Home Office

Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted January – June 2022 that require a retrospective assessment

Contents

1. Evaluation of neuroprotective agents in experimental stroke models

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
    - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
- Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

Stroke, Neuroprotection, Middle cerebral artery occlusion model, Coagulation model

Animal types	Life stages
Mice	adult
Rats	adult

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 central aim of our research is to provide service to companies to test the efficacy of potential new therapies for stroke.

A retrospective assessment of these aims will be due by 18 August 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 it's 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?

We are a contract research organisation (CRO) offering *in vivo* services to companies as per their requirement. This work will support pharmaceutical companies and academic institutions to advance their drug discovery and test new agents to treat stroke. The information generated from these studies is necessary to design studies in human. In order to evaluate drug efficacy, we will provide various outputs such as brain staining, behavioural assessment and mechanistic work depending on short- term or long-term studies.

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

The outputs of the project will look at the efficacy and safety of the drug candidates. The information generated from these studies could potentially be used to design studies in human. In order to evaluate drug efficacy, we will provide various outputs such as brain staining, behavioural assessment and mechanistic work depending on short-term or long-term studies. The data generated will be helpful to progress the potential candidates into clinical trials. These outputs will also help us to understand the complex mechanisms/ pathways involved in stroke. The data will be useful for scientific publications in peer-reviewed journals or patent applications.

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

A new therapy for stroke that could benefit large number of patients is desperately needed. In the first instance, the clients will be benefited from these outputs as the experimental models will be validated and established within our laboratory. The short-term goals of the project will be to test and identify potential candidates that can be advanced to the clinical trials. This will include optimisation of the pharmacokinetics of the initial compound received from the company followed by identification of compounds that can elicit a pharmacological response at well tolerated doses. The person/ team experienced in these models will be carrying out these studies and will be aware of any relevant adverse effects and humane end points.

The ultimate long-term objective of this project is to generate preclinical data that will in turn support the development of new therapies for the benefit of stroke patients.

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

In agreement with the client, publications of results in peer-reviewed scientific journals will be a major critical element in the dissemination strategy of this body of work, therefore making the research available for exploitation by stroke and pain researcher worldwide. To generate maximum output from these studies for the clients, each protocol has been efficiently designed to measure various outcomes. A record of unsuccessful approaches will be documented, reported, and will not be used in the future.

Wherever possible we will collaborate with other academic institutions/ companies for successful completion of the client's work. Furthermore, to widen the scope of knowledge transfer, visits and collaborations with laboratories across the UK will be organised during the duration of this project.

Species and numbers of animals expected to be used

- Mice: 2750
- Rats: 1950

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.

Once the *in-vitro* efficacy and toxicity of a drug has been determined, the preclinical testing becomes necessary for the drug to proceed to the next stage. In case of stroke models, the outcome measures that need to be tested include functional and behavioural testing which unfortunately cannot be tested in cells. The complex interplay of cerebral blood vessels, different brain cell types and chemical mediators underpin stroke pathophysiology. It is very difficult to mimic this complexity *in vitro* and, which makes *in vivo* testing critical.

Because adult mice and rats show a similar pathophysiological response to humans, these species are widely used for stroke studies.

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

Most of the animals that will be used in this project will undergo induction of ischemic stroke. Some animals will undergo transient or permanent occlusion of the middle cerebral artery (MCA) by inserting a nylon microfilament into the MCA, through the common carotid artery. Some other animals will undergo permanent MCA occlusion by direct occlusion of the MCA by electrocoagulation.

All animals will be tested for functional outcome, including neurological evaluation, motor-sensory evaluation, development of pain and cognitive impairment.

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

Following transient and permanent MCAO, animals will show transient weight loss and neurological impairment because of the surgery and the experimental stroke. Activity can be subdued for a few hours after recovery from anaesthesia. The weight loss recovers around 48 hours. The functional impairment starts to improve significantly after 48 hours and is very mild after 72 hours and specialised functional tests such as the Garcia scale are used to detect the functional deficits because they are so mild.

For short term studies, animals are expected to be sacrificed at 48 hours post MCAO. The rest of the animals will survive to about 30 days post-surgery to look for long term functional

improvement.

We have recently discovered that animals develop a mild hypersensitivity to pain in the affected limb which appears to persist to about 30 days. However, this pain is mild and does not appear to affect activity, feeding or other behaviours.

The therapeutic agents that are being tested could potentially have toxic effects which may manifest in weight loss, reduced activity and feeding. If toxicity is encountered, then these animals will be culled.

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 stroke model we are using mimics the human disease and over 50% of ischaemic strokes are due to MCA occlusion. After induction of stroke, animals will develop neurological deficits. Most of the transient middle cerebral artery animals are expected to reach severe level early after surgery (48hrs) and recover very well after that and have little observable deficits subsequently. In the permanent stroke models the deficits are very mild and animals have excellent recovery - all the animals reach moderate severity. All animals will be closely monitored and any animals exceeding the expected severity level will be terminated.

