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Genetically modified animals in the biomedical sciences: The challenge of rapid advances & ethical demands

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Abstract

Technologies which permit targeted manipulation of genetic material not only provide new opportunities to explore the organization, regulation and biological or pathological function of molecular processes but, in so doing, have revolutionised the development and validation of animal models. In a very short time, we have seen rapid escalation in the development of new models, most often in mice, and the pace and scope of these changes is likely to continue for the foreseeable future.

With the introduction of these technologies, community concern focused on the risks to human health and the environment. Thus, public policies seek to restrict access to or release of such genetically modified (GM) organisms including GM animals or animals infected with GM material; agreed criteria define the risk and thus the level of containment which determines the conditions of housing and animal husbandry.

Despite the potential benefits, there are significant ethical and logistic challenges in the development and management of GM animals with implications for both their welfare and scientific outcomes.

This paper will explore key issues around the demand to house, manage and monitor large numbers of GM animals, the definition of GM animal models, and the management of models where welfare is compromised.

Keywords: genetic modification, animal models, 3Rs, housing

Introduction

The development of technologies which permit the targeted manipulation of genetic material has enabled the possibility of exploring new frontiers in biomedical and agricultural sciences including the development of new therapies targeting specific disease processes and species of a prescribed genetic background to enhance productivity and disease resistance. We also have seen a rapid escalation in the development of animal models which target specific genes or genetic interactions to study the organisation, regulation, biological function and pathology of molecular processes. A range of species are used; of the traditional laboratory species, most commonly, the mouse but there is increasing use of other species such as zebra fish.

Despite the potential benefits of this technology there has been continuing public disquiet about its use (Einsiedel, 2005). A range of issues are raised including fundamental ethical questions about the use of GM technology and notions of the sanctity of life and the autonomy of the individual as well as concerns about risks to human health and the environment. Questions about the welfare of the animals involved also has been a recurring issue and has been addressed in a number of reports (CCAC, 1997; ECVAM Working Party, 1999; Boyd Group, 1999; Royal Society, 2001; Animal Procedures Committee, 2001; Robinson et al., 2003; Wells et al, 2006).

Against this background, the use and management of GM animals presents new challenges not only to address ethical and animal welfare issues but also to effectively manage burgeoning numbers of animal strains, mostly mice.

In Australia, GM technology was the subject of a Federal parliamentary enquiry in 1992 (House of Representatives, 1992) which, whilst concluding that this technology held prospects for significant benefits, recommended the development of Commonwealth legislation to govern its use. Australia has since implemented the *Gene Technology Act* (2000) and established Biotechnology Australia, a federal agency with responsibilities for the development and oversight of a National Strategy (2000) which is aligned with international standards and committed to the development of biotechnology with appropriate safeguards. This agency monitors and reports on community attitudes and concerns about the use of GM technology (Cormick & Ding, 2005). A consistent finding in these reports identifies concerns about animal welfare.

This paper discusses how the ethical and animal welfare issues which arise from the use of this technology have been addressed in Australia.

Gene technology legislation

After a number of years under a self-regulatory system, the *Gene Technology Act* (2000), was implemented as national legislation; protection of humans and the environment being the primary focus. Under this Act, institutions are accredited to use GM technologies as part of which they must establish an Institutional Biosafety Committee to advise on (i) the suitability of facilities, (ii) procedures in relation to containment and (iii) specific risks associated with a given project; risk assessment being categorised at three levels. The Office of the Gene Technology Regulator (OGTR) is established under this legislation with responsibilities for the administration of this Act including, accreditation of institutions, approving high risk projects and monitoring the activities within accredited institutions and the development and review of relevant regulations, polices and guidelines.

This legislation also mandates the establishment of several committees whose role is to advise the Gene Technology Regulator. One such committee, the Gene Technology Ethics Committee (GTEC), must include on its membership a person with expertise in animal welfare.

Thus, this legislation requires the evaluation of risks in relation to both the operation of a facility and specific details of a proposed project but also promotes consideration of the ethical issues – a separation of risk and ethics. A blurring of risk assessment and ethics has been a major concern in the management of GM issues (Levidow & Carr, 1997).

