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ICLAS Working Group on Harmonization: International guidance concerning the production care and use of genetically-altered animals

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Abstract

Replacement, Reduction and Refinement, the 'Three Rs' of Russell & Burch, are accepted worldwide as fundamental to the ethics of animal experimentation. The production, care and use of genetically-altered animals can pose particular challenges to the implementation of the Three Rs,[1] necessitating additional considerations by those responsible for overseeing the ethical use and appropriate care of animals involved in science. The International Council for Laboratory Animal Science brings representatives of the international laboratory animal science community together to recommend acceptance of guidance documents. The harmonization of guidance concerning genetically-altered animals was seen as a priority because of the increasing globalization of research involving these animals.

Keywords

Genetically-altered animals, reduction, refinement, breeding colonies, experimental procedures

The International Council for Laboratory Animal Science (ICLAS) harmonization committee reviews national guidelines relating to the care and use of animals for scientific purposes. In reviewing the guidelines, the Committee develops international guideline principles, and recognizes those guidelines which are suitable for implementation internationally. International guidance for animal care and use is important to facilitate the conduct of appropriate animal-based science on a global level and to protect the welfare of animals used in science.

The ICLAS harmonization committee decided that the increasing numbers of genetically-altered animals being used in science warranted consideration of guidance currently available worldwide related to the production, care and use of genetically-altered animals. The aim was to derive general principles and collect reference documents which may assist in improving the quality of scientific data and the wellbeing of these animals.

A working group was initiated at the Third International ICLAS Meeting for Harmonization of Guidelines, held during the 10th Federation of European Laboratory Animal Science Associations (FELASA) meeting in Cernobbio, Italy, in 2007. The working group also met in November 2008 at the Fourth International ICLAS Meeting for Harmonization of Guidelines held in Indianapolis, USA during the 58th American Association for

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Laboratory Animal Science (AALAS) meeting. All other work was conducted electronically, including distribution of the documents for peer review by experts during 2009/2010. The ICLAS Working Group on Harmonization: International guidance concerning the production care and use of genetically-altered animals was finally accepted by the ICLAS governing board in Bangkok, on 9 October 2012.

Technologies that enable the targeted manipulation of the genome have created new opportunities for studying the role and interplay of specific genes both in the regulation and function of physiological and behavioural processes and in the development of pathological conditions. Through the development of new or novel animal models, these techniques enable new insights into the molecular basis of disease processes and provide opportunities for developing targeted therapeutic approaches.

In recent years, there has been a rapid escalation in the development of new genetically-altered animal models. In the biomedical sciences mice are by far the most often used, but other species have also been genetically altered, including zebrafish, pigs, rats and non-human primates. The pace and scope of the development of new genetically-altered animal models are likely to continue for the foreseeable future, presenting logistical challenges for the effective management of these animals, especially when this involves significant numbers of animals and many strains or lines with differing phenotypes.

The generation, breeding and use of genetically-altered animals present particular ethical and welfare challenges. Primarily these relate to the large number of animals that are required to establish a genetically-engineered animal line, the procedures involved in creating new strains, and the potential for unanticipated pain and or distress, resulting from the genetic modification. Many genetically-engineered animal lines once created do not exhibit any particular welfare problems. However, in those cases where welfare is compromised, the concerns may arise from any of the three stages involved in the establishment of genetically-altered animal models: development, production and use.

Since the first steps in the development of genetically-altered animals, there have been several advances in genetic engineering techniques and in the care of these animals, which if properly employed should assist in minimizing any potential pain and distress. Examples include the development of techniques to generate conditional modification of genes, localizing the deletion or activation of a gene to a particular developmental stage or a particular tissue. In addition, it is now possible to create genetically-altered animals using a single, predefined insertion site, which has well-understood effects.[2–4] Examples of improvements in care include the validation of tools to evaluate the welfare of genetically-altered animals and a greater awareness of the risk of unforeseen complications and of the need to develop strategies to manage and report these events; a better understanding of the role of the housing environment in promoting animal welfare; and the development of a number of relevant guideline documents and reports.

