Supplementary resources for lay members of Local Ethical Review Processes

Projects involving genetically modified animals

Natasha Lane
Maggy Jennings

RSPCA
Research Animals Department
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The RSPCA and the Ethical Review Process

The RSPCA as a matter of policy is opposed to all experiments or procedures that cause pain, suffering or distress to animals including those involving the manipulation of the genetic constitution of animals. As in other areas of its work, the Society adopts a constructive, practical approach to this issue, developing and promoting initiatives that lead to greater application of the 3Rs – replacing animals with humane alternatives, reducing animal use and refining husbandry and procedures to reduce suffering and improve welfare.

The RSPCA is a long-standing advocate of Ethical Review Processes (ERPs) as a means of promoting ongoing consideration of ethical aspects of animal use, wider involvement in decisions regarding the justification for animal use, and advancing more active implementation of all of the 3Rs. This document is one of a series of resources that are being developed to facilitate the work of ERPs, and particularly lay members, in the UK and equivalent bodies worldwide.

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Contents

1 Introduction

This guidance is a supplement to the ***Lay Members' Resource Book*** (Smith and Jennings 2003, available from erp-laymembers@rspca.org.uk) and is part of a series of resources being developed for members of local ethical review processes (ERPs), or their equivalent, to help facilitate constructive ethical review of animal experiments. This supplement specifically addresses research involving the genetic modification of animals; more general ethical and animal welfare issues are dealt with in the main resource book.

The use of genetically modified (GM) animals in scientific research has increased exponentially over the past ten years. Fewer than 75,000 procedures involved GM animals in 1992 whereas that figure was over 700,000 in 2002. This has occurred against a general decline in the overall numbers of animals used in scientific experiments. GM animals are considered to be powerful research tools by scientists and they are used to try to address a wide range of scientific questions (see Section 2.4). It is expected that the use of GM animals, and GM mice particularly, will continue to increase in the life sciences and hence there will be an increasing number of GM animal projects coming to the ERP for review**.** The technology can be complex and its application and related terminology can all be difficult to follow for non-specialist members of ERPs - a point made by many of the lay members who attend the RSPCA Lay Members Forum.

This supplement aims to facilitate the ethical review process by providing an easily accessible guide to the technology and terms relating to GM animals (see Section 2). It describes the animal welfare issues specific to genetic modification (see Section 3), and then provides more detail of practical ways of implementing the Three Rs, again specific to GM animals (see Section 4). It does not attempt to describe all the likely uses of GM animals. This would be a daunting task and there are useful summaries of information available elsewhere. Nor does it discuss the general issues relating to the ethical aspects of animal experiments, the cost/benefit assessment and the 3Rs, since these have already been covered in the Lay Members Resource Book.

The focus throughout the document is on mice, since these are by far the most common animals to be genetically modified, but much of the information is applicable to other species and some implications for these other species are also discussed.

The document is designed and produced in such a way that it can be easily updated and we would appreciate your comments to help us make it as useful as possible. Please send these to **erp-laymembers@rspca.org.uk**

1.1 A key to the presentation of material

Similarly to the *Lay Members' Resource Book,* the text contains boxes (blue outlined) and italicised red arrow bullet points.

Blue outlined boxes list points to think or enquire about when:

I. participating in review of projects involving GM animals, or

II. reviewing the systems in place at the establishment that relate to GM animals.

➤ *Italicised red arrow bullet points are intended as information or action points. These provide information or make suggestions for practical steps that ERP members can take to obtain more information, devise questions and/or think further about the issues raised within the ERP and their role within it.*

A **glossary** containing some of the commonly used genetics terms is provided in Appendix A. These terms are highlighted in blue where they are first found in the text.

2 Guide to the technology and terms

The aim of this section is to provide sufficient background information to help non-specialists become familiar with the processes involved in the creation, care and use of GM animals and so more easily follow the information given in the project licences they are asked to review. It covers:

- the genetic status of animals used in research;
- common terminology;
- methods of producing GM animals;
- why the research is done;
- how the ASPA applies to GM animals.

Animal welfare issues are addressed later, in Section 3.

2.1 The genetic status of animals used in research

Animals used in research are either **normal (inbred or outbred), genetically modified (GM)** or **mutant.**

2.1.1 Normal animals

Normal animals are exactly that – they have a normal genotype (gene structure) and phenotype (appearance) and are often described as "wild-type". In the laboratory normal animals are often highly inbred, in that, in the case of mice, brothers have been mated with sisters over many generations, leading to the formation of many different strains. Different inbred strains often have distinct characteristics, but within each inbred strain individuals are very similar in their genotype and phenotype. Scientists argue that this similarity reduces experimental variation associated with differences between animals and so fewer animals will be needed to gain statistically significant results in experiments.

Outbred animals are the result of breeding programmes that try to minimise or avoid breeding between highly related individuals. They are less related to each other than inbred animals and have more diverse genotypes and phenotypes.

2.1.2 GM Animals

GM animals are derived from normal animals. They arise from embryos whose DNA (genetic code) has been altered by;

- adding **extra copies** of a normal gene or DNA sequence;
- **adding new DNA** or a gene that would not normally be present; or
- **removing normal DNA** (knock-out) or **altering normal DNA**.

Once a GM animal has been produced, it is often possible to breed from that animal to generate a line of similar GM animals. All of the animals in the line will have similar genotypes and phenotypes because they are normally bred as inbred strains.

Although their genetic material has been altered, many GM animals look and behave exactly the same as the normal animals they were derived from; i.e. they have the same phenotype as normal mice. However, the phenotypic effect of genetic modification ranges from no effect, or just very subtle differences, right through to more obvious and dramatic differences.

2.1.3 Mutant animals

Mutant animals are also derived from normal animals. They have one or more genetic changes in their DNA; either naturally occurring or artificially induced.

- **Naturally occurring mutants** carry a random genetic error that arose spontaneously in their DNA.
- **Artificially induced mutants** carry random genetic error resulting from the deliberate administration of mutagenic chemicals or exposure to radiation.

All animals, including humans, are born with some naturally occurring random mutations in their genome and it is largely due to these mutations that species can evolve. Most natural mutations make no difference to the overt phenotype of the animal. However, over the years many animals have been born with mutations to their DNA which have brought about phenotypic changes that are interesting to scientists and they have been bred as lines of natural mutants.

To generate mutant animals artificially, an adult male is treated with chemicals or radiation that causes random genetic mutations in his sperm. The treated male is then mated and some of his offspring inherit the random mutations in their genomes. Many of the inherited mutations lead to no change in phenotype and the animals appear normal, but some of the offspring will have phenotypes that scientists want to study further and a line of similar animals will be bred from them.

2.2 Common terminology

- The terms **genetically modified** and **transgenic** are often used interchangeably, although strictly speaking an animal is only transgenic if DNA from another strain or species has been added to their genome.
- The terms **line** and **strain** are also used interchangeably, but in this document ‘strain’ will be used to refer to an inbred but non-GM population and ‘line’ will be used when referring to a GM population.
- The Home Office has introduced a new term - **‘genetically altered’ (GA**), which includes all GM animals, mutants (both natural and artificially induced), and animals resulting from nuclear transfer (cloned animals - see Section 2.3.3).

The focus of this resource is on GM animals, but much of the information equally applies to mutant animals.

➤ *Appendix A contains a glossary of some commonly used terms. Further explanations of terms can be found in:*

- *The 6th report of the Joint Working Group on Refinement – 'Refinement and reduction in production of genetically modified mice' Robinson et al., 2003*
- *The National Institutes of Health (NIH), USA, genetic glossary site* - www.genome.gov/10002096

➤ *Terminology in the whole area of genetic modification is often difficult, for some biologists who are outside of the genetic field as well as lay persons. Even summaries can be jargon-laden. Therefore do ask for clarification of any term – this is likely to be helpful for some other members of the ERP as well.*

2.3 Methods for creating GM animals

The method selected to create animals with genetic changes depends on the type of genetic modification that is required. It can also be influenced by available expertise and equipment in the establishment, time constraints and cost.

Two of the most commonly used methods of producing GM animals are:

- **pronuclear microinjection** (often simply called microinjection), and
- **gene targeting** in embryonic stem (ES) cells and blastocyst microinjection.

Other methods are also used to introduce new DNA into cells, including use of viruses and liposomes. Another technique, known as RNA interference (RNAi), is also becoming more frequently employed. However, only microinjection and gene targeting are described here, because they are currently the most widely used.