Ethical framework

A *National Framework of Ethical Principles* (GTEC, 2006) was published by the GTEC as a "national reference point to promote on-going dialogue on values and ethical principles relevant to the development of gene technology". Underpinning this document is the view that a transparent decision-making process which clearly shows the reasoning used is most likely to result in well-informed ethical decisions.

To guide our reflection on these issues, the National Framework discusses nine ethical principles including one to minimise the risk of harm or discomfort to humans or animals. Concerning the ethical issues relating to animals, it is noted that respect for animals is an agreed value in our community and, although there are differing views as to the obligations which flow from such respect, it is argued that consideration of the consequences of our actions must follow. The National Framework recognises that ethical issues in relation to the use of animals is addressed in more detail in the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes* (NHMRC, 2004); the principles in this Ethical Framework complementing the principles of this code.

Australian code of practice

The *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes* (the Code) is the national public policy which governs all use of animals for research, teaching and product testing. The Code establishes a set of principles which govern decisions as to when and how animals are used for these purposes and defines animal welfare outcomes. Through the establishment of institutional Animal Ethics Committees (AECs) the Code sets out a framework for ethical decision-making with the involvement of the wider community through AEC membership.

To be satisfied that a project is justified, the AEC must be convinced there is evidence of scientific merit and to support the need to use animals (Replacement), using the minimum number of animals to achieve valid data (Reduction) with minimal impact on their wellbeing (Refinement). Concerning studies involving GM animals, the proposal to the AEC must justify the need to create GM animals, include details of the predicted impact of introducing the new gene or altering expression of existing genes in all animals in the breeding program, identify potential side effects on parents and offspring and detail a plan to manage and monitor such effects. In cases where pain or distress is predicted, how the special needs of these animals will be met must be discussed and humane endpoints defined.

The Code also addresses the question of when GM animals transition from an 'experimental animal' to 'breeding stock' and requires that both new and introduced lines are deemed 'experimental', i.e. under a research protocol, until the stability of the phenotype is established over several generations and there is full documentation of morbidity, mortality, population health and adverse side-effects.

National guidelines

To provide additional guidance to members of AECs and researchers on the special ethical and welfare issues relevant to the use of GM animals the

Guidelines for the Generation, Breeding, Care and Use of Genetically Modified and Cloned Animals for Scientific Purposes (NHMRC & OGTR, 2007) were developed by the Animal Welfare Committee of the NHMRC, in consultation with GTEC, and published jointly by NHMRC and OGTR. These Guidelines complement the Code and, recognising that GM animals may have specific welfare needs which will extend over their lifetime and into subsequent generations, aim to promote the 3Rs in the management of these animals.

These Guidelines apply to all GM animals including laboratory species, livestock and companion animals. It is recognised that there are a number of ethical and welfare issues around the development of new GM strains, but it is noted that these have been comprehensively addressed in other recent publications (Robinson et al., 2003; Wells et al, 2006) and so are not discussed in detail in this document.

The GM Guidelines highlight a range of ethical and welfare issues, discuss matters in relation to monitoring and reporting and include specific examples of ways by which the 3Rs, Replacement, Reduction and Refinement, can be promoted.

In relation to specific ethical concerns, the GM Guidelines identify the production of large numbers of animals to achieve a desired phenotype, high culling rates, the effect of GM on the integrity of the animal including its interaction with con-specifics and the environment and the unpredictability of phenotypic expression as being major issues. Further the possible tensions between the goals of Reduction and Refinement is noted as an emerging issue.

A number of specific animal welfare issues which follow from these ethical concerns are highlighted and ways to ameliorate or manage the impact on the animal discussed. In this regard, the impact of techniques used to produce and monitor GM animals and the expression of modified or deleted genes and interactions between gene products with the potential to disrupt physiological processes or resulting in a poor fit between the new strain and its environment are noted as major animal welfare risks compounded by the unpredicted nature of these effects.