There are other broader issues related to the production and maintenance of genetically-engineered animals that require consideration, such as issues relating to biosafety and biocontainment. A risk assessment should be carried out prior to the generation of a new animal line to consider the potential impact of the use of recombinant deoxyribonucleic acid (DNA), and the potential for the release of the genetic material into the environment. Where there is any potential for impact on human or environmental health, the relevant regulatory agencies should be contacted, and may require certain conditions to be fulfilled, prior to the commencement of the study (see for example FDA Guidance for Industry: Regulation of Genetically Engineered Animals Containing Heritable rDNA Constructs [USA];[5] and the Gene Technology Act 2000 [Australia]).[6]

Issues to be considered in the production, care and use of genetically-altered animals

1. Appropriateness of an animal model

1.1. Prior to the generation of a genetically-altered animal, the appropriateness of the generation of such an animal should be established taking into consideration both scientific as well as animal welfare/care concerns

It is recognized that the 'advancement of scientific knowledge is important for improvement of human and animal health and welfare, conservation of the environment and the good of society. Animals play a vital role in these scientific activities and good animal welfare is integral to achieving scientific and educational goals'. Animals should only be used when necessary and only when their use is scientifically and ethically justified.[7]

Many countries have a requirement for ethical review and approval prior to the generation of genetically-altered animals. Ethical review should involve the consideration of whether the generation of a genetically-altered animal is the only and ethically acceptable means of addressing the research question.

Weighing the predicted value of research against the potential effects on animal welfare is of particular relevance when genetic engineeering and cloning are involved, because:

- other animals may need to be generated in order to produce animals of the required genotype;
- although it has been suggested that the use of genetically-altered animals may be a Refinement due to the increased accuracy of the animal model, a significant number of animals are required to generate a new animal line, thus bringing the principles of Refinement and Reduction into conflict;
- the integrity of animals may be affected by genetic engineering and cloning in that the nature of the animal and how it interacts with other individuals and its environment may be altered;
- the impact of genetic modification on population robustness and wellbeing may be important for particular species of animals; and
- the unpredictability of phenotypic expression may result in an adverse animal welfare impact.

While there is a need to balance the cost to the animal and the benefit of the research, the possibility of adverse impacts on animals or the environment may be regarded as unacceptable even if there is a significant research benefit.

2. Generation of new animal lines

2.1. A new animal line should not be generated if similar suitable animal lines are reasonably and practically available, or if a relevant in vitro method can be employed to address the same scientific question. Where new lines are generated the most effective genetic engineering method that provides the best opportunities to achieve the goals of Refinement and Reduction of animal use should be employed

A significant number of animals are required to generate a new animal line. While it may be important to compare the same genetic modification on different background strains, the generation of new animal lines should not be undertaken if the research question can be answered through the use of animal lines already available to the investigator. The generation of new animal lines also involves a certain degree of pain and distress for the animals used (e.g. the embryo donors, stud males and surrogate dams), which can be circumvented by the use of animal lines already in existence. Section 5.4 includes a table of examples of repositories of animal lines, which may be consulted to determine whether a suitable line already exists. This table is not an exhaustive list of databases. In addition, searching the scientific literature may also identify published animal lines.

In many cases, methods used to introduce a new DNA construct into the germline of an animal do not control the site in the genome where the construct will insert. The site at which the construct is located can affect the health of the animal (as well as the effectiveness of the construct); leading to uncertainty about the level of pain and distress that may be experienced by the first genetically-engineered animal of the line (founder), as well as subsequent generations.

There may be difficulties in accessing new technologies; however, problems related to intellectual property, patents, etc. should not be given priority over strategies to achieve Refinement or Reduction.

2.2. The effectiveness of techniques used for genetic engineering of animals should be established through benchmarking

Genetically-altered animals can be produced using a variety of different methods. Decisions as to which techniques to use depend primarily on the species and the type of genetic modification required. The techniques chosen should aim to minimize the impact of the modification on the animal, for example by the use of conditional or inducible mutations.[8] Robinson et al.[9] have provided a list of targets and intervention points for the production of genetically-altered mice using different methods. These targets provide a means of distinguishing between the inherent wastage of animals due to the inefficient nature of the technology, and the wastage of animals resulting from poor technique, lack of skill or carelessness.

Anaesthesia and analgesia should be used for any surgical interventions.

2.3. The least invasive methods of tissue collection for genotyping should be used

FELASA Working Group on Refinement of Genotyping Methods in Rodents has developed recommendations for these procedures, while emphasizing the refinement and harmonization of the techniques, aiming for better science and better animal welfare.[10]

The use of oral or faecal samples should be encouraged as non-invasive sources of DNA for genotyping. For mice, tissue from ear clipping should be used in preference to tail tipping. Only when there are scientific or practical reasons that ear clipping is not suitable should tail tipping be considered. For mice, based on FELASA guidelines,[10] tail biopsies should be performed preferably between the ages of 14–17 days. In older animals the benefits of using analgesia or anaesthesia should be evaluated.