For the Pharmacokinetic studies, the animals are expected to reach mild to moderate severity only.

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

- Killed

A retrospective assessment of these predicted harms will be due by 18 August 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 overall objective of this project is to develop new therapies for acute stroke. *In-vitro* studies are not sufficient for evaluation in humans as ethically it would be unacceptable and potential toxic agents would be given patients who are already suffering from a disease. Before commencement of *in-vivo* studies, the potential issues associated with the drug will be discussed with the client to design potential *in-vivo* studies. Only the best candidates eliciting neuroprotective ability with minimal toxicity *in-vitro* will be considered for preclinical studies.

These studies are critical for companies to understand drug tolerability and efficacy before advancing to the next stage.

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

We will use primary cell culture models to assess the efficacy and toxicity of a drug *in-vitro*. We will induce excitotoxicity (an established feature of ischemia) *in-vitro* using N-methyl-D-aspartate (NMDA) and test for neuroprotective efficacy in cortical neurons. Another in vitro model of cerebral ischemia is the combined oxygen and glucose deprivation (OGD). Retaining glucose in the hypoxic chamber is less suitable for modelling an ischemic event, which always is accompanied by breakdown of the nutrient supply. Additionally, compared to *in-vivo* models, there is a need for a longer episode of energy deficiency to induce neuronal death. This will help us to identify candidates and design the *in-vivo* studies.

Why were they not suitable?

Although *in-vitro* experimental research has been an important component of our overall development plan, it is not, however, sufficient on its own because stroke involves complex interplay of multiple mechanisms in various organs. The complex situation of ischemic stroke cannot be modelled in an in- vitro system with single cells or brain slices with the absence of blood vessels and blood flow as well as lack of infiltration of leukocytes.

Furthermore, an important part of stroke efficacy and safety evaluation is the study of functional and behavioural outcomes which is not possible using in vitro experiments or computer simulations.

A retrospective assessment of replacement will be due by 18 August 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?

Based on previous experience and study requirements we feel this is a reasonable number over a period of 5 years. We will use the minimum number of animals to achieve statistically meaningful results. The number of animals will vary depending on the type of study, end points and client's requirements. We will be continuously monitoring the number of animals used in the study and carefully consider 3R's wherever possible.

What steps did you take during the experimental design phase to reduce the

number of animals being used in this project?

We will ensure that the experimental designs are rigorous to keep the animal numbers to minimum and also make sure that the all personal licensees working on the licence are appropriately trained and competent. We will follow the standard proven experimental design which has been previously published to minimise any errors associated with research.

This will help us in reducing variability, yield robust and reproducible data. The allocated personal licensees working on this project will be appropriately trained and suitably competent. We will regularly carry out literature search to identify human and/or animal cell-lines/ assays derived to assess pharmacokinetic parameters to negate the need to use *in-vivo* models and when appropriate we will revise the numbers requested down if an alternative to testing in animals is developed.

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

Wherever possible, we will follow published literature to guide our experiments. We will utilise *in silico* modelling techniques to assess toxicity prior to *in vivo* experiments. By adequately designing experiments, we aim to achieve robust and reproducible data and ensure that every animal is used to its full potential.

A retrospective assessment of reduction will be due by 18 August 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.
Species

To mimic clinical stroke lesions, we require an experimental species that closely resemble human stroke condition. Mice and rats are a common and desirable choice of mammalian species and have been used in scientific research for several years. Their circle of Willis is well defined and closely resembles that in humans. The proposed models are standard stroke models which have been used in the scientific research for over 30 years.

Models

Middle cerebral artery occlusion (MCAO) model - filament model

This model involves introduction of monofilament into the common carotid artery and advanced along the internal carotid artery to occlude the middle cerebral artery (MCA). The monofilament can be withdrawn before 90 mins to induce transient ischemia or left in place to induce permanent ischaemia.

Cauterisation model

Another way of introducing permanent ischemia without significant damage is by exposing the MCA by drilling a small hole and cauterising MCA. Even though these models were developed in 1980s, subsequent refinements have led to reproducible and reliable infarct volume. To further reduce the variability in our stroke models we will be using Laser Doppler to ensure that there is occlusion of MCA.

Throughout the surgical procedures, core body temperature of the animal will be maintained at ~ 37 degrees using the heat pad. This is a crucial parameter to obtain consistent infarct volume. For the filament model, the animals will be in an incubator/ heat box to allow better recovery of the animals for a maximum of up to 60 minutes post-surgical procedure.

Severity

The filament model causes significant brain infarction which results in functional deficits such as paralysis of contralateral limbs and sensory loss. We aim to minimise any mortality by humanely killing experimental animals that do not show signs of recovery. Post-operative sheets will be kept in place and the progress of each animal will be monitored carefully. Animals will receive fluid supplements immediately post-surgery, followed by mash/ wet gel. Since the loss of body heat could be a potential hallmark of ischemia post-surgery, cages will be housed on a heating blanket for 24- 48 hours.