➤ *Different techniques have different success rates, involve different numbers of animals and are generally used to answer different scientific question. This has a bearing on questions about the possibility of reducing numbers and therefore it is important for the ERP to know what the proposed method of genetic modification is and why that method has been selected.*

➤ *A helpful introduction to RNA interference can be found at* www.wellcome.ac.uk/en/genome/thegenome/hg02f003.html

2.3.1 Pronuclear microinjection

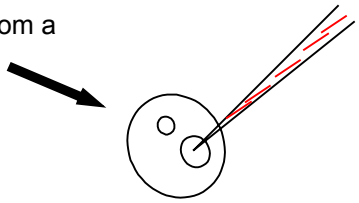
This method of producing GM animals involves injecting a small piece of DNA directly into a recently fertilised egg. The method is illustrated in Figure 1, Page 11. Female mice are mated so that they produce fertilised eggs; often they are given hormone injections to increase the number of eggs obtained. The females are then killed and the oviduct (which contains the eggs) removed. The eggs are then collected

by flushing them out of the oviduct with culture medium. A piece of DNA called a construct, or transgene, which has been made in the laboratory and contains the DNA sequence of interest, is then injected into one of the two pronuclei (where the DNA is located in the egg), hence the name of the technique - pronuclear microinjection. The injected fertilised eggs are then surgically implanted into new females who act as surrogate mothers.

Only some of the animals born will be successfully genetically modified (typically between 5 and 50%) because the injected DNA construct integrates into the DNA of the fertilised egg randomly, often in multiple copies, but sometimes not at all. It is important to know which these animals are, so to detect whether or not the genetic modification has been successful a small piece of tissue is taken from each of the offspring and their DNA is analysed for the presence, location and number of copies of the construct. Those animals that contain the modification, and at the desired copy number, will then be bred to produce a line of GM (or transgenic) animals. The rest will be killed.

Figure 1. Pronuclear Microinjection

Fertilised eggs are taken from a female



The new gene is injected into the fertilised eggs (also called one-cell embryos)

The injected embryos are surgically placed into the uterus of another female (surrogate mother)

A few of the mice born will have the added DNA in all of their cells. A piece of tissue is analysed from each offspring to test for which ones contain the added DNA.

Those with the new gene are called transgenics. They are bred from and their offspring are used in experiments.

2.3.2 Gene targeting in embryonic stem (ES) cells

This method is illustrated in Figure 2 overleaf. Gene targeting in embryonic stem (ES) cells, like pronuclear microinjection, also involves a DNA construct. But instead of the construct being injected into fertilised eggs, it is put into ES cells - cells originally derived from very early mouse embryos and grown in culture. ES cells retain the ability to turn into all the different tissue types of the animal, e.g. blood cells, muscle and liver, but cannot form a whole new animal on their own.

The construct integrates into a specific point in the genome of the ES cells. It integrates correctly because its ends have been made with the same sequence as the point in the genome where it is wanted (termed homology), which helps to 'target' it to the correct spot. Those ES cells which have the construct inserted at the correct site are correctly 'targeted' and can be selected whilst they are still growing in culture. The ability to target the DNA to a specific point in the genome, and in a single copy, provides some advantage over pronuclear microinjection, as the resulting modification is more specific and less likely to produce side-effects in the animals.

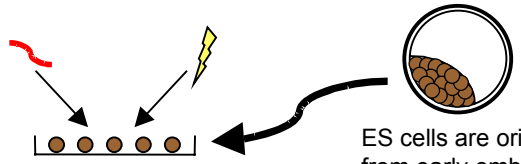
In the next stage of the process pregnant females are killed 3.5 days after mating and their early embryos are removed. Correctly targeted ES cells are injected into these embryos, which are then surgically placed into the uterus of surrogate mothers. The targeted ES cells and the injected embryos are obtained from animals of strains which have different coat colours as well as different genetic backgrounds. When the animals are born it is usually possible to see immediately which animals have a high contribution from the targeted ES cells because they will have a mixed coat colour. This makes it easy to tell when the experiment has worked well.

The two-coloured animals produced are called chimeras. If the ES cells have contributed to the sex cells (germ cells) of the chimera, then their offspring can inherit the genetic modification and will have the modification present in all of their cells. This is what the researchers are aiming to achieve.

Approximately 15 to 25% of manipulated embryos survive to birth. Success rates vary, but about 50% of the mice born are chimeric and about half of these transmit the modification to their offspring. The additional mouse generation, and additional breeding required to obtain fully GM animals, is one disadvantage of using an ES cell based approach over pronuclear microinjection.

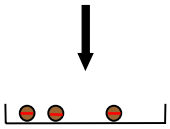
True ES cells – those that can contribute to all the tissues of the new animal, including the germline, have only been demonstrably obtained in mice. Therefore, making GM animals by ES cell gene targeting is, so far, only possible in mice.

Figure 2. Gene targeting

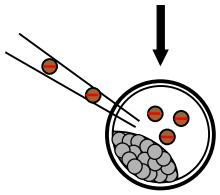


DNA sequences are introduced to the ES cells

ES cells are originally derived from early embryos. They can be grown in culture for many cell divisions whilst still retaining their ability to turn into all the different cell types



Cells that have the new DNA correctly added (targeted) are selected



Females are mated and killed in order to provide early embryos (blastocysts)

The targeted ES cells are injected into a new blastocyst

Injected blastocysts are implanted into surrogate mothers

Some of the offspring are chimeras, as a proportion of their cells are derived from the injected ES cells. Chimeras with a large proportion of coat colour (hence genetic contribution) derived from the injected ES cells are bred

A fraction of their offspring will have the modification in all of their cells

2.3.3 Making GM animals by reproductive cloning

In reproductive cloning, a new animal is made from a single cell (e.g. a skin cell) of another animal (either an adult or an embryo) and the two animals are therefore genetically identical. The process involves removing the nucleus (containing the genetic material) out of the adult cell, and putting it into an egg cell that has had its own nucleus removed – this is called nuclear transfer. The eggs are taken from females who have usually been killed, either in the laboratory or, often in the case of livestock, in an abattoir. Once the adult or embryo cell's nuclear material has been added to the recipient egg, development into an embryo is artificially started by using an electric current, and the embryo is then placed into a different female animal who acts as a surrogate mother. The process is highly inefficient in terms of the numbers of manipulated embryos required to obtain a cloned animal. Nevertheless, since the birth of Dolly the sheep in 1996 (the first animal cloned from an adult cell) a wide range of species including cows, horses, pigs, goats and cats have been cloned.

It is possible to make targeted genetic changes in the normal cells growing in culture before their nuclei are removed and put into the eggs. The cloned animals are then also GM. Cloning by nuclear transfer is therefore one way to introduce targeted genetic changes into the DNA of species for which there are no good ES cells. This currently applies to all species other than mice.

2.4 Reasons for the genetic modification of animals

The range of applications of GM technology in the biosciences is enormously diverse. The technology is considered to be a powerful 'tool' by scientists and there are very many different reasons for creating genetically modified animals, far too numerous and specific to go into in this document. However, a fundamental principle underlying the use of GM mice in research is that mice and humans (and in fact all mammals) are genetically very similar; nearly every human gene has a mouse equivalent. Scientists can find out more about the function of genes in general by studying them in mice and then apply this knowledge for a variety of purposes.

Note: This document does not set out to comment on the scientific validity of using GM animals, i.e. whether the intended benefit is likely to be achieved or whether the approach suggested is the best for the question being asked. Nor does it address the justification for GM animal creation and use. These are questions that must be reviewed on a case by case basis for each and every application.

Some of the broad purposes for genetically modifying animals given by scientific investigators include:

- **answering questions relating to gene function and regulation**. The normal genetics and physiology of mice is well documented, so removing a gene with unknown function may lead to changes in the mouse's physiology that indicates

what the removed gene actually does. Also, studying DNA sequences involved in turning genes on and off can help to add to the overall understanding of how gene expression (gene activity) is controlled during animal development and in response to the daily demands placed on cells.

- **identifying and understanding the causes of disease**. Creating a GM animal by deleting or altering a gene thought to be involved in human disease may help to show whether and how the gene is involved.
- **creating animal models of disease to evaluate potential therapies**. Giving a potential new drug or treatment to a GM mouse showing signs of a particular disease may help to evaluate whether it is effective.
- **providing 'improved models' for use in toxicology or vaccine testing.** Creating animals who are particularly susceptible to particular chemicals or diseases may help to reduce both the duration of experimental procedures and the number of animals used. In addition, it may be possible to 'humanise' mice, for instance by adding genes present only in humans, in order to make them more predictive models.