3. The care and maintenance of genetically-engineered animal lines

3.1. Good communication between the scientific team and the animal care team, including the veterinarian, should be established prior to the generation of a new animal line, or to the arrival of a new animal line within an institution

Large numbers of animals are required to maintain breeding colonies of genetically-engineered animals, in particular those species with relatively fast generation times (i.e. mice and zebrafish). Good communication (ideally encouraged by an ethical review system) involving members of both the scientific and animal care teams is needed to ensure that sufficient space will be available to house the anticipated numbers of animals, prior to their generation or arrival within the facility. In addition, the maintenance of genetically-altered animals requires additional time and resources from the animal care staff. Adequate care and accommodation includes not only appropriate housing (which may involve the use of bioexclusion systems) but also environmental complexity both to improve animal welfare[11] and also to facilitate the identification of abnormalities in the phenotype of the animals, while recognizing that such environmental changes may also alter the progression of a disease;[12] and also requires preparation for likely interventions resulting from the genetic modification (for example genetically-altered mice with facial malformations may require softer food) as well as preparation for pain/distress mitigation.

3.2. The care and maintenance of genetically-altered animals requires the involvement of sufficient, adequately trained animal care staff, in particular for carrying out welfare assessments (see Section 4.1 below)

3.3. Due to the considerable numbers of animals involved, and the potential difficulties in establishing and maintaining genetically-engineered animal lines, good breeding colony management is essential (including cryopreservation)

Maintaining a genetically-engineered animal line by breeding heterozygotes produces non-genetically-engineered wild-type animals, hemizygotes/heterozygotes and homozygotes. Unless the wild-type animals and heterozygotes are required for comparison with the mutant homozygote, surplus animals are generated. In some cases, where there are no severe adverse phenotypes observed in the homozygotic state, it may be preferable to maintain the line through breeding homozygotes. Typically however, it is preferable to maintain genetic diversity by maintaining the line through breeding heterozygotes. See Rülicke *et al.*[13] for details on the maintenance of congenic strains, and the potential impact of the background strain of animal on the phenotype (mice).

Cryopreservation should be used where possible to (1) provide backup, ideally multiple, in case of catastrophic loss of a genetically-engineered animal breeding colony, (2) overcome problems associated with loss of fertility due to ageing, (3) archive a genetically-engineered animal line which is not currently being used, or (4) prevent genetic drift. Cryopreservation may involve freezing sperm, and ovaries as well as embryos. While the use of cryopreservation is becoming more standard, there may be issues with the retrieval of complex constructs and particular lines.

4. Welfare assessment and humane endpoints

4.1. A practical, strategic approach to species-specific welfare assessments and on-going monitoring based on risk should be implemented

The description of the phenotype of the animal should be complemented by the establishment of animal welfare indicators.[14] As described in Section 2.2 the techniques employed to create the genetic alteration, such as use of conditional or inducible promoters, should aim to minimize any detrimental effect on the animals' phenotype. The phenotype description should provide an accurate time course and characteristics of a phenotype with relevant indicators of potentially negative effects on an animal's welfare. This should facilitate the determination of specific settings for interventions to ameliorate negative effects or to implement humane endpoints, including euthanasia.

Wells *et al.*[15] proposed a structured assessment of the welfare of new genetically-altered lines in order to create a 'welfare profile' so that once a line is established, monitoring would focus on welfare indicators specific to that line. The assessment should also ensure that expected and unexpected phenotypes are identified and should extend to longer-term monitoring and include crosses and change of background strain. Continuation of monitoring/surveillance of genetically-altered animals and lines is important as problems may not be exhibited in the initial period of observation. It is also important to take account of the construct used and the background strain.

Adequate welfare assessment requires considerable human resources. Therefore, it is important to employ the precautionary principle and to focus on those animals where there is reason to believe that there will be a detrimental impact on their welfare.

Proposals to create a genetically-engineered animal line present a unique set of problems with regard to establishing humane endpoints. For animals with a well-characterized genome, principal investigators are

expected to list anticipated experimental outcomes and describe how situations involving pain and distress will be addressed (as outlined in the ICLAS harmonization of guidelines on endpoints[16]). Appropriate endpoints should be established before the generation of genetically-altered animals, recognizing that some phenotypic effects may not be known in advance. The endpoints should then be revised in light of the observed phenotypic characteristics, particularly those with a negative impact on the animals' welfare. These agreed endpoints should also become part of the documentation accompanying the animal strain, so that institutional ethical review committees have the information available when considering protocols involving the use of these animals. The *CCAC guidelines on: choosing an appropriate endpoint in experiments using animals for research, teaching and testing*[17] may assist in establishing endpoints. When pain and distress cannot be mitigated, and are not the object of the approved protocol, the animals should be euthanized.