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

To model stroke preclinically, we need to use species that closely resemble humans in many ways, such as resemblance in Circle of Willis and have central nervous system like humans.

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

We will be closely monitoring the animals post-surgery, in particular for initial 72 hours period, to make sure animals have recovered well from the ischemic surgery. We will also be in close contact with Named Veterinary Surgeon (NVS) or Named Animal Care & Welfare Officer (NACWO) to improve/ refine the post-operative care in order to minimise the distress.

This method has been refined over the last few years to reduce the severity of the procedure on the animals.

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

The best practice guidance published by National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) clarifies the responsibility in the use of

animals in scientific research in the most refined way. We will follow the STAIR guidelines and continuously look for improvements to the animal welfare.

In addition, for surgical procedures aseptic technique will be used to minimise the risk of infection and guidance on this can be obtained using http://www.procedureswithcare.org.uk and follow the recommended IMPROVE guidelines.

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

We will continuously be following the literature, check NC3Rs portal, and have conversations with the NC3Rs Regional Programme Manager.

A retrospective assessment of refinement will be due by 18 August 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?

2. Breeding and maintenance of rodents for drug discovery platform

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
    - 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 05 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 it's 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 and Glanzmann mice sometimes have surface bleeds that can be treated, and these usually resolve over 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 05 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 05 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 05 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 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 05 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?

3. Molecular mechanisms in cardiometabolic disease: effects of diabetes on the heart

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
    - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
    - Assessment, detection, regulation or modification of physiological conditions in man, animals or plants

Key words

Diabetes, Heart failure, Myocardial infarction, Skeletal muscle

Animal types	Life stages
Mice	adult

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?

This project aims to identify the molecular mechanisms which lead to the development of diabetes and cardiovascular disease. It focuses on how diabetes affects the heart after a heart attack and worsens the effects of heart failure on the body.

A retrospective assessment of these aims will be due by 24 November 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?

Cardiovascular disease is the commonest cause of death in people with diabetes. Although much is known about some of the links between diabetes and diseases of the heart and circulation, we still do not fully understand why people with diabetes remain at high risk of heart failure and do not respond as well to treatment. Increasing our knowledge in this area is vitally important at this time, because changes in human lifestyle have led to large numbers of people with obesity and are predicted to cause a huge increase in the number of people worldwide with diabetes over the next 15 years. In combination with the tendency for people to live longer, there will be many more people living with the consequences of diabetes and heart failure in future.

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

We expect to have increased our understanding of the causes of heart disease and how these are worsened by diabetes. In particular we will understand the actions of insulin and related proteins within the heart and muscles and how these are altered by both diabetes and cardiovascular disease. We hope to have identified new genes or proteins which link diabetes with cardiovascular disease and discovered how they affect the body.

Our short term outputs will be scientific papers published in scientific journals and presentations to the scientific community at meetings. We hope that our research findings will allow us generate longer term outputs with new ways to diagnose, prevent and treat cardiovascular disease in people with or at risk of diabetes.

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

In the short term the scientific community will benefit from these outputs, which will increase understanding of the basis of cardiometabolic disease. In the longer term we hope that our outputs will improve the lives of people living with, or at risk of, diabetes and heart disease.

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

We will maximise the value of our outputs by dissemination through a variety of means. These include presentations at scientific meetings, publications in open-access scientific journals and release of key findings through our institution's websites and social media streams. We have close links with networks of researchers and clinicians working in this field. Our institution has strong support systems in place to facilitate translation of research findings through to clinical application. We work very closely with colleagues in other disciplines - for example to allow us to develop new drug-like molecules to explore the findings from this research.

Species and numbers of animals expected to be used

- Mice: 5850

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.

We use mice to study the links between diabetes and cardiovascular disease. This is because it is relatively straightforward to alter the genes of mice to study how that gene influences diabetes and its effects on the heart. Many lines of mice are available to the scientific community in which selected genes have either been deleted or increased. Because genes encode proteins in the body, this allows us to study the effect of specific proteins. One example is that we study the receptor by which insulin exerts its effects on the cells of the body. By reducing or increasing the numbers of insulin receptors in certain cells within blood vessels and the heart, we can see how insulin and its effects on those cells might influence the susceptibility to recovery from heart attacks and the development of heart failure.

Mice are amenable to studying most of the diseases that affect humans. Because mice are mammals, findings can be used to mimic what happens in humans. For example, we can study diseases such as heart attacks and heart failure as well as studying the effects of treatments. We can also examine how the influence of diabetes on blood vessels affects the body's capacity to repair and heal itself after a heart attack. Because the physical limitations experienced by people with heart failure is caused by the effects of their condition on their muscles as well as their heart, it is important that we study the influence of diabetes and heart failure on skeletal muscle.

