Home Office

NON-TECHNICAL SUMMARY

Genetically altered rodent generation, breeding and maintenance.

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Physiology, Genetic Manipulation, mice

Animal types	Life stages
Mice	adult, embryo, pregnant, juvenile, neonate

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The creation, breeding and supply of novel and established lines of Genetically Altered (GA) mice. A GA mouse is a mouse that has had its genome altered through the use of genetic engineering techniques. Genetically Altered mice are commonly used for research as animal models of human diseases, and are also used for research on genes. Mice will be of high health status and defined genetic quality for use in research projects at this and other establishments. Cryopreservation of such lines provide health and genetic security. Mouse embryos or sperm are frozen in liquid Nitrogen, to avoid loss by contamination, unexpected disasters eg fire in a facility and genetic drift. Genetic drift is where genetic changes happens constantly, even small changes might have an impact on future research and replicability.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

It is recognised how important genetically altered mice are in understanding the pathophysiology of many disease conditions including cancers, cardiac and metabolic diseases, as well as improving our knowledge of basic physiology. Creating, breeding and maintaining genetically modified animals we will ensure that such animals generated are produced to the highest standards of health and welfare enabling more reproducible and publishable research. Thus we are able to recruit top quality research scientists.

Cryopreservation provides security against genetic drift and health status contamination whilst meeting the 3R’s.

What outputs do you think you will see at the end of this project?

Provision of GA models to researchers, resulting in new knowledge of physiological and disease mechanisms and dissemination via peer reviewed publications

Who or what will benefit from these outputs, and how?

Researchers here and their collaborators, other research facilities who do not have the technical expertise to create GA mice.

How will you look to maximise the outputs of this work?

Peer reviewed publication of the subsequent work on these animals, including encouraging publication of any failures of the approach, collaborations with other institutions.

Species and numbers of animals expected to be used

- Mice: 17700

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

Mice are a well recognised species for work involving genetic alterations and there are standard protocols, methods and reagents used that have been optimised for this species and acknowledged benefits for use.

Typically, what will be done to an animal used in your project?

The majority of the animals will be used for the breeding and maintenance of genetically altered or mutant animals. Some animals will undergo surgery as follows-

Typically female mice will be mated with vasectomised (sterile) male mice to induce pseudo-pregnancy (in mammalian species, pseudo-pregnancy is a physical state whereby all the signs and symptoms of pregnancy are exhibited, with the exception of the presence of a foetus, creating a false pregnancy). They will then undergo a minor surgical procedure, under general anaesthesia, to implant previously genetically modified embryos into the oviduct. During this procedure analgesia will be administered. The females will be allowed to recover and give birth. Aseptic technique will be observed throughout. The resulting offspring will have a small piece of ear tissue removed to confirm the genotype.

What are the expected impacts and/or adverse effects for the animals during your project?

Superovulation -There may be momentary pain on 2 occasions when an I. P injection into the abdomen is administered.

Recipients -Surgical: A female mouse will have a small, less than 1 cm incision, to expose the Uterus, enabling delivery of embryos into the uterus. Mice recover rapidly and, in our hands, without complications. Anaesthesia, analgesia and aseptic technique used to prevent pain and infection. -Non-surgical: A female mouse will have a small speculum inserted in the vagina and positioned around the cervix, the catheter is then inserted into the speculum and through the cervix, at this stage embryos are expelled into one of the uterine horns. Anaesthetic will be used.

Vasectomy -Minor surgery from which mice recover rapidly and, in our hands, without complications. A small incision approximately 5mm, is made into the scrotum and the Vas deferens is cauterized to render the mouse infertile. Anaesthesia, analgesia and aseptic technique used to prevent pain and infection.

Breeding of genetically altered animals with mild phenotype (the observable traits eg eye colour is a phenotype). These animals are not expected to show any deviation from normal wild type mice. A

typical breeding female will have 6-8 litters in her lifetime.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

The majority, approximately 60%, of the mice are perfectly normal and will suffer severity which is sub-threshold. Approximately 30% of the mice will have 2 injections into the abdomen which is mild severity. Some mice, approximately 10%, will undergo minor surgery will have moderate suffering which is expected to be brief and resolve without complication.

What will happen to animals at the end of this project?