New areas of research and new methods of GM animal production are being investigated all the time and this can make it hard for non-specialists to gain a good understanding of the purpose – and hence intended benefit – of the individual project licences coming to the ERP for review.

2.4.1 Reasons for the genetic modification of species other than mice

Although mice are by far the most commonly genetically modified animal, the ERP may encounter projects involving the modification of a wide range of other species. For example zebrafish (*Zebra danio*) are commonly used to look at mechanisms of development and gene control, as are *Xenopus* frogs. Genetic modifications that were only possible in mice are now becoming possible in rats and so the use of GM rats is set to increase, particularly in fields such as cardiovascular and behavioural research. GM rabbits may also be used where larger animals are required, for example in some experiments involving surgery.

The reasons for genetic modification of larger (livestock) animals are more likely to relate to increasing their productivity or to enable study of a characteristic or disease common to that species, rather than to involve fundamental investigation of gene function. For instance, there are several reasons why scientists might argue that it is important to produce GM livestock, including to:

- provide a better understanding of disease processes and mechanisms of disease resistance in livestock;
- remove a gene linked to a harmful disease - such as the Prp gene involved in the sheep disease scrapie;

- produce pharmaceuticals in their milk, for example blood clotting factors in sheep's milk to treat haemophilia or cow's milk nutritionally enhanced to be more like human milk; or drugs for humans produced in chickens eggs;
- increase their productivity, e.g. by increasing growth rate of farmed salmon or the quality of sheep's wool;
- decrease the likelihood of rejection of source organs or tissues for animal to human transplantation (xenotransplantation).

➤ *It is important to ensure that you are clear about the reasons the research is being done, what the expected benefits are, and why it is considered necessary. Make sure that this is explained in non-technical language, preferably by the researcher attending the ERP meeting and talking through his or her proposals.*

➤ *The Report of the Animals Procedures Committee (APC*): Review of cost-benefit assessment in the use of animals in research (2003) *provides further discussion of the general principles that apply to assessing scientific validity and benefit (see www.apc.gov.uk).*

➤ *Information on the potential applications of biotechnologies to livestock is provided in Clark and Whitelaw (2003). The APC's* 'Report on Biotechnology' (2001)*, provides some guidance on what are not acceptable as applications of the technology (see www.apc.gov.uk).*

➤ *General questions relating to benefits in the context of project review are given in the* Lay Members' Resource Book. *Some general questions the ERP could consider for projects involving GM animals are given in Box 1.*

Box 1: General discussion points regarding reasons for research

- What is the reasoning or driving force behind the research (for example, are the GM mice to be used to probe gene function, as models of disease or to enhance animal productivity)? The answer to this question is likely to lead to additional questions about why the research is considered valuable and necessary.
- What techniques have been used for the research up until now and, if GM animals have not been used previously, why are they to be used now?
- Why have mice (or other mammalian species) been selected as most appropriate for the research, for example over invertebrate species?
- Will the project utilise existing GM lines, or will it require the creation of new lines?

2.4.2 The impact of genome projects

The Human Genome Project (HGP) is an international effort to elucidate the entire sequence of the human genome so that the information is freely available for all researchers in all countries to use to find out more about how humans 'work' and to use this knowledge to develop treatments for medical conditions. The basic sequencing project was completed in 2003 and the human genome was found to contain in the region of 30,000 genes. The aim now is to understand the function of the genes that have been discovered. This could impact on the work of the ERP because projects developed as a part of the sequencing project may come to it for review. For instance, the Eumorphia project (www.eumorphia.org) has been set up by a group of scientists to produce genetic mutations in every gene in the genome (either in whole mice or ES cells) to find out which genes are of biomedical interest. This has resulted in, and will undoubtedly continue to lead to, the creation and use of a huge number of GM and artificially induced mutant animals, both in the UK and elsewhere. The mouse genome has also now been sequenced and other genome projects are underway including the cow, rat, dog and chimpanzee. This is all likely to lead to an increase in the creation of GM animals.[*]

2.5 The Animals (Scientific Procedures) Act 1986 and genetic modification

All production, breeding and use in procedures of GM animals is covered by the Animals (Scientific Procedures) Act 1986 (ASPA). GM animals are not classified as a special case and the ASPA applies to them in the same way that it applies to any other experimental animals - see the *Lay Members' Resource Book* for a brief summary of the legislation. However, there are several specific points with regard to GM work that need to be considered.

Firstly, there are two different types of project that may be encountered involving:

(i) the **creation** of the GM animals;
(ii) the **use** of the GM animals.

Thus a project licence may be just for production of the animals, or may involve the use of GM animals already available or obtained from elsewhere, or may cover both production *and* use.

There are several other important points:

- A licence is required to produce and use GM animals in procedures, and also to breed them. Every GM animal born is currently recorded as a procedure (as are any GM embryos and fetuses past the mid-point of gestation), even if no other regulated procedures are carried out on them.

[*] Chimpanzees are not used in the UK – there has been a voluntary ban on Great Ape use since 1997.

- The Home Office (or the ERP) may suggest that a 'stop-point' be added to the project licence just after the creation of the GM line. At the stop-point, research will not continue until the welfare of the new GM animals has been assessed. This allows the *actual* adverse effects of the genetic modification to be assessed and weighed against the predicted effects (which should be included in the licence) and against the benefits of the research once the full costs to the animals are easier to see. Suitable end-points can then be added to the project licence at this point, before the new GM line is widely bred.
- The Home Office is likely to require that the establishment and individual project licensees make a statement in the project licence to the effect that systems are in place to ensure that the welfare of new GM lines will be properly assessed (see Section 4.3.1).
- A statement on the expected efficiency of the techniques should be included in the project licence and records of efficiency should be kept. For example, percentage success rates of microinjection leading to birth of transgenic offspring should be recorded.

➤ *Additional guidance relating to projects involving GM animals has been produced by the Home Office and is available at:* http://www.homeoffice.gov.uk/docs/sub_transgenic.html

2.5.1 Ethical issues and the cost-benefit assessment

General information on how to approach ethical discussion and the weighing of harms and benefits is provided in Section 4.5 and Appendix C of the *Lay Members' Resource Book*. However, genetic modification of animals is, in itself, an issue that tends to raise strong feelings in many people. It is not possible to set out here all the relevant ethical arguments, but it is important to be aware of some of the questions raised about the technology as a whole. These include:

- Fundamental changes can be made to the genome of an animal that would not be possible, or would not readily arise in nature. The technique is therefore considered by some people to be 'unnatural' and scientists are thought to be 'playing God'.
- There may be unforeseen consequences of genetic modification, e.g. for the welfare of the modified animals, or for human health or the environment. For example, GM salmon, engineered to grow faster and larger than normal salmon, may out-compete wild salmon if they escaped into the wild. Also, greater changes in phenotype can occur in just one generation than would normally be achieved in conventional breeding, therefore there is less time to assess the implications of those changes for the animals concerned. (Note: the selective breeding methods employed in modern farming can also cause substantial welfare problems for animals and are not subject to the same controls.)

- GM animals who are created to be predisposed to disease may be literally 'born to suffer'.
- The techniques are inherently highly inefficient and healthy GM offspring are only gained from a few percent of manipulated embryos. The remaining offspring are killed and this is considered to be an ethical cost because animal life itself is of value.
- The GM animals may be created purely for a non-medical commercial purpose (e.g. goats made to produce silk protein in their milk). Such applications of the technology are comparatively rare at the time of writing.
- The application of the technology to some species (for example primates – including humans) or for some purposes (e.g. to make non-allergenic cats as companion animals) raises additional ethical questions.
- The sheer number of GM animals used in research might lead them to be regarded as laboratory consumables rather than sentient individuals, particularly in the case of the mouse, which is the species most frequently genetically modified.

➤ *A list of useful references regarding ethical aspects and differing views on GM animal use can be found in Section 5.*

The cost, or harm, benefit assessment for projects involving genetic modification should be approached in a similar way to any other project. The potential harms of genetic modification are described in Section 3. The benefit – or purpose – of projects involving genetic modification was addressed briefly in Section 2.4 and should be clearly described in the project application.

➤ *Make sure there is enough information specific to the project with respect to both the harms and the benefits to enable you to form a view and contribute to discussion.*

3 Animal welfare issues – a summary of costs to animals associated with genetic modification

In this section the costs (or harms) to animals that need to be considered when assessing and monitoring projects that involve the creation and/or use of GM animals are described. The costs are explained in relation to mice, but most are equally applicable to rats and, to greater or lesser extents, to other animals. Some specific welfare concerns for other animal species are also given.

