reconcile the scientific objectives of the research with the need to minimise potential harm to the animals involved, in accordance with relevant legislation, such as European Directive 86/609 (Article 7.5): "When an experiment has to be performed, the choice of species shall be carefully considered and, where necessary, explained to the authority. In a choice between experiments, those which use the minimum number of animals, involve animals with the lowest degree of neuro-physiological sensitivity, cause the least pain, suffering, distress or lasting harm and which are most likely to provide satisfactory results shall be selected."

The main issues arising from application of the three R's to questions of species selection are as follows.

*Refinement*: substituting a lower species (phylogenetic reduction) is often considered to be refinement, but such a judgement can only be made if assessment of the available scientific evidence suggests that the lower species is less sentient and therefore less likely to suffer. It has been argued (for example) that animals of a lower phylogenetic order (e.g. fish as opposed to mammals) have a less developed nervous systems so may not suffer pain. However, recent evidence would cast grave doubts on this assumption (Sneddon et al., 2003). This suggests that we should not act on such assumptions in the absence of definitive proof. Judgements about whether it is more humane to use one species over another are particularly difficult where the species are closely related phylogenetically (e.g. species within the class Mammalia or order Primates). Nonetheless, some countries afford special protection to certain higher mammals in their legislation, reflecting increased public concern for these species. For example, the UK Animals (Scientific Procedures) Act states that: "The Secretary of State shall not grant a project licence authorising the use of cats, dogs, primates or equidæ unless he is satisfied that animals of no other species are suitable for the purposes of the programme to be specified in the licence or that it is not practicable to obtain animals of any other species that are suitable for those purposes."

It is possible to make inferences about the likely relative impact on animal welfare of research on different species from what is known about their natural history, behaviour and welfare needs (e.g. importance of companionship, response to laboratory housing, habituation to humans) and the potential stressors involved with their use (e.g. capture from the wild, long and multi-staged transport and degree of restraint required), and then to make decisions about species selection based on the sum total of harms involved. This is the approach endorsed in this report.

*Replacement*: this is usually defined as the replacement of a living animal with a non-animal method or a method using cells, tissues or organs taken from dead animals. The latter is considered by some groups to be only 'relative' rather than 'absolute' replacement since animals are still used to provide the tissues. Use of a species not protected under legislation on the protection of animals used for experimental and other scientific purposes (i.e. use of an invertebrate instead of a vertebrate) is also considered by some groups a replacement, but this definition is a matter of debate. For instance, the horseshoe crab (*Limulus*) is now in general use as the pyrogen (endotoxin) test for microbial contamination of biological fluids, a procedure that was previously carried out in rabbits (Watson and Levin 1982).

The term relative replacement can properly be applied to the development of *in vitro* techniques with cell cultures derived from

animal tissues, since this would result in animals being replaced in the future. Thus while we do not accept that the substitution of one mammal (e.g. the mouse) for another (the monkey) constitutes replacement, we do accept that if the minipig could be shown to be a suitable model for the development of *in vitro* techniques, then this would be a step in the right direction.

*Reduction*; species selection can impact on reduction in various ways. For example, choice of the most appropriate species might help avoid the need to repeat studies in the future and, in so doing, avoid use of additional animals. Use of a well characterised species instead of a less familiar one may also permit reduction of animal numbers. Use of a species with large as opposed to small litters (e.g. rodent or pig instead of macaque) may permit reduction of the total number of animals required for a given study, e.g. reduction of the numbers of animal mothers if each infant in the litter (rather than each litter) is considered to be an 'experimental unit'.

The use of inbred, specific pathogen free (SPF), or genetically modified (GM) animals can, in some circumstances, lead to the need for smaller sample sizes in scientific procedures (Festing 2004, Morton 1998). We are advised however that sample numbers in toxicology and drug testing are already close to the lowest possible number consistent with the statistical needs of the regulators. Thus use of the minipig may offer limited scope in this regard. However, (see Bode et al., 2010-this issue) the capacity of the minipig to produce two large litters per annum does provide opportunities for reduction, generally in the numbers of animals kept for breeding purposes and specifically in relation to "Reprotox" tests (studies of the impact of test substances on the offspring of the challenged animal).

*1.4.4. Traditions and other values unsupported by scientific evidence*

It may be argued that, according to common morality, it is more acceptable to use the minipig than dogs or primates as a "second species" in toxicity testing on the basis that it will cause less distress within society, presumably on the basis that the pig is already accepted in most cultures as a food animal. We have already drawn attention to UK legislation that states "The Secretary of State shall not grant a project licence authorising the use of cats, dogs, primates or equidæ unless he is satisfied that animals of no other species are suitable for the purposes of the programme to be specified in the licence or that it is not practicable to obtain animals of any other species that are suitable for those purposes." (ASPA 1986) Just application of the ethical matrix requires us to show proper concern for all concerned parties, which include the animal care staff who might find it less distressing to carry out toxicology studies with minipigs than with dogs or primates. If this relative lack of concern were based on an impression that the minipig was somehow less sentient, and thus less likely to suffer than the other species, it would be invalid. However if it could be demonstrated that minipigs, maintained to the highest standards of care in research laboratories, were less likely to develop close personal bonds with individual members of the animal care staff than selected breeds of dog (e.g. Beagles) or species of monkey, then this might become a valid argument for inclusion within the overall debate concerning choice of species. Nevertheless, we repeat our assertion that we do not accept that substitution of one mammalian species for another constitutes replacement within the context of the three R's. We therefore reject the premise that the minipig can 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.

*1.4.5. The ethics of species selection: conclusions*

*The capacity of an animal to experience suffering must be defined in terms of its own sentience, not its status in human society*. We therefore reject the general argument 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. 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 and 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". We recognise areas where case-by-case analysis will favour the use of the minipig in toxicology and drug trials. We also recognise the potential of minipigs in new studies designed to improve the implementation of the "three R's" and we would encourage any sound proposals for specific developments in this regard.

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