Home Office

NON-TECHNICAL SUMMARY

Breeding and maintenance of rodents for drug discovery platform

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

Rodent breeding, Drug discovery

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

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Contains severe procedures

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 aim of this project is to provide and maintain breeding colonies of genetically altered (GA) rodents to support our projects.

A retrospective assessment of these aims will be due by 5 July 2027

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve its aims and if not, why not?

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?

This work supports a substantial research program that we have for our clients, for studies that cannot take place without information that animals will provide. Each client is developing drugs which will hopefully contribute to helping people with various diseases with an unmet clinical need. For example, we have a large program of work studying haemophilia, a rare (1 in 30,000 people) but life-changing disease that affects the blood's ability to clot. Treatments are difficult as the body can produce ways to fight against them (raise antibodies) and treatments usually involve long stays in hospital. Looking for treatments that are more effective and convenient would significantly improve patients lives.

Having carefully managed in-house breeding programs provides animals for study on an on-demand basis; in doing so it reduces production of excess animals and refines the breeding strategies for subsequent projects.

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

The output of this project will be animals produced and provided to other projects we hold.

Wider outputs from subsequent projects will be a) a better understanding of the disease area being studied and how close it is to human disease, and b) pre-clinical data that will contribute to getting

drugs further along the drug discovery pipeline. Our science-driven approach and focus to our plan of work will enable milestones to be met more efficiently for our sponsors (academics, the Pharmaceutical and biotech industry, clinicians) so key ‘go’ / ‘no go’ decisions can be made using the minimal number of animals possible and assure a better success rate in the drug discovery process than has been seen previously.

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

In the short-term research projects will be fulfilled by generating animals crucial to the study of valuable research into several disease areas. This work is all within our own projects for clients seeking to develop drugs for human diseases, meaning that all animal experiments will directly contribute to the knowledge needed to discover new treatments.

In the long-term the supported projects will continue to contribute to developing and improving treatments for diseases, in particular drug discovery that could significantly improve patients' quality of life. For example, new haemophilia treatments would significantly improve the lives of patients, by avoiding the triggering of the body's defences against the drugs and making treatments much less complicated, or by changing the way the treatment is given, making it more convenient and preventing long stays in hospital.

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

Colony managers within the Team will maintain good communication in their established networks. This will help to disseminate information about breeding strategies and management of complex strains discovered throughout the duration of this project.

Through good communication with Named Animal Care and Welfare Officers (NACWOs), any wildtype animals that are surplus to project requirements will be made available for other projects across the University network where appropriate.

Similarly any post-mortem tissues banked that can be utilised by other projects will be made available. As a group we have a very comprehensive sample record system which makes it very easy to access the tissues we have banked and know the conditions of how they were collected and stored.

Species and numbers of animals expected to be used

- Mice: 7650
- Rats: 5000

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.

As this is a breeding and maintenance project, mice and rats of breeding age (adults) are needed to maintain the animals for studies in several disease areas of interest. All life stages (except aged) are required to create the genetically altered mice and rats.

Each genetically altered strain of rat and mouse is needed to provide other projects with animals specifically for the study of different diseases.

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

The vast majority of mice and rats on this project will be either involved in a natural mating, or offspring transferred to other project licences for animal (*in vivo*) research. Rarely (less than 1%), these animals may need a small procedure to determine whether they have a mutation or not, by taking a hair sample, or a very small piece of ear.

A small group of mice may undergo minor surgery, for example a vasectomy, where the males are made sterile (unable to breed). This will be done while the animal is unconscious (under anaesthetic) and won't feel the pain of the procedure.

A small subset of mice will undergo a blood test to check how much of a certain marker of interest they have (for example, a protein). This will then be used to decide if the animals are used in a study or used for breeding.

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

The majority of mice and rats will not suffer any pain or distress under this project as they will only be used for breeding and will not show any signs that they have a mutation.