The specific costs associated with the creation and use of GM animals include:

(i) suffering associated with the procedures used to create and maintain the GM animals;

(ii) negative welfare effects of genetic modification (either predicted or unpredicted);

(iii) large numbers of animals are used to create and maintain the GM line.

There are also the more general harms associated with keeping animals in a laboratory situation and using them in procedures whether or not the animals are GM, for example, the effects of housing and husbandry, restraint, marking for identification and euthanasia. Information on these general harms is covered in detail in section 4 of the *Lay Members' Resource Book*.

➤ *A review of the welfare implications of genetic modification of laboratory animals is provided in Buehr and Hjorth (2003) and aspects of animal welfare with respect to implications for ethical decision making are discussed in Olsson and Sandoe (2004).*

3.1 Costs associated with procedures used to create and maintain GM animals

The standard procedures involved in the creation and maintenance of genetically modified mice and their potential impacts on the animals are listed in Table 1 below. The suffering caused by these procedures can be minimised, for example by the use of appropriate anaesthesia and analgesia. However, it is not just the regulated procedures that can impact on animal welfare; every step in the genetic modification process should be carried out such that the least possible number of animals is used, with the least suffering.

➤ *See Section 4 for ways in which animal suffering can be minimised and for examples of current best practice with respect to applying the 3Rs.*

Table 1. Standard procedures used to create and maintain GM mice, with their impact on the animals

Procedure	Reason carried out	What it involves	Effects/Costs
Superovulation	To increase the number of eggs produced by the females	Administration of hormones to females by intraperitoneal injection	- Stress from handling - Pain from injection - Potential for hormonal side effects, e.g. pain - Potential stress and damage to young and/or small females during subsequent mating - Death, as females are killed to allow egg removal
Vasectomy	To gain sterile males for mating with females to induce a state of false pregnancy (pseudopregnancy)	Surgery under general anaesthesia	- Stress from handling - Discomfort or stress from anaesthesia - Post operative pain or discomfort
Embryo transfer	To place the genetically manipulated embryos into the pseudopregnant female recipients	Surgery under general anaesthesia	- Stress from handling - Discomfort or stress from anaesthesia - Post operative pain or discomfort
Genotyping	To test the genetic status of offspring resulting from genetic manipulation	Most often involves cutting a piece of the tail (tail biopsy) to use in DNA analysis. Alternatively might involve ear biopsy, blood sampling or other approach (e.g. saliva)	- Stress from handling - Discomfort or stress from anaesthesia - Potential acute and chronic pain at the time of and following cutting - Abnormal balance or gait if too much tail taken

3.2 The effects of genetic modification

Introducing genetic change has the potential to result in the birth of animals whose welfare is compromised. Several different types of adverse effect are possible, including:

- physical abnormality that directly causes pain, or causes suffering due to mental stress from an inability, e.g. resulting from limb defects, to carry out natural behaviours such as moving around freely;
- physical abnormality that indirectly causes pain, suffering or distress, such as an abnormality leading to increased sensitivity to sound;
- genetic effects that lead to disease or symptoms of disease, e.g. Huntington's Disease;
- abnormalities that compromise the animals so they are more susceptible to disease, such as abnormalities of the immune system in immunocompromised animals;
- mental abnormalities that may or may not manifest physically, for instance in performing repetitive behaviours, such as constantly spinning around (see Nelson and Young (1998) for an overview of behavioural effects).

It may be more difficult to judge harms in projects involving creation of new GM animals compared with other studies because the effects of genetic modification are not wholly predictable. So if a new line is to be created, it is important to be aware that there is always a potential for unexpected adverse effects. Effects can be very subtle and difficult to recognise so the assessment of the animal's welfare and hence identification and relief of any suffering can be difficult (information about assessing welfare of GM animals is given in Section 4). It will be easier where the project requires the use of GM lines that already exist, as information about the animal's phenotype, and hence welfare, should already be known. In addition, any harmful effects are a result of the animal's fundamental genetic make-up and may in fact be an integral part of the scientific objective, as for example in disease models. This means there is a need to carefully balance care for the animal's wellbeing with the introduction of humane endpoints, and the need to meet the scientific objectives.

All ERP members need to have a full understanding of any likely adverse effects of the modification and of the steps that can and will be taken to prevent or alleviate animal suffering. It is thus very important that the predictable adverse effects are clearly described in the relevant sections of the project licence application. It is also good practice to introduce a "stop-point" into the project licence, in which animals are assessed for unpredictable adverse effects and the harm-benefit assessment reconsidered if necessary.

3.3 Numbers of animals

Large numbers of animals are needed for the production of GM lines, both for:

- **creating** the line - as many genetically manipulated embryos fail to develop;
- **breeding** the genetic modification onto the desired genetic background and maintaining the GM line - because many of the animals created as 'by-products' of the breeding programme will be killed as surplus to requirements.

The number of animals involved may be increased by a number of factors, which may or may not be avoidable. DNA constructs can fail to function for scientific reasons, which cannot be foreseen, or because they have been poorly prepared, which is preventable. Failing to keep track of the breeding processes leading to over-breeding can also lead to avoidable increases in numbers.

Regardless of whether or not suffering occurs, the relatively high numbers of animals used during genetic modification and subsequent breeding is an ethical cost that has to be taken into account by the ERP during project review.

➤ *It is important to make sure everything possible is done to reduce the number of animals used, by investigating and following current best practice. Section 4 works through some ways in which the number of animals used can be minimised.*

3.4 Specific costs associated with genetic modification of species other than mice

The method generally employed to genetically modify all species other than mice is pronuclear microinjection. The costs to animals from the procedures are very similar for all species, as essentially the same steps are followed. However, some of the steps that require killing animals to obtain eggs or surgical intervention in mice may not require the death of an animal when larger species (such as cows) are involved. For example, eggs may be obtained from animals already killed for human consumption in abattoirs. It is also often possible to transfer the genetically manipulated embryos into host cows without using surgery due to the larger size of these animals.

Harms relating to the possible effects of genetic modification and the large numbers of animals required are universal concerns that apply to all species. However, where commercial livestock are concerned there is the potential for other kinds of harms that also need to be considered. Some examples are included below: -

- Where the intention is to increase production, the extra physiological burden on the animal could lead to stress on related organ systems or have other serious

welfare impacts. For example, engineering beef cattle to develop more muscle mass has been shown in one case to cause the calves to be so large that they cannot be born normally and require caesarean delivery (Webster 2002).

- Where the aim is said to be to improve animal welfare, for example by producing disease resistant livestock, it may be that the eventual outcome would encourage the continuation or adoption of husbandry systems that make the animal fit the environment, rather than fitting the environment to the animal. Opinions differ as to whether this is acceptable.
- When reviewing a project that involves genetic modification, the ERP should always carefully consider the wider welfare and ethical implications of what is being proposed – how will it affect the whole animal, and what is the ultimate application?

GM animals are also often housed in specified (defined) pathogen free (SPF) environments (see Section 4.4). In the case of the larger species such as pigs, cattle or sheep it may be thought more difficult to provide environmental enrichment or group housing in such environments. This need not be the case – many establishments have found ways of providing complex, enriched environments for SPF animals of a range of species. Housing in barren environments, or (for social animals) without companions, will seriously limit the animals' ability to express their natural behaviour and will have a big impact on their welfare.

When housing animals, the default should always be to provide environmental enrichment and group housing (for social species). Animals kept in SPF units are sometimes individually housed and/or not given adequate enrichment in the belief that this is necessary to maintain their health status. Any such restrictions on group housing and enrichment should always be questioned, as they may not be necessary or justified. For example, many cage additions can be autoclaved, so that they can be given to animals without compromising their SPF status.

3.5 Costs associated with cloning to produce GM animals

It is now possible to make targeted genetic changes in many species (including cows, goats, horses, sheep, pigs and cats) via reproductive cloning (see Section 2.3.3). Creating GM animals with the inclusion of a cloning step raises welfare concerns additional to the use of microinjection - the techniques involved are even less efficient (i.e. more animals are involved) and cloned animals have associated problems of viability and health quite apart from the effects of their genetic modification. Using an adult (specialised) cell to try to make a whole new animal appears to result in some of the genes not being turned on and off correctly during development and this can cause problems. For example, cloned animals are often larger at birth than normal animals (probably because of inaccurate control of growth genes) and caesarean sections are therefore often needed, with consequent discomfort and pain for the surrogate female

even with the use of appropriate anaesthetics and analgesics. There is also published scientific evidence that some clones die shortly after birth or die early, and they have been known to suffer from a range of pathologies including respiratory defects and tumours (Cibilli *et al*., 2002, Cohen *et al*., 2003, Ogonuki *et al*., 2002, Rhind *et al*., 2003).