The majority of our research is carried out in adult mice as this is the life stage at which most humans develop heart disease.

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

Most mice used in this project will be genetically altered and will be bred under a separate licence held by the applicant for breeding of genetically altered animals. The genetic alterations affect the animal's molecular and cellular processes but do not themselves cause direct harm or disease. We use them to examine how specific genes affect the mouse's susceptibility to develop diabetes and heart disease.
We will compliment information obtained from genetically altered mice in some cases by treating the animal with a drug or infusing it with cells from another animal.

In many of the mice in our project we induce type 2 diabetes by feeding a high calorie and high fat diet. This leads to obesity and diabetes just like in humans. We induce type 1 diabetes by injection of a drug which damages the insulin-producing cells in the pancreas. We assess diabetes in mice by taking blood samples after giving an injection of glucose or insulin. We can measure energy usage, activity and metabolic rate by housing mice temporarily in a special cage.
We gain more detailed information on diabetes and the heart in groups of mice in which we study particular human diseases. We assess the effects of heart attacks in mice by placing a suture around one of the coronary arteries in an operation performed under anaesthesia. This blocks the blood supply to the heart just like in a human with a heart attack. The

resultant damage to the heart muscle can be assessed by ultrasound, MRI or CT scans. We can reproduce other heart muscle diseases seen in humans, such as those caused by viruses, drugs or alcohol, by infusing drugs into mice which reduce the pumping of the heart. We can also mimic the effects of high blood pressure or diabetes, which cause thickening of the heart muscle, by placing a constriction around the main artery at the outflow of the heart. All of these methods of heart injury in mice predispose to the development of heart failure and allow us to see how the body responds in the presence of diabetes.

Although we know that both diabetes and heart failure affect the way the body's muscles work, and reduce the ability to exercise, little is known about how diabetes and heart failure together affect muscles. Inability to undertake physical activities in people with heart failure can also cause a deterioration in muscles because the are not being used. To examine this in mice, we can place a tape around one of its hind legs so that mouse cannot bend its ankle. This results in the mouse not being able to use muscles in the calf and allows us to study the effects of disuse on these muscles.

We gain information on the heart and circulation by measuring blood pressure with a cuff around the tail, by taking blood samples and by taking scans of mice using ultrasound, MRI or CT. Heart tissue and muscle tissue can be studied in more detail in the laboratory after the animal has been humanely killed.

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

The genetic alterations themselves affect only the molecular and cellular processes in the body but are not expected to cause direct harm to the animal. Our research looks at how these genetic alterations affect the mouse's tendency to develop diabetes or heart disease when exposed to the experimental approaches discussed above.

As in humans, diabetes can lead to thirst and increased urine production and high fat diets can lead to an oily coat in addition to obesity. Blood sampling and blood pressure measurements lead to temporary discomfort. Ultrasound, MRI and CT scans are performed under anaesthesia from which mice recover very quickly. Metabolic testing requires animals to be temporarily housed in single cases which can sometimes cause distress.

Surgical procedures, for example to place a suture around a coronary artery or around the aorta, are performed under a general anaesthetic from which most mice recover rapidly. However, they are invasive procedures with up to 10% risk of mice dying during or shortly after surgery. As in humans, causing a heart attack or heart muscle damage in a mouse can lead to sudden death. The risk depends on the type of experiment, but can occur in up to 30% of mice studied. Death occurs instantly and is usually caused by rupture of the heart muscle or an abnormal electrical rhythm. Around 10% of mice develop signs of heart failure after a heart attack or heart muscle damage. Like in humans, the signs include rapid breathing and inability to exercise normally. If these persist for 24 hours the animal will be humanely killed. Taping the lower limb prevents the mouse from being able to bend the ankle joint in that leg. Mice can still move around adequately and the tape is applied for no more than two weeks.

Because we need to study how diabetes, heart disease and muscle impairment all contribute to the symptoms experienced by humans with diabetes and heart failure, some

animals will be subjected to all three conditions of diabetes, heart muscle damage and limb immobilisation.

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)?

Three of the protocols in this licence are moderate severity and four are severe. However, some animals not exposed to serial optional steps may experience mild severity. Overall the following proportions of animals are expected to fall within each severity:

mild: 15%

moderate: 73%

severe: 12%

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

- Killed

A retrospective assessment of these predicted harms will be due by 24 November 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 influence of diabetes on the cardiovascular system is complex. Diabetes comprises not just elevated blood sugar levels, but in most cases raised insulin levels, resistance to the effects of insulin and activation of the immune system contribute to its effects on the body. All of these affect the function of cells in blood vessels and in the heart. In obesity, factors are released from fat deposits into the circulation which also affect the heart. The complex interactions between these various processes and the communications between individual cells as heart diseases develop means that these diseases can only be effectively studied in animals or in humans.