Where possible rats and mice will be moved onto new projects at weaning, where they will enter into studies. However, before being transferred to other projects (or if being used for breeding) some genetically altered animals may develop symptoms of the human disease condition they are designed to model, such as developing bleeds under the skin or signs of a metabolic disorder.

Haem A, Haem B, and Glanzmann mice sometimes have surface bleeds that can be treated, and these usually resolve over 24 hours. Sometimes Haem A mice can have bleeds from the ears that also can, in most cases, resolve within 24 hours. In general if these animals have a bleeding event it will be internal (for example in the digestive tract). The external signs of these bleeds can be difficult to spot before the animal is suffering, so changes in behaviour such as reduced movement around the cage must be relied upon. Unfortunately some of these animals may die in between welfare checks if the bleed happens suddenly and is severe enough, although this is not common (less than 5%).

So far we have not had a Haem A rat die directly of a bleed; we have always been able to treat it, or if the treatment isn't working we have humanely killed the animal before suffering is too great. Before discovering the bleed and while waiting for treatment to work (usually over several hours), some discomfort and pain can be experienced by the animal, although pain relief can be given if needed. Bleeds are usually mild (such as a bleed under the skin) but can sometimes be more complex and therefore cause more suffering, such as bleeding within a joint, or on top of the head or in the cheek. Around 75% of homozygous rats experience a bleed in the first few weeks during and after weaning

(3-7 weeks old), so these may occur before the animals move on to other projects. Even after experiencing bleeds before the study starts, these animals are still very much of scientific value as they can usually take part in studies after treatment and recovery.

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)?

Mouse: Sub-threshold or mild 83%

Mouse: Moderate 2%

Mouse: Severe 15%

Rat: Sub-threshold or mild: 70%

Rat: Moderate: 30%

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

- Used in other projects
- Killed

A retrospective assessment of these predicted harms will be due by 5 July 2027

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

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?

The aim of this project is to provide animals for study in other projects, and animals are therefore vital to the success of the work. In order to understand the effects of modifying genes and the effect of potential drugs in treating disease, the whole "system" must be studied which means live animals are needed to see how these changes affect an organism as a whole.

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

The aim of this project is to provide animals for research projects, and therefore there is no non-animal alternative to this.

More widely in the connecting projects, cells grown in the lab or computer models are used where possible. Sometimes, however, this does not answer the research question and studying the whole animal is necessary.

Why were they not suitable?

Alternative methods are not suitable in the context of this project, but they are significantly intertwined with the work which this project will supply animals for. For example, tissues taken from these animals can be analysed in the lab, or even used to grow cells from.

A retrospective assessment of replacement will be due by 5 July 2027

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

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?

We have analysed the number of animals used on previous projects and typically how many animals it takes to fulfil each kind of study, using the most up-to-date breeding methods. This was then used together with a prediction of likely demand of future projects.

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

For breeding design we use several online resources, especially when bringing in a new strain. For example, we frequently refer to "Worked example of calculating breeding numbers for an experimental cohort" on the NC3Rs website, or the Jackson Laboratory "Colony Planning" page, including the colony worksheet. Where appropriate we have also sought advice from researchers or technicians who have maintained the particular colonies before.

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

Efficiency of breeding to fulfil the projects whilst generating as few animals as possible is at the heart of this project.

One project licence to cover all breeding for these projects enables significant control over the numbers of animals produced.

Each strain will have a dedicated colony manager who will communicate regularly with the project managers to ensure that breeding levels are kept closely in line with the demands of each project. These teams will discuss animal numbers for the studies to ensure that numbers are kept to a minimum.

Where project demand falls in the long-term, a breeding colony will be frozen down as embryos or sperm.

A retrospective assessment of reduction will be due by 5 July 2027

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

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.

The majority of rats and mice on this project will have a genetic alteration that will not cause them harm, and they will not undergo any regulated procedures while on the project.

Some of the animal models on this project may suffer harm because of the mutation they carry, but this is important in order to study the disease they mimic. For example, in models we have of haemophilia, the rats may experience spontaneous bleeds in their limbs or under the skin. Similarly, the mice may have internal bleeds and suffer. These models are currently the most effective way to study treatment of this disease.