- Killed
- Kept alive
- Used in other projects

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

New technologies are improving the field of genetic engineering in animals and they will allow the generation of new mouse models to be applied in biomedical research. First steps in a scientific project will involve in vitro approaches, the final characterisations and applications will require the use of genetically altered animals. The different animal models will integrate the complete range of molecular, cellular, physiological and behavioural interactions necessary to fully understand how genetic modifications result in normal or abnormal processes

Which non-animal alternatives did you consider for use in this project?

We have an established cell engineering service as a potential alternative to in vivo models in vertebrates.

Why were they not suitable?

In-vitro assays cannot adequately model the complete array of molecular, cellular, physiological and behavioural interactions necessary to fully understand how genetic modifications result in normal or abnormal processes.

Invertebrates are a useful adjunct to animal studies in that large throughput of gene alterations can be screened but the differences in circulatory, neurological systems etc limit their use.

Prior to importing or creating a new strain, consideration will be given to the scientific evidence gathered from in vitro data e.g. receptor binding assays to identify appropriate targets and PCR analysis of gene expression. The breeding method and proportion of affected animals produced will also be considered.

All of these factors will be used to justify the introduction and creation of strains under this licence with advice and discussions taken with the researcher and NVS (Named Veterinary Surgeon) as required

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Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

These numbers are predicted, based on analysis of the number of animals used in the last five years to meet the demand of our researchers.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Switching to an IVF (In Vitro Fertilization) -based approach to generate mouse embryos in place of traditional overnight mating's reducing the number of mice used by half.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

Using efficient breeding techniques including using the Home Office genetically altered animal breeding guidelines

Switching to an IVF-based approach to generate mouse embryos in place of traditional overnight matings reducing the number of mice used by half

Integrating CRISPR-Cas9, a gene editing technology, which has greatly reduced the numbers of mice required to make a new transgenic strain for many allele types.

Developed our own method to generate high efficiency long single stranded DNA (lssDNA) donors for gene knock in projects.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

Mice are a well-recognised species for work involving genetic alterations and there are standard protocols, methods and reagents used that have been optimised for this species and there acknowledged benefits for use.

Embryos, gametes, sperm and ovarian tissue will be collected from donor strains. They may be cryopreserved or used for the following purposes.

- Fresh or frozen embryos and sperm will be used for rederivation of infected mouse strains to improve health status.

- Gametes, embryos/sperm or/and tissue will be archived by cryopreservation for strain storage in support of the breeding colonies.

- Gametes, embryos/sperm or/and tissue will be used to replace a strain for storage where there is no longer a research requirement to avoid wastage from maintaining ‘tick over’ breeding programmes.

- Gametes, embryos/sperm or/and tissue will be used where possible instead of live animals to transfer strains to other establishments within the United Kingdom and abroad

Why can’t you use animals that are less sentient?

It is fundamental that the GA embryos are implanted into a receptive female and she is then allowed to complete gestation. The resulting offspring will be used in research projects for which the whole mammalian body systems are required.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

One of the chief benefits of undertaking this work under a service licence is that all the techniques to be used are undertaken by a small group of highly experienced technical staff which minimises suffering. It also ensures that the highest standards of asepsis are maintained and that appropriate and effective analgesia is always used.

We are looking into using NSET (non-surgical embryo transfer) for some of our projects, but at present NSET is not suitable for the majority of our pipelines as these involve microinjecting 1-cell embryos. For NSET these would have to be cultured to blastocyst, the problem we experience is that 1 & 2-cell embryos culture well to the blastocyst stage (80 to 90%) but when they are transferred into the uterus the implantation rate is quite poor. Therefore, we feel that surgical transfer of 1-cell embryos is still the most refined method.

We are a part of an NC3R's funded project to develop a genetically sterile male mouse which could replace the vasectomy surgery.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

I will consult with colleagues across the field and sources such as

AWERB

International society for Transgenic Technologies

Animal Welfare and Management Discussion Group

NC3R's Efficient Breeding Strategy

Institute of Animal Technology

Efficient Breeding of Genetically Altered Animals Assessment Framework(Home Office)

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

Advice has been sought from NC3R's project manager on the development of this PPL application and will continue to be sought during visits to this facility on a weekly basis.