The GM Guidelines emphasise the key roles of monitoring and reporting to ensure effective management of the welfare of these animals. In relation to monitoring, the need for a whole of life approach is emphasised. Although it is recognised that the intensity, nature and frequency of monitoring will need to be tailored to the circumstances, never the less, it must include steps to actively seek identification of adverse events. Observations need to cover both anticipated and unexpected outcomes and comprehensive data to reliably assess the impact of genetic change is essential. Further, the involvement of both animal care and research staff in the monitoring program is strongly advocated.

Regular reports must be provided to the AEC which document the welfare of both the mother and offspring, including first and subsequent generations. Such reports should include mortality rates, the cause(s) of unexpected deaths and the success rate in achieving the desired phenotype. The final report to the AEC which will validate the transition of the strain from 'experimental animal' to 'breeding stock' must include a comprehensive phenotype report documenting the health, production performance and fitness of the GM animals, the nature and time of onset of adverse effects, the proportion of animals affected, and, using a range of relevant biomarkers, a comparison between the GM animals and their unmodified counterparts.

Finally, the GM Guidelines provide a number of practical examples as to ways by which the principles of the 3Rs can be applied to the production and management of GM animals. For example, using *in vitro* techniques rather than implanting a GM embryo in recipient animals, testing the effects of GM *in vitro* using embryonic stem cells, the strategic use of heterozygous or homozygous breeding to reduce the impact of GM or the numbers of animals bred, the use of knock-out/ knock-in systems to control expression of phenotypes and refinement of methods to determine genotype. In all cases a pilot study is mandated to demonstrate the effects of the proposed GM and to assess whether such animals will be suitable for subsequent studies.

Issues & challenges

There are significant logistic consequences to the rapid expansion in the development of GM animal models. Two major issues concern providing housing and care which meets species-specific needs and validation of the range of methods used in phenotypic screening so that such information is universally transportable. The following comments highlight the challenges in meeting these demands in relation to the laboratory mouse.

The escalation in the number of GM lines has created unprecedented demand to house numbers of laboratory mice with additional pressures on the capacity to manage complex breeding programs under specific bio-containment conditions. The introduction of the system of individually ventilated cages (IVCs) has been an important technological development to meet this challenge. However, questions remain as to how well the design of such systems meets species-specific needs (Barthold, 2002).

Until recently there have been few studies into how species-specific needs should be addressed in the design and management of mouse housing but there is some indication of factors which should be considered. For example, comparing four types of cages, Baumans and colleagues(1987) showed mice have a significant preference for the "Cambridge" mouse box, which, by the design of the lid, enables mice to compartmentalise their living space with a designated, protected nesting area (Wallace, 1982). Related to this is the evidence that mice show a strong tendency to build nests, most often under the feed hopper (Sherwin, 1997). Also relevant to cage design, is the observation that climbing on the cage lid is a significant component of their locomotor activity (Buttner,1991).

Two recent reports into the effects of cage design on emotionality in mice have highlighted the need to give serious consideration to these issues. Pietropaulo et al (2007) found that depriving mice of climbing behaviour by not using a grid roof results in impaired fear-conditioned learning in mice of both sexes and anxiety behaviours in females. Kallnik et al (2007) found that IVC housing, compared with conventional boxes, enhanced anxiety-related behaviours in male mice. The findings of effects on emotionality in female mice may have further implications given the evidence of the effects of maternal stress on the behaviour and emotionality of offspring (Wurbel, 2001).

It is common practice to provide mice either with materials to build a nest or a structure such as a tube or an igloo as a shelter. Whether or not the solid structure as a functional shelter is a satisfactory alternative to nest building activity is not known. However, a recent study which showed significant differences in the emotional and sensory responsiveness of two strains of mice associated with their being given a cardboard tube or a plastic mouse house (Tucci et al., 2006) indicates the urgent need to further investigate these kind of questions.

There have been several major international initiatives to standardise the methods used and reporting of phenotypic expression in GM mice. However, recent reports have identified major challenges in achieving consistency between laboratories (Wahlsten et al., 2003) and the significance of gene-environment interactions, emphasising the influences of the laboratory environment (Chesler et al., 2002).

Given the emerging evidence of the important, and often subtle, effects of gene-environment interactions and their significance to both the design of housing systems and the validity of phenotypic screening, there is an urgent need to address these key issues to inform both animal welfare and scientific outcomes.

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