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

For a breeding and maintenance project, essentially all life stages of rats and mice are needed in order to deliver the animals to the various projects.

Mature rats and mice are essential for studying complex diseases in our downstream projects, such as haemophilia (a rare bleeding disease) and alpha- anti-trypsin deficiency (a disease that causes breathing and liver problems), where all systems must be similar enough to humans to find the most effective drugs. A study will always include the fewest number of procedures in order to answer the research question.

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

Each of our strains have a dedicated colony manager which enables us to combine years of knowledge and experience and tailor strategies for husbandry (general care) and breeding. Open and regular communications with other colony managers throughout the Establishment alongside unit technicians, Named Animal Care and Welfare Officers (NACWOs) and Named Veterinary Surgeons (NVS) further enables relevant and specific care for our strains and to identify any new and better treatments that could be utilised.

When a new strain is to be imported on this licence, details of the expected phenotype will be obtained from the supplier and sought in any published data, which will help plan the breeding and maintenance strategy and determine any specific husbandry needs. We also keep in regular contact with suppliers after import of the animals to inform them of our experience and learn any updated information that might help our colonies.

Changes to husbandry are adopted as required by each strain. This can involve increased general monitoring or at certain life stages for particular strains. We also tailor enrichment to alleviate stress (such as over-grooming) or fighting where a strain is found to be susceptible. Examples of past/ongoing refinements:

NZM mice, a model for a disease that causes inflamed joints and skin (lupus), can start to have symptoms from 4 months of age. The best, non-invasive way to monitor disease progression is to test the urine for protein. This could require a brief scruff (while supported, holding the animal by the skin along the neck and back); mice will usually urinate when handled, allowing to catch the few drops needed to test. However, sometimes just allowing the mouse to run freely on a clean glass-like surface may be enough to get the sample.

Haem A, Haem B, and Glanzmann mice that are used to study bleeding disorders need special daily checks that monitor for bleeding events; this can pick up any bleeds that can be treated with a powder (styptic) if it is on the skin. They also require a change from the usual bedding to a softer variety and the removal of certain enrichment due to their activity levels and propensity to bleed when knocked. Refined handling techniques also prevents bleeds in the scruff. Good communication with technical staff is essential; the most significant refinement for these mice has been creating score sheets for all to use, these set out a standard way of grading of health observations which significantly helped to assess any harm to these mice.

Haem A rats require monitoring over and above regular colony checks; rats vulnerable to having bleeds (homozygous) are handled each day and given a thorough health check, checking all over their bodies for signs of bleeds under the skin. Signs of bleeds within the body (internal bleeds) are also observed, for example pallor (very pale extremities like the ears). Their body weight is checked and

recorded if there are any signs of ill health. The daily checks pick up bleeds early on, meaning that appropriate action can be taken very soon in the progression of a bleed. Rats with a bleed can be given a treatment to help the blood clot (Factor VIII), and any suffering can be eased with pain relief given in Nutella (this means the rats will readily eat it from a syringe tip). Generally the first Factor VIII treatment and any pain relief reduces any suffering to a minimum and the bleed disappears over a day or two.

All animals are normally housed in social groups; the only exception to this would be if males are fighting or if the experiment requires temporary separation to measure individual outputs such as feeding.

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

We use PREPARE guidelines for the planning of animal experiments; these complement the latest version (2020) of the ARRIVE guidelines that are a checklist of important information to include when reporting animal research. Taken together these ensure that animal studies are reproducible, and as translatable to human diseases as possible.

We will also consult Laboratory Animal Science Association (LASA) publications for more general topical advice.

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

All members of the in vivo team are active members of LASA, so are well-placed to keep informed of new developments in the 3Rs.

We also regularly consult the Laboratory Animal Science Association (LASA), Federation of European Laboratory Animal Science Associations (FELASA) and National Centre for the 3Rs (NC3Rs) websites for new developments.

A retrospective assessment of refinement will be due by 5 July 2027

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?