4 Practical ways of implementing the Three Rs and minimising costs to animals

4.1 Introduction

It is critically important to ensure that all opportunities for applying the three Rs of Replacement, Reduction and Refinement are fully explored and implemented within all projects at every establishment and this is one of the central roles of the ERP. All of the general issues, points and ideas for questions discussed in detail in Section 4 of the *Lay Members' Resource Book* will be applicable to projects involving GM animals and serve as a good starting point. In this supplementary resource, we therefore focus on additional possibilities for implementing the three Rs that are specific to the creation, maintenance and use of GM animals.

- *Information on what has been done to implement the Three Rs during the planning of the project should be described in the relevant section of the project licence application. The NVS and NACWO should also be able to provide information on this.*
- *The points in Boxes 7 and 8 of the Lay Members' Resource Book can serve as a useful starter with regard to general questions regarding the Three Rs and more specific questions may lead from there.*

4.1.1 Replacement – alternatives to using GM animals or to creating new GM lines

The ASPA requires that alternatives to animals are used wherever possible and that researchers document the efforts they have made to search for alternatives in their project application. It is always difficult for non-specialists to judge whether the search has been rigorous enough, for both normal and GM animal projects, because the science is usually highly specialised. GM projects often include examining what a gene actually does in a living animal, and this may severely limit opportunities for using replacement alternatives. Nevertheless, the general questions provided in the *Lay Members' Resource Book* (Section 4, Box 7), such as: why animals need to be used; the extent to which non-animal methods (such as cell based assays) could be used as alternatives; and whether *in vitro* research has gone as far as possible prior to *in vivo* work, are valid and relevant and serve as a useful starting point.

Where the purpose of the research is to *create a new GM line*, it is important to investigate whether an appropriate line exists elsewhere that could be brought in, rather than deriving a new line in-house. This option should always be explored and used in preference wherever possible. Establishments such as the MRC at Harwell in the UK, the Jackson laboratory in the U.S.A. or other groups in the same field of research elsewhere may have lines available.

➤ *It is useful to ask what alternatives to using animals and to creating a new GM line have been investigated, for instance whether databases of existing GM lines have been checked. This information should also be included in the relevant section of the project application.*

➤ *More information about obtaining existing GM and mutant mouse lines from the MRC Harwell mouse mutant archive, and its links to the* 'European Mouse Mutant Archive' (EMMA) *can be found at:*
http://www.mgu.har.mrc.ac.uk/fesa/fesa.html

4.1.2 Reduction and refinement

There is great potential for applying both reduction and refinement to work with GM animals. This applies to all of the following processes:

- *the initial production* of the specific GM animals (See Section 4.2);
- *the breeding* of these animals (the founders) to produce a GM line; and
- *the maintenance* of the GM line (See Section 4.3).

Opportunities for reduction and refinement at each stage are examined in detail in a report produced by the BVAAWF/FRAME/RSPCA/UFAW* Joint Working Group on Refinement. The report, '*Refinement and reduction in production of genetically modified mice*' (Robinson *et al*., 2003) contains a great deal of background information, together with many recommendations, which provide a useful basis for the review of all aspects of GM animal work. It is rather detailed for non-specialists, but in Sections 4.2 to 4.5 below, we have summarised some of the issues it covers where it is likely that lay members could most usefully have an input.

➤ *Boxes 2-5 in this document contain additional suggested topics for ERP discussion and are designed to complement the Joint Working Group on Refinement's recommendations.*

➤ *The ERP should have copies of the BVA(AWF)/FRAME/RSPCA/UFAW Joint Working Group on Refinement report available for members and relevant staff in establishments where GM animals are created and/or used.*

* British Veterinary Association Animal Welfare Foundation (BVAAWF), Fund for the Replacement of Animals in Medical Experiments (FRAME), Royal Society for the Prevention of Cruelty to Animals (RSPCA), Universities Federation for Animal Welfare (UFAW).

Box 2: Ways in which the ERP can ensure the 3Rs are implemented for projects involving genetic modification

- Ensure that all the issues that might affect animal welfare have been thought through and the best system for the animals that can still allow scientific objectives to be achieved is put in place.
- The ERP could carry out periodic reviews of aspects of GM animal work to see if standard practices could be refined. The ERP could consider asking for such a review, basing it on the Joint Working Group on Refinement report.
- Think about retrospective review of projects involving genetic modification – especially at the time of project renewal. For example, if the project applicant has a background in genetic modification, how successful have they been? How have their constructs performed and have they gained high rates of GM offspring? If not then perhaps they need to re-evaluate their techniques or undergo more training before more work is undertaken.

4.2 Applying refinement and reduction to the creation of a GM line

There is potential for reduction and/or refinement of all the procedures and techniques used in the creation of a GM line (see Section 2.3 and Figures 1 and 2; Section 3.1 and Table 1) from the making of the construct through to the identification of the individual animals produced and their subsequent breeding. The overall aims should be: for the experiment to be well designed; to avoid wastage of eggs or embryos; and to reduce as far as possible the number of animals required.

4.2.1 Reducing wastage when creating a GM line

The efficiency and success of the genetic modification process – and hence the number of animals required - depends on a number of factors, in particular:

(i) The nature of the construct – In pronuclear microinjection the DNA construct needs to be well purified, not degraded, and must be suspended in a good quality solution before it is microinjected into the fertilised eggs. Nevertheless, some constructs may work poorly however well they are produced, because of other factors such as the DNA sequence itself, or the length of the construct.

(ii) The quality of the ES cells – For gene targeting the rate of production of good chimeras (ones that successfully transmit the genetic modification to their offspring) can be extremely variable when using different ES cell lines for a gene targeting based approach. It is also essential that the ES cells are good quality, e.g. they should be maintained in high quality specific media and should not be kept in culture for too long.

(iii) The strain of the "donor" mice - The strain of mouse selected (and therefore the genetic background of the eggs or embryos into which the construct or targeted ES cells are injected) is important, regardless of the genetic modification method. Some inbred lines of mice produce embryos that are more likely to survive following genetic manipulation and embryo transfer than others.

(iv) Technical skills - The skills of the staff who are carrying out the genetic manipulations can dramatically affect success rates, so good training and assessment of competence is essential.

➤ *Few members of the ERP would be expected to be able to evaluate technical aspects of the experiments, such as whether one DNA purification protocol is better than another, or which ES cell line is best to use. However, since efficiency has a direct bearing on animal numbers, the ERP will want to check that there is a system in place to maximise and monitor success rates, such that sub-optimal conditions of construct production and use are noticed and addressed.*

4.2.2 Refining experiments by creating less severe phenotypes

Recent technological advances have enabled researchers to have greater control over inserted DNA (Lewandoski 2001, Lobe & Nagy 1998). In some cases it is possible to make and insert the DNA so that the new gene is produced *only in the relevant tissue or organ*, instead of being produced throughout the whole animal, or it is made so that it is produced *only at the relevant stage of development* instead of continuously. For example, a GM gene that causes tumours is likely to cause numerous cancers if it is produced throughout the animal's tissues, but if the aim is just to look at lung tumours, then the construct could be made so the altered GM gene is only active (i.e. only 'expressed') in the lung. This is known as 'tissue specific expression' through a 'conditional approach'. In this example, it leads to fewer additional tumours occurring and hence less suffering. Advances such as these can and should be used as refinements to reduce or avoid animal suffering.

➤ *It may well be worth the ERP checking whether a conditional approach has been considered in the project under discussion, especially if the anticipated effects of the modification are likely to cause moderate or substantial suffering.*

➤ *More information about conditional technologies (and further references) can be found in the BVAAWF/FRAME/RSPCA/UFAW Joint Working Group Report on* 'Refinement and reduction in production of genetically modified mice'.

4.2.3 Reducing and refining the process of obtaining embryos

Many embryos are needed for the genetic modification process and, to reduce wastage, it is important that there is an efficient system of egg and embryo production that provides for the needs of the research, but does not lead to overproduction. It is equally important that researchers always use the eggs and embryos that they request and do not waste them.