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

We use a wide range of non-animal approaches to address our research aims. We use tissues from humans to identify genes which contribute to diabetes and cardiovascular disease. We perform much of our research in cultured cells from fat depots, blood vessels,

muscles and the heart to dissect out individual genes, proteins and pathways which influence their function. We mimic the context of diabetes by culturing cells in high glucose or high fat conditions. We generate proteins in cultured cells to assess how these behave and interact with receptors. We use computer-based modelling to design molecules to mimic the effects of these proteins. Finally we conduct clinical studies in humans to investigate the effect of diabetes on clinical outcomes and interrogate genetic databases and tissue banks to identify new targets. All of these approaches feed into the design of the experiments in this project.

Why were they not suitable?

These approaches complement and inform animal-based studies but unfortunately cannot replace them. As discussed above, the complex interaction between circulating and cellular factors implicated in the development of cardiovascular disease in diabetes means that this can only be studied in an intact animal.

A retrospective assessment of replacement will be due by 24 November 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 estimated the number of animals needed for each protocol based on our experience of using these approaches in previous projects and plans for continued and new work in this project. In most cases we have based our assessment on statistical approaches to calculate the minimum number of animals to obtain significant results. However, as new genetic alterations will be studied as informed by ongoing research, we have made assumptions on future requirements based on our best assessment of the science and our previous experience.

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

We utilised available online resources such as the NC3Rs experimental design assistant to plan experiments and perform power calculations to determine sample size. These were based on knowledge of the mean values and variability of the primary outputs for each protocol based on our prior experience and on published data. We designed experiments so that multiple experimental readouts can be derived from a single animal. We use imaging when possible so that disease development can be tracked non-invasively and confirmed by tissue approaches after humane killing.

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

We work collaboratively with other researchers at our institution, so that we can share tissues between projects and avoid duplication of animal use. We optimise breeding of genetically altered animals (performed under the authority of another licence) so that breeding is fully aligned with planned experimental requirements. We use an electronic animal management system so users can track animals remotely and plan experiments to reduce waste. We keep updated with advances in scientific techniques and with ideas for reduction in animal use from the NC3Rs newsletter.

A retrospective assessment of reduction will be due by 24 November 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.

We will use mouse models of diabetes and cardiovascular disease. Typically, genetically altered mice will be used to study individual genes (or combinations of genes) in disease development. This approach will be supplemented by administration of drugs, viruses or cells when required to address scientific questions. We will use a wide range of methods and models to study the full range of human vascular disease. These are described in detail in the 'Project Harms' section of this application. Our general principle is to use the model with the least likelihood of causing suffering to address the scientific question.

We have gained substantial experience of surgical techniques during the course of our previous licence. We have performed >200 surgeries for coronary artery ligation to induce myocardial infarction and >100 for transverse aortic constriction. This has allowed us to develop a number of refinements described in the section below.

We avoid single housing of animals unless essential for scientific reasons or animal welfare. We perform surgical procedures under general anaesthesia with routine use of analgesia. Longer procedures are covered with adequate hydration, warming tables, application of eye lubrication and post-operative warming.

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

It is necessary to use a mammal to study the complex interactions involved in the development of diabetes and cardiovascular disease and to translate the findings to

humans. Although certain genetic factors implicated in blood vessels or heart development can be studied in zebra fish, it is not possible to model type 2 diabetes and more complex cardiovascular pathologies in fish. Because cardiac pathologies typically develop over days to weeks, is not possible to study the entire process under terminal anaesthesia in mice. Adults will typically be used as this is the life stage at which the cardiovascular diseases we are studying usually occur in humans.

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

Surgical procedures will be performed under general anaesthesia. Animals will be recovered in a warmed chamber following longer procedures. Analgesia will be administered routinely to avoid pain developing.

We have taken the opportunity to develop a number of refinements over the last five years during the course of our previous project licence. We have reduced the duration of surgery for coronary artery ligation from 1.5 hours at the beginning of our experience to 35-45 minutes currently. This allows more rapid recovery from anaesthesia. We have optimised the site of the thoracotomy depending on how proximally the coronary artery is ligated and the size of the infarct required to address scientific objectives. We access the thoracic cavity between 2nd and 3rd ribs for larger infarcts and between 3rd and 4th ribs for smaller infarcts. For transverse aortic constriction, we have reduced the duration of surgery to 25-40 minutes. We routinely employ endotracheal intubation which has been reported to improve survival. In our hands mortality in mice undergoing transverse aortic constriction with endotracheal intubation was 11.6% (compared to 20-25% in the published literature). We restrict transverse aortic constriction surgery to mice weighing 23g or more to reduce the risk of surgical mortality.