5. Information capture and data sharing

5.1. At the institutional level good record keeping practices should be implemented, including use of the correct nomenclature and of breeding performance and animal usage data to minimize potential for production of animals surplus to requirements

It is essential to use an effective standardized nomenclature system. Rigorous identification of genetically-engineered rodent lines is indispensable if investigators are to understand and interpretate findings in the animals they are working with. It enables precise communication of scientific findings arising from research involving transgenic animals. In addition, it facilitates incorporation of information concerning the animal model into databases and its subsequent retrieval to avoid unnecessary repetition of models already available.[13]

5.2. Investigators should properly document the phenotypic and welfare characteristics of the genetically-engineered animal line generated

New lines should undergo the appropriate level of animal welfare assessment. Imported lines which have not been assessed previously or phenotyped, or for which this information is lacking, should also undergo the appropriate level of assessment. Information gained from this process should be used to develop genetically-engineered animal welfare score sheets and to devise mitigation strategies, if needed. Once the score sheets and mitigation strategies have been evaluated for effectiveness by those responsible for the welfare of the genetically-engineered animals, this information may be added to the genetically-engineered animal documentation.[15,18,19]

5.3. Information concerning the quality of the animals should be collected and this documentation (animal passport) should accompany animals if transferred to another institution

Genetically-engineered animal documentation or 'passports' should contain: information on the phenotype of the animal, with indices relating to potential animal welfare concerns; any special requirements in terms of behavioural needs, feed, sensitivity to temperature, etc.; special conditions for husbandry; mitigation strategies for pain and/or distress, including humane endpoints; and method of genotyping used. For detailed examples of what to include, see the RSPCA UK document on 'GA passports: the key to consistent animal care'.[20]

5.4. The animal line should be archived and made available to others through publication, distribution and/or promotion via an online database

Investigators should archive their animal lines via the cryopreservation of gametes (embryos, sperm and ovaries). Details of why and how to archive animal lines are provided by RSPCA UK in their document on 'Sharing and archiving of GA mice'.[21]

In addition, investigators should publicize the availability and characteristics of their genetically-engineered lines in international databases so that animal lines are not unnecessarily duplicated.[14,22] Further, comparative analysis of normal strains versus genetically-engineered strains in susceptibility to disease, stress and reproductive abnormalities should be reported. Generation protocols should include a resource sharing plan, indicating how the animal model will be made available to the broader research community (for example: when the line will be made available [after publication of a first paper or the end of the project, whichever is first]; how the line will be made available [through repository, through his/her laboratory, through his/her institution, etc.]; and the method of publication [through repository, through IMSR, through GenBank, http://www.ncbi.nlm.nih.gov/Genbank/, etc]).

The following table gives examples of repositories of animal lines (table adapted from Rosenthal & Ashburner):[23]

Species	Database	URL
Zebrafish	Zebrafish Model Organism Database	http://zfin.org
	Zebrafish International Resource Center	http://zebrafish.org/zirc/home/guide.php

(continued)

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Species	Database	URL
Mouse	Mouse Genome Database (MGD)	http://www.informatics.jax.org
	Mouse Phenome Database (MPD)	http://www.jax.org/phenome
	International Mouse Mutagenesis Consortium (IMMC)	http://www.informatics.jax.org/mgihome/other/phenoallele_commun_resource.shtml
	Trans-NIH Mouse Genomics and Genetics Resource Coordinating Group	http://www.nih.gov/science/models/mouse
	International Mouse Strain Resources Initiative (IMSR)	http://www.informatics.jax.org/imsr/index.jsp http://www.findmice.org//index.jsp
	International Mammalian Genome Society	http://www.imgs.org/news.html
Rat	Rat Genome Database	http://rgd.mcw.edu/wg/physgenknockouts
	The national bioresource project for the rat in Japan, NBRP	http://www.anim.med.kyoto-u.ac.jp/nbr/
Farm animals	Databases for farm animals generated for the purposes of biopharmaceuticals or production of proteins are limited; however, published reports or networking through international organizations may assist in minimizing duplication of effort	

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