The number of females that are required can be reduced by using a process of superovulation. This involves injecting the females with gonadotrophins (hormones) at precise time intervals, which induces them to produce higher numbers of eggs. The females are then mated and killed soon afterwards, so that the early embryos can be collected. Once the embryos have been genetically modified, they are surgically placed (under general anaesthesia) into the uterus of new females who act as surrogate mothers. These surrogate mothers will not accept the transferred embryos unless a state of false pregnancy (pseudopregnancy) has been induced first, by mating them with a sterile, usually vasectomised, male.

There are a number of potential refinements to this whole process of superovulation and mating, some of which are listed below.

Females: It is essential to select the females for superovulation and as recipients of embryos very carefully, taking into account the strain, age and size. The strain is important as some strains produce more eggs (and so embryos) following superovulation than others, and some make better mothers. They need to be at the right age to be fertile and receptive and not so small that the males will hurt them during mating. Females who receive embryos from the same experiment should be housed in pairs to provide them with company.

Males: The stud males used for mating should be carefully selected to show a good mating performance but without being aggressive to the females.

Production of sterile males:

There are two ways of producing sterile males – either by vasectomising normal males, or by breeding sterile animals. Both methods have advantages and disadvantages in terms of animal welfare. The former involves surgery with associated suffering, the latter avoids surgery but results in the production of excess females who may then be killed without being used in any scientific study.

If vasectomy is selected as the best method, this in itself can be refined. The procedure involves cutting either the abdominal wall or the scrotal sac. The latter technique is considered to be a refinement because the surgery required is likely to cause less pain. Appropriate anaesthesia and analgesia is essential in either case.

➤ *The ERP may enquire about different methods of production of sterile males and discuss them in order to decide on the most appropriate to adopt as the default technique.*

4.3 Applying refinement and reduction to the breeding of new lines and maintenance of the GM colony

Once new GM animals have been produced, their phenotype will be assessed for scientific value and for adverse effects. It is then likely that researchers will want to breed from the animals to produce a line for use in experiments. The line will be maintained, used in experiments and possibly passed to different research establishments. There are a number of possibilities for reducing the numbers of animals used and animal suffering throughout the many stages of the project, for example when carrying out the initial assessment of the adverse effects, checking which offspring of each generation carry the genetic modification, breeding up the new GM line and when generally caring for and using the GM animals in experiments. These stages, and associated reduction and refinement measures, are described below in Sections 4.3.1 to 4.3.5 and questions for the ERP to consider are provided in Boxes 3-5.

4.3.1 Refinement: by assessing and reducing adverse effects

Once the founder GM animals have been produced, it is vitally important that they are carefully observed to ensure that any adverse effects of the genetic modification are noticed and alleviated. (Note that new lines brought in will also have to be carefully observed.) Signs of pain, suffering or distress may be very subtle and animals will require very careful assessment over and above the standard daily welfare checks that animal care staff carry out on established GM lines and normal animals. This is facilitated by having a defined system for welfare assessment within all establishments.

A good welfare assessment is also likely to provide scientific benefits, as it will give additional information on the phenotype of the animal that may be scientifically valuable. To be useful and effective it should be practical and informative. It should include analysis of factors such as posture, gait, activity and body condition, and will require additional time to be spent with the animals. Control animals (usually non-transgenic littermates) should be used for comparison, and all animals should be housed and cared for in the same way whilst their assessment is ongoing. Throughout the breeding programme special attention must be paid to any phenotypic effects that might arise and cause suffering. The results and consequences of the monitoring programme, for example any special care or additional monitoring necessary, should be accurately recorded and disseminated to all involved in caring for and using the animals, including the NVS, NACWO and the relevant personal and project licensees.

If the line is transferred to another animal unit, all the welfare information should be sent in advance (to allow necessary provisions to be made). It should also be sent with the animals when they are transported. Similar information should also be requested when a new line in brought into the unit; this may take the form of a 'mouse passport', which gives details about the origin of the line, the nature of the modification and phenotype and welfare effects etc.

- *The APC's* 'Report on Biotechnology' *provides some guidance on the aims and contents of a welfare assessment (see* www.apc.gov.uk).
- *Box 3 provides several useful points for the ERP to consider with respect to welfare assessment.*
- *This issue is addressed in a more detail in a report on* 'Assessing the Welfare of Genetically Altered Mice' *which can be obtained from the MRC Centre for Best Practice for Animals in Research (CBPAR, contact details in Section 5). It is important for all establishments using GM animals to have a copy of this easily accessible to relevant staff. Also see Jegstrup* et al., *(2003) for a review of assessment protocols.*

Box 3. Discussion points with respect to welfare assessment of GM animals

- Is there a comprehensive system in place for assessing the welfare of any newly derived lines or lines brought into the unit from elsewhere?
- Do staff have sufficient time to carry out welfare assessments and if not how will this issue be addressed?
- Are the outcomes of the specific welfare assessments recorded so that they are easily accessed and updated and are they disseminated to all relevant staff?

4.3.2 Reduction: by avoiding wastage when breeding GM lines

The breeding protocols used to produce a GM line from the GM founder animals or to maintain a line can be very complex and use many animals, so it is critically important to implement strategies that help to avoid overproduction and wastage.

Breeding to produce a line

Part of the initial breeding protocol often involves inbreeding the line so that all the individuals within the new line are genetically highly similar. Any difference in phenotype seen between the GM and normal animals (used as experimental controls) is then more likely to have resulted from the genetic modification itself, rather than from random genetic differences between the mice.

One inbreeding system commonly used for this purpose is backcrossing. This involves many animals and takes many months, since mice of a particular GM line are mated with normal mice from a selected inbred strain for at least 10 generations. It is used in order to change the genetic background once founder animals have been obtained. This is often necessary because strains that are easy to genetically modify are frequently poor breeders – hence it is hard to produce a viable GM line from them – or they may be inappropriate for studying the effects of the modification. The strain of mice selected for the backcrossing is likely to be one that has previously been well characterised (genetically and phenotypically) or one that breeds well and has good mothering ability.

Breeding to maintain a line

Once the line has been bred onto a suitable background for study it needs to be maintained in a way that provides for the scientific requirements without too many or too few animals being born. This is often complicated by the fact that some projects involve complex crosses of different GM lines. These crosses all need to be carried out without mistakes being made.

A further potential problem is that the inbred strains used throughout the process of creating the line, and the resulting GM lines themselves, often have different breeding characteristics and may have reduced fertility or libido. For example, some inbred strains are very sensitive to vibration and will not breed if they can feel vibrations from sources such as building works.

In summary, there are three critical points for full implementation of reduction within breeding programmes:

- breeding systems need to be set up and run by experienced staff who are aware of the nuances of each strain and line;
- those using the animals also need to be aware of, understand and follow the breeding program so that they do not disrupt the system by failing to carry out timely genotyping, or request animals that they fail to use, as both can lead to wastage of animals; and
- users must communicate any changes in their requirements in good time to avoid breeding animals unnecessarily thus causing needless wastage.

4.3.3 Refinement: reducing suffering when checking whether animals are GM

Not all the offspring produced following genetic manipulation, or during backcrossing or normal maintenance breeding will be genetically modified, and it is important to establish which ones are. It may be possible to identify GM animals visually if they have a visible phenotype (e.g. a different coat colour or substantial growth impairment), but for most GM lines it will be necessary to do this by analysis of the individual animal's DNA. This requires taking a small piece of tissue from the animal

(a tissue biopsy) and testing it for the presence of the desired genetic change. The tissue taken is most often a piece from the end of the tail. Some ongoing studies indicate that this is painful for the animals, particularly if more than 5mm is taken. Less painful methods should therefore be used in preference. Other commonly used methods include taking a piece from the ear with a small 'punch' (or scissors to make a notch) and blood sampling, both of which may be less painful. There are also methods of genotyping using saliva or hair follicles. These are minimally invasive but are not yet widely used because of concerns about reliability (e.g. from false results associated with cross-contamination) (see Robinson *et al.,* (2003) for more information and references).

The genotyping procedure can be refined by using techniques which require much less DNA, and hence much less tissue, such as PCR (Polymerase Chain Reaction), rather than Southern blot analysis. Combining tissue sampling with the process of identification of the animal will also reduce the amount of tissue needed overall. Ear punches or notches can be taken from different locations, according to a predetermined/standard code.

Sometimes, the need to test for the presence of the modified gene can be avoided altogether if the animals are maintained as homozygotes. This is where both DNA strands have the required modification and it will therefore be passed on to all the offspring automatically. It may be preferable to maintain lines as homozygotes, however, it is important to check that neither the animals' welfare nor the scientific outcome will be unduly affected by having the genetic change on both copies of the DNA.

➤ *Even apparently simple procedures like genotyping can have significant welfare costs, so the ERP needs to be aware of all of these and to review what has been done to reduce the associated suffering.*

4.3.4 Refinement: when identifying animals

Identification of each individual in the GM colony, or within each cage is often required so that the results of genotyping analysis mentioned in Section 4.3.3 can be linked back to the individual mouse. This may also be necessary to keep track of the animals generally.

When deciding on whether and what identification method to use, the impact on the animal must be taken into account. Non-invasive methods of identification should be used where they are available – using their coat colour or sex is the simplest and best for animal welfare. More invasive methods, such as ear notching, ear tags, microchipping or tattoos are however commonly used, and as mentioned above, a significant refinement would be to use any tissue removed for identification purposes

as a source of DNA for genotyping as well. This negates the need to take two pieces of tissue from each animal.

4.3.5 Reduction: by cryopreservation of GM lines

One problem often encountered is that GM lines that are only occasionally used in scientific studies still need to be maintained through continuous breeding. This results in animals being produced who are not used and are just killed. One way of avoiding this problem is to preserve the lines by freezing germ cells or early embryos in such a way that they are still viable when thawed. This is called cryopreservation. Maintaining lines by cryopreservation can also be used to provide an 'emergency supply' of the lines in case of disease in the colony, or if a line is failing to thrive or breed.

4.4 Welfare issues arising from the need to keep the GM colony free from disease

The prospect of infection entering a GM animal unit is extremely serious - if disease does break out all the animals may have to be killed in order to eradicate it. Infection is a particular problem for two reasons; experimental results may be influenced by the health status of the animals, and/or the phenotype of the GM animal may be adversely affected by disease. The latter is especially relevant when using immunocompromised lines as the compromised immune system of these animals makes them particularly vulnerable to infection. High health status, usually specified pathogen free (SPF), animals are therefore commonly used at all stages in genetic modification programmes, combined with some form of 'barrier system' designed to restrict the likelihood of infection entering the unit.

The nature and the extent of the barrier system will depend on the individual establishment. A range of precautions may be taken, from limiting the number of people who have access to the animal unit, through to filtering all the air that enters the building and requiring all entrants to shower and change their clothes completely when entering it. (These sort of animal units are often referred to as barrier units.) In addition, the animals may be housed in individually ventilated cages (IVCs), where clean filtered air is delivered to each cage.

There is a particular risk of infection when new animals are brought in and so the facility will have a quarantine area, together with some form of microbiological 'screening' to ensure that animals are 'clean' before they leave quarantine.

The specific welfare issues that arise as a result of requiring 'clean' environments and animals are:

Housing and husbandry: It is very important that housing and husbandry systems satisfy the complex needs of the animals as well as allowing for the research to be

carried out. In the case of IVC systems there are several specific concerns. For example, some cages have very large food and water hoppers that can significantly reduce the space available for the animals. Additionally, most IVC systems prevent the transfer of sound and odour cues between cages. The animals in any one cage will not therefore be able to smell (or hear) others in the room and, if singly housed, will be completely isolated. Providing cage mates and environmental enrichment is therefore of even greater importance than with normal open caging. For an overview of the key welfare issues associated with IVC use and suggested refinements, see Hawkins et al., (2003)

Quarantine: Microbiological screening involves some mice being killed and autopsied to check for infection. The new animals are often very valuable or in short supply, so normal ('sentinel') mice may be kept with them for this purpose. An alternative approach is for all new lines entering the unit to be rederived by embryo transfer. This involves taking embryos from the mother and transferring them into a new female of known health status so that any previously undetected diseases in the imported mother are not transferred to her offspring. The latter approach involves additional surgery, the former involves using additional animals. The ethical and welfare advantages and disadvantages of each approach need to be carefully weighed.

Box 4: Questions with respect to applying the 3Rs to maintenance of the GM colony

1. Avoiding wastage when breeding up a GM line

- How is communication organised and established between the users and animal facility to remove the large potential for animal wastage by inappropriate or over-breeding? The ERP may wish to request and review data on animal wastage, for example the number of animals killed without being used.
- Do researchers always use the eggs and embryos that they have requested?
- Has the maintenance of GM lines in the homozygous state been considered for its potential to reduce the number of animals used during breeding and negate the need for tissue biopsy for genotyping?

2. Checking whether animals are GM

- Has the possibility of using less invasive tissue collection methods for genotyping, such as saliva, or hair follicles, been investigated and weighed against more invasive methods of tissue collection, such as ear notching/punching, tail biopsy, or taking blood?

3. Refining the identification of animals

- Do all animals in the colony actually need to be individually identified (above the level of their home cages)?
- Does the establishment use a method of identification that causes the least possible distress and suffering to the animals whilst still remaining practical?

4. Cryopreservation to reduce the use of animals

- Is the use of cryopreservation possible at the establishment and, if so, under what circumstances?

5. Maintaining welfare whilst keeping the GM colony free from disease

i) Avoiding barrier breakdown

- How is it ensured that all personnel take any precautionary measures seriously and are well trained in the procedures?
- Are all animal deaths within the colony recorded and are post mortems conducted to establish a cause of death, which might provide an early warning of disease within the unit?
- The ERP may consider periodically reviewing the systems in place to avoid negative impact of the laboratory environment on the animals

ii) Ensuring good animal welfare within the housing system

- In order not to compromise their welfare, are all animals within barrier units still given:
  - environmental enrichment (including nesting material),
  - adequate space,
  - cage-mates?

6. Minimising impact when moving animals between units

- What safeguards are taken to ensure the well-being of animals transported long distances to and from other establishments?
- Does the establishment insist that phenotypic and welfare information is sent prior to shipment of the animals and that a hard copy arrives with the mice?

4.5 Minimising the adverse effects of transport

Transport of animals has the potential to cause stress whether the journeys involved are short and in-house, or from other facilities in the UK and abroad. GM animals may be less able to cope with transport stress and may require specialist care. Transporting fresh or cryopreserved embryos, or cryopreserved gametes, instead of live animals, can avoid such problems and should be the preferred option wherever possible. Wide consultation between consignor, carrier and user is necessary when planning journeys for GM animals, including those in embryonic form. It is vitally important to make sure that there are effective contingency plans to cope with likely delays.

In addition, the particular needs of a GM line should be discussed prior to import into the facility so that the welfare of the animals is not compromised at any stage. A particular line may have special requirements (e.g. a different feed) or need specialist expertise for monitoring and care, and this needs to be in place before the animals arrive. Comprehensive information including the nature of the phenotype and any specialist management needs with respect to husbandry and veterinary care should be sent with any animals sent out from the unit. (See Section 4.3.1)

- *For more information about transport of GM animals see* Awasthi et al. (2003).
- *LASA has recently updated its transport guidelines, which apply to animals in general and all facilities should have a copy of these readily accessible.*

4.6 Reduction and refinement through periodic review of common procedures

Large establishments carrying out a lot of GM animal work may have animal units dedicated to GM animal production and maintenance. Many of the processes and procedures (e.g. superovulation, vasectomy, identification) listed in individual projects coming to the ERP for review will be standard for most projects (see Table 1 and Section 3.1.) Instigating a periodic review of the common procedures would therefore be a useful goal for the ERP. Good practice can then be defined and implemented across the establishment, and this will save time that would have been spent asking the same questions repeatedly at every project review involving GM animals.

However, where GM projects come to the ERP only infrequently, it may be a more effective use of ERP's time to review establishment practices with each project.

Many of the techniques used (e.g. blood sampling, injections) will be common to other types of project not involving GM animals and periodic review of these in terms of the potential for refinement is essential.

Surgery is another procedure that may be common to other projects within the establishment. There is a need for the establishment to have a comprehensive pain management regime in order to minimise adverse effects.

- *The ERP could instigate periodic reviews of the establishment's strategies for identification of potential adverse effects; monitoring the animals; ameliorating the suffering when it arises and; wherever possible, incorporating strategies to avoid the effects in the first place.*
- *Information and recommendations about pain and surgery are given in the Joint Working Group on Refinement's report, and a future RSPCA lay members resource will also be covering this topic.*

As a general rule, it is important to remember that current best practice is exactly that – current – and as new information becomes available all techniques, protocols and practices should be re-evaluated and updated where necessary.

- *It is important for the ERP to ensure that there is a system in place to help staff keep informed of new developments in genetic modification technology and associated refinements and changes in best practice.*

4.7 Training issues relevant to genetic modification

Well trained and empathetic staff will have a very significant positive impact on animal welfare and on the numbers of animals used at all stages of projects involving genetic modification. Individual establishments are likely to approach staff training differently in order to satisfy the requirements of their particular research, and opinion differs within the field as to whether and what specific training is required in order to care for and use GM animals appropriately.

As a basic principle all staff should have a working understanding of the processes involved in the genetic modification of animals. In establishments where GM lines are generated, some will need specific skills in the techniques routinely used in their creation, such as superovulation of mice. Acquiring these skills will require dedicated training over and above the current Home Office modular training. A system of dissemination of information also needs to be in place to help staff and the ERP keep up to date with relevant new developments and guidelines.

➤ *The ERP needs to maintain a good level of awareness of training needs and new training resources as they become available. This requires an effective mechanism for surveying staff training requirements, and for ensuring that staff know what training is available to them.*

➤ *The ERP could discuss whether there is a need for a specialised course for GM animal users and/or carers over and above the HO modular training course. Some aspects to consider with respect to training needs are provided in Box 5.*

Box 5: Training needs

The following tasks are commonly associated with creation, care and use of GM animals and may require specialist training:

- Specific procedures including embryo transfer, superovulation and vasectomy
- Welfare assessments of newly derived or imported GM lines
- Complex breeding strategies, including backcrossing and continuation of the line in a way that takes account of patterns of genetic inheritance, without overproduction and wastage
- Selection of suitable inbred strains to provide: good rates of superovulation, good breeding performance and desirable characteristics in the background strain
- Accurate record keeping to satisfy Home Office requirements for records of GM animal use and to keep track of complex breeding programmes and genetic identity

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End note

We want these Lay Members' resources to be as useful as possible so feedback is important. Please let us have your comments, either positive or negative, to help us update and improve this and subsequent documents. Comments can be sent by email to mjennings@rspca.org.uk or to the RSPCA, Research Animals Department, Wilberforce Way, Southwater, Horsham, West Sussex, RH13 9RS

5 Sources of additional information and references

5.1 Sources of information

Information on ERPs

Smith, J.A. & Jennings, M. (2003). Lay Members Resource Book. Available from the RSPCA via erp-laymembers@rspca.org.uk

The Home Office: http://www.homeoffice.gov.uk/comrace/animals/reference.html

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Robinson V, Morton DB, Anderson D, Carver JFA, Francis RJ, Hubrecht R, Jenkins E, Mathers KE, Raymond R, Rosewell I, Wallace J, Wells DJ (2003). Refinement and reduction in production of genetically modified mice: Sixth report of the BVA(AWF)/FRAME/RSPCA/UFAW Joint Working Group on Refinement. *Laboratory Animals* 37 (Supplement 1)

Other sources of information or opinion about genetic modification

Agriculture and Environment Biotechnology Commission (AEBC) –
www.aebc.gov.uk
The Government strategic advisory body on biotechnology issues affecting agriculture and the environment. Has produced a report on animals and biotechnology.

Animal Procedures Committee (APC) – www.apc.gov.uk
An independent, public body that advises the Home Secretary on matters concerned with the ASPA and investigates and reports on matters relating to the use of animals in research. Has produced a report on biotechnology and animal use and another on the cost-benefit assessment.

Biotechnology and Biological Sciences Research Council (BBSRC) – www.bbsrc.ac.uk
A UK funding body for academic research and training in the biosciences at universities and science institutes. Has produced information booklets about GM animal use.

British Union for the Abolition of Vivisection (BUAV) – www.buav.org
An anti-vivisection organisation that campaigns for an end to all animal experiments. Has various fact-sheets on-line and has produced, *'Designer mice',* a report on GM mice.

European Centre for the Validation of Alternative Methods (ECVAM) - http://ecvam.jrc.cec.eu.int/index.htm
An international centre that develops and validates alternatives to the use of animals in testing, provides information, and convenes workshops relevant to the use of animals in research and testing. Information available includes a report of a workshop convened by ECVAM to discuss the production and use of transgenic animals.

Eumorphia - www.eumorphia.org
An integrated research programme involving the development of new approaches in GM animal phenotyping, mutagenesis and informatics. The web site provides information about the aims of GM animal research.

Genewatch – www.genewatch.org
A not-for-profit group that monitors developments in genetic technologies from a public interest, environmental protection and animal welfare perspective. Has produced a report on GM and cloned animals and responds to relevant consultations and developments.

Medical Research Council (MRC) Centre for Best Practice for Animals in Research (CBPAR) - www.mrc.ac.uk/public-cbpar
A centre acting as a resource for the scientific community which develops, disseminates and implements information on best practice in the use and welfare of laboratory animals and to applying the 3Rs. Site contains information and a useful links page.

The Jackson Laboratory- www.jax.org
The world's largest mammalian genetic research facility. A non-profit institution providing information, genetic resources and training for scientists.

The Royal Society – www.royalsoc.ac.uk
An independent academy of science that promotes and funds scientific work and communication. Has produced a report on GM animals.

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Webster, A.J. (2002) Rendering unto Caesar: welfare problems in Belgian Blue cattle. *Veterinary Journal*, 163(3), 267-82.

Appendix A Glossary of useful genetic terms for lay members of ERPs

Chimera
An animal composed of tissues containing two or more different genotypes. This can be the result of normal cross-breeding methods or by mixing cells of different types in early embryos.

Conditional transgenic technology
Where the effects of genetic modifications are restricted to certain cells or tissues, or to different points in the embryo's/animal's development, or to occur in response to a signal – e.g. following the administration of a chemical agent.

Construct (or transgene)
A piece of artificially created DNA that contains the DNA sequence of interest.

DNA (deoxyribonucleic acid)
In most organisms, DNA carries the primary genetic information and the full complement of an organism's DNA is called its genome. DNA comprises of a long chain of sub-units called bases that can be read as a code - the genetic code. Certain bits of the DNA, the genes, can be copied and interpreted to make proteins. Other bits of the DNA act as control elements, so that the proteins which the genes code for are made at the right time and quantity, i.e. they control the expression of the genes.

Embryonic stem (ES) Cells
Cultured cells that are derived from early embryos (3.5 days post fertilisation) called blastocysts. ES cells have the advantage that they can be genetically modified *in vitro* and they can be injected into new blastocysts to create chimeras.

Gene
A gene is a piece of DNA within the genome that codes for a protein. It is composed of a region that is read to make the protein and regulatory sequences that control when and where the gene is turned on within the organism, i.e. when it is expressed.

Genotype
The genetic make-up of the animal.

Genotyping
The process of determining an animals genotype by analysis either of visible phenotype (what the animal looks like e.g. mouse coat colour markers), or by DNA analysis of a tissue biopsy.

Germ cells
The sperm and egg (oocyte) cells, and their precursors. Germ cells have only one set of chromosomes while somatic cells (all other cell types) have two sets of chromosomes.

Germline
The cells of an animal that give rise to the germ cells**.**

Heterozygote
A cell or animal with different forms (alleles) of one or more specific genes. For example, a person with brown eyes would be heterozygous if they had one allele for blue eyes and one allele for brown eyes, and would be homozygous if both their alleles were for brown eyes. Either way, they still have brown eyes because the brown allele is dominant over the blue allele.

Homology
Where the DNA sequence is identical, or very similar, between the animals or species under study. Regions of homology can swap over (recombine) in cells and this phenomenon is used as the basis of gene targeting in ES cells.

Homozygote
A homozygous animal is one with identical forms of one or more specific genes. For example, a transgenic mouse will be referred to as homozygous if both copies of the gene under investigation contain the introduced genetic change.

Immunocompromised
An animal is immunocompromised if it has an immune system that is weakened by disease, stress, drugs, malnutrition, or genetic modification.

In vitro
Literally meaning, "in glass", a biological or biochemical process occurring outside a living organism. An *in vitro* experiment is one that is carried out in test tubes or on petri plates rather than on, or within, a whole living animal/organism.

In vivo
Literally meaning "in life", a biologic or biochemical process occurring within a living organism. An *in vivo* experiment is one that is carried out on, or within, a living whole animal/organism.

Isogenic
Isogenic animals have the same genetic background, as a result of inbreeding for many generations. For example, all mice that are inbred strain C57BL/6J will have an almost identical genome and therefore be termed isogenic mice.

Knock-in
Describes an animal in which one particular DNA sequence has been replaced by another sequence or that has had additional sequences added at a specific point in its genome.

Knock-out
When one particular gene sequence has been modified to block gene expression.

Phenotype
The visible characteristics of an animal (within that particular environment).

Specified Pathogen Free (SPF)
Animals regularly tested to be free from a defined list of pathogens.