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

We will use the following resources in planning and conducting experiments: ARRIVE Guidelines 2.0. https://arriveguidelines.org/arrive-guidelines PREPARE Guidelines. https://norecopa.no/prepare
NC3Rs Experimental Design Assistant. https://eda.nc3rs.org.uk/

NC3Rs guidance on blood sampling in mice. https://www.nc3rs.org.uk/3rs-resources/blood- sampling/blood-sampling-mouse

NC3Rs guidance on microsampling, including the microsampling decision aid. https://www.nc3rs.org.uk/3rs-resources/microsampling

NC3Rs Mouse Grimace Scale. https://www.nc3rs.org.uk/3rs-resources/grimace-scales/grimace-scale- mouse

NC3Rs guidance on anaesthesia. https://www.nc3rs.org.uk/3rs-resources/anaesthesia
NC3Rs Guidance on analgesia. https://www.nc3rs.org.uk/3rs-resources/analgesia
NC3Rs Guidance on handling and restraint. https://nc3rs.org.uk/3rs-resources/handling-and-restraint

LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery. https://www.lasa.co.uk/wp- content/uploads/2018/05/Aseptic-Surgery.pdf

EFPIA/ECVAM good practice guide to the administration of substances and removal of blood, including routes and volumes.
https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/abs/10.1002/jat.727s.

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

Our group will stay informed through the NC3Rs website. Relevant information, including the NC3Rs newsletter, is circulated within our institution by email to all personal and project licence holders. We will attend local events organised by our Animal Welfare and Ethical Review Committee and information sessions on NC3Rs funding streams organised by our institution's Research & Innovation Service. We will share best practice within our institution and have well developed interdisciplinary networks to facilitate this. We will hold regular local user-group meetings for project licence holders at which the group will receive updates on any changes to best practice or requirements.

A retrospective assessment of refinement will be due by 24 November 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?

4. Neural circuits for movement

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
    - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
    - Assessment, detection, regulation or modification of physiological conditions in man, animals or plants

Key words

spinal cord, brain stem, dystonia, motor neuron diseases, movement disorders

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

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 overall aim of this project is to understand how the brain and spinal cord control movement. The specific aims are: (a) to understand how the spinal cord coordinates behaviours such as locomotion and grasping; (b) to understand how the brain stem “turns on” and modulates these target spinal circuits; and (c) to identify the mechanisms by which these circuits adapt (or not) in neurological diseases or injuries.

A retrospective assessment of these aims will be due by 24 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 it's 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 research is the foundation for the development of strategies aimed at improving quality of life in people with neurological diseases or injuries affecting movement, such as spinal cord injury, movement disorders, motor neuron diseases, traumatic brain injury, multiple sclerosis, stroke, cerebral palsy, movement disorders, and other neurodegenerative diseases.

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

We will create new knowledge about how circuits of nerve cells in the brain and spinal cord are wired to produce movement. We will learn how these circuits are affected in some neurological diseases. This knowledge will be presented at appropriate conferences, and will result in publications.

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

In the short term, the global scientific community that focuses on the neural control of movement will benefit from the new knowledge, which will have significance to researchers whether their approach is cellular/molecular, systems neuroscience, or disease neuroscience. In the longer term, this research will have impact for human diseases and suggest new approaches to address movement impairment in those with neurological diseases.

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

We will maximise outputs through publication (open access) and making our data available to others (open data). We will continue to publish negative data. Furthermore, we are involved in a number of collaborations that impact the efficiency and significance of our research. In addition to publications, we typically present early stage research at conferences and seminars: these opportunities provide feedback that can lead to more efficient progress. We will engage in public outreach activity as appropriate.

Species and numbers of animals expected to be used

- Mice: 28,500

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.

To understand how neural circuits produce movement, it is necessary to use an animal that has limbs, and a nervous system that includes a brain stem and spinal cord. Furthermore,

to identify and manipulate specific types of neurons, it is important to have genetic access to these neurons. Mice fulfil these criteria.

A critical period in the development of neural circuits for movement is in the first 2-3 weeks after birth. We thus investigate developing circuits during this time period, and mature circuits in adult mice.

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

We use genetically modified mice so that specific types of neurons can be seen (e.g. through expression of fluorescent proteins) and manipulated (e.g. by eliminating their neurotransmission). In addition, we use genetically modified mice for disease models, such that we can understand how these circuits change in diseases.

The techniques we use include: movement training (e.g. walking on treadmills), assessment of movement parameters (e.g. how the limbs move during walking), recording of muscle activity during movement ("EMG" recordings), stimulating nerves (e.g. by surgically implanting wires around nerves), labelling of neurons (e.g. with dyes or non-infectious viral vectors requiring injections into muscles, spinal cord, or brain), and lesioning areas in the brain, spinal cord, nerves, or muscles (often requiring surgery) to determine how these regions contribute to movement.

The training occurs over several days to weeks, behavioural assessments may be done repeatedly (e.g. before, during, and after training). The quantification of muscle movement may be done with implanted wires (lasting several weeks) or with wires implanted only for hours (for single use).

While injections are usually singular, there are occasions when 2 or 3 injections will be given at staggered time points.

At the end of experiments, the animals will be anaesthetised and tissue harvested for analysis. This is sometimes done without any of the above procedures, and sometimes following training, assessments, injections, and/or lesions.

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

The vast majority of animals will not experience any significant adverse effects, and will be housed in enriched environments.

In genetic models of diseases (e.g. movement disorders such as dystonia) or in animals with nervous system lesions, mice can have abnormal behaviour (reduced movement) and may grow less rapidly than their counterparts. We have not seen signs of pain in these animals. The duration of these effects is under a month in the more affected mice (in fact, we generally do not wean these mice), but could be longer (e.g. 2-3 months) when there are mild signs. Mice are observed for signs of distress, at which time they are euthanised.

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 of our breeding mice will be mild, and some will be of moderate severity (e.g. those with genetic manipulations such as dystonia). The remaining protocols are mostly of moderate severity, although those involving lesions may be severe (<5% of all animals).

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

- Killed

A retrospective assessment of these predicted harms will be due by 24 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?

In order to understand neural circuits and how they go awry in movement, it is necessary to use an animal that has these circuits (and multi-joint limbs that they can control). It is not possible to understand circuits using computer models without having knowledge of how they are wired to function in the first place. And cell culture techniques do not replicate neural circuits. Finally, while we can understand the phenomenology of movement and movement disorders in humans, we cannot understand the underlying mechanisms (including the circuits) of movement in humans.

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

When possible, we do use cell culture techniques (e.g. embryonic stem cells, iPSCs) instead of animals. Furthermore, when we gain sufficient knowledge, we use computer models to help us understand how neurons function together to produce movement. And of course, we study movement in humans (e.g. our patients) when possible.

Why were they not suitable?

While these alternatives have a role, they cannot shed light on how neurons function together, in circuits, to produce movement.

A retrospective assessment of replacement will be due by 24 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 are basing this number on the number required for our specific aims. Many of our experimental mice require 2, 3, or 4 genes (alleles) to be present, which requires multi-generational breeding. We have met with statisticians to define, for example, the appropriate experimental unit for different types of experiments. And we have developed processes to maximise efficiency of data collection from each mouse.

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

Most importantly, we have adopted a new animal breeding strategy ("staggered tickover") that significantly results in fewer mice being bred. We have also met with a statistician to discuss experimental design and experimental units. And we have designed experiments for optimisation of data collection.

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

We will keep mouse colonies to a minimum size, depending on their capacity to breed, the number of different genes or copies of genes needed in experimental animals, and the number of experimental animals needed. In addition, we significantly refined our breeding strategy to reduce the number of mice bred and to maximise the experimental yield. Moreover, using animals bred in colonies of other research groups and reciprocally sharing animals from our own colony with other research groups avoids the establishment and maintenance of duplicate lines.

We make every effort to obtain the maximum amount of data from each animal. Our research is discovery research concerning the circuits and properties of the neural circuits involved in the production of movement. This type of research does not often allow for a statistical design where the number of animals can be calculated in advance, making it difficult to accurately predict numbers needed for each sub-project. However, obtaining the maximum amount of data from each animal minimizes the number of animals used. We will also combine different methods of tissue harvesting (e.g. saline perfusion for sampling of fresh tissue followed by fixative perfusion for sampling of fixed tissue), allowing for several different types of analysis that would otherwise require the use of supplementary animals.
A retrospective assessment of reduction will be due by 24 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.

While we have learned many principles regarding motor circuit function from the study of invertebrates, a limbed vertebrate model is necessary to study how the nervous system produces limb movement such as walking or grasping. There are significant differences between mammalian and non- mammalian species, both in how they move their limbs and in their spinal circuits. Therefore, we have selected a mammal. Mice are used because of the availability of genetically modified lines needed to identify and manipulate specific populations of identified neurons within the spinal cord and brain stem.

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

Our aim in this proposal is to study neural circuits for movement, with the ultimate goal being to lay a foundation for new therapies that will address movement and quality of life in people who suffer with neurological diseases and injuries. Given that we study circuits and how diseases disrupt circuits, it is necessary to study an organism that has circuits that produce movement. That is, neither computer models nor cell culture methods are sufficient to study motor circuits: we need a multicellular animal. We also have excluded invertebrates as they have no spinal cord we need to use animals with spinal cords (chordates). Next, we note that diseases severely impact limb movement, so we need a limbed animal - in fact, an animal that can produce complex movements with their limbs. Thus, we have ruled out fish and amphibia; while some birds and reptiles have amazing limb movements, these are biomechanically quite different from ours; we are thus left with mammals. We need genetic access and manipulability. We do not have the genetic tools in rats. So we are left with an excellent model that fulfils our criteria: the mouse. We use young (pre-weaned) mice when possible and appropriate.

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

We will use the most refined methods according to published literature. Suffering will be minimised at all points. While some of the genetically modified mice may have disorders affecting movement, we will ensure they (as all our mice) are in enriched environments, and that they have no issues with feeding or other daily functions. Mice will be regularly examined by experienced and trained staff. Veterinary advice will be sought where and when necessary. Humane endpoints will be used to ensure any suffering is kept within moderate limits.

Whenever possible, we will use genetic systems that are designed to confine mutations to specific neurons within the circuits under study, so as to minimise widespread expression. Furthermore, when possible, we will use genetic systems that require a drug to activate them, so as to confine the expression to a particular set of neurons at a specific developmental stage, for example early post-natal mice. This strategy, too, will minimise the phenotype to the particular time point being studied, prior to which the mice will have no abnormal behaviours.

For all surgical procedures described, general anaesthesia is used, and post-operative analgesia will be used with consultation of the Named Veterinary Surgeon.

Certain protocols within this licence have been categorised as severe because surgical procedures will result in significant loss of movement function and some discomfort during the post-operative recovery period. However, as pain is a negative outcome for both the welfare of the animal and the results of the experiments, every effort will be made to ensure that pain and discomfort are minimised. Staff who are experienced (e.g. >2 years) in post-operative animal care and in recognising pain and discomfort will monitor mice closely throughout the experiments. Analgesics will be administered post-surgery, and any animals displaying signs of excess discomfort will be euthanised using approved schedule 1 methods. These lesion models are essential for furthering our understanding of how neural circuits function after CNS injuries and will underpin the development of specific strategies to improve functional recovery. Currently there are no alternative, less severe models which are able to faithfully model CNS dysfunction.

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

The work in this project will be undertaken in accordance with the principles set out in the Code of Practice for the Housing and Care of Animals Bred, Supplied or Used for Scientific Purposes (ISBN 9781474112390) and in the Guidance on the operation of the Animals (Scientific Procedures) Act 1986. Other sources of information regarding the application of the 3Rs and animal research may include http://nc3rs.org.uk, https://www.gov.uk/guidance/research-and-testing-using-animals and http://www.lasa.co.uk. We will continue to undertake and report our data according to ARRIVE guidelines.

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

We continue to be involved with the animal research community, and receive regular updates from NC3Rs. Our lab manager liaises with them, and keeps up to date on advances through courses, for example. She then communicates these new advances with me and the whole lab group.

A retrospective assessment of refinement will be due by 24 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?

5. Bioaccumulation of Nanomaterials and Related Substances

Project duration

5 years 0 months

Project purpose

- Basic research
- Protection of the natural environment in the interests of the health or welfare of man or animals

Key words

Fish, Bioaccumulation testing, Nanomaterials, Oral exposure, Environmental hazards

Animal types	Life stages
Zebra fish (Danio rerio)	adult, juvenile, neonate
Rainbow trout (O. mykiss)	juvenile, adult
Rats	adult

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?

Engineered nanomaterials are novel substances used in many products and are being released into the environment, but there are concerns about the bioaccumulation in humans and wildlife. The overall aim is to develop a tiered approach to bioaccumulation testing, so that only the materials of most concern are put forward for testing in vivo on vertebrate animals.

A retrospective assessment of these aims will be due by 28 August 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 it's 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?

Nanotechnology is identified as a priority for economic growth and prosperity in the EU, and this work will enable safe, responsible innovation. The data will provide the supporting evidence to help eliminate and reduce several aspects of animal testing, especially on bioaccumulation. This will have wide benefits within the EU and in countries involved in the consensus building on nanomaterial testing at the Organisation for Economic Cooperation and Development (OECD), and a step change to move away from animal testing.

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

The outputs will include new data and scientific publications to provide the evidence-base on the bioaccumulation testing strategy for nanomaterials, and related aspects where uptake into organisms is important to inform on environmental or human safety. The scientific documents will also include evidence submitted to international bodies, specifically to the OECD.

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

The outputs will be used by the OECD to inform on how best to proceed with the testing of nanomaterials, whilst minimising the use of vertebrate animals, and seeking alternatives to animals. In the short term, this will contribute to the work at the OECD and the wider global community involved in the standardisation of methods for nanomaterials, and in the medium term contribute to new technical guidance documents, and hopefully, eventually, legal changes in the need for testing animals. The beneficiaries will be all the companies involved in the nanotechnology sector, government departments, regulatory agencies, and the wider public who can benefit from nano-enabled products.

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

The work is mainly funded by collaborative EU projects, and the outputs will be disseminated at conferences, in scientific publications, via newsletters, social media and other aspects of the dissemination plans in those EU projects. We can ensure the scientific evidence is presented to the OECD and international community, with our proposals to minimise vertebrate animal testing.

Species and numbers of animals expected to be used

- Zebra fish (Danio rerio): 4700
- Other fish: No answer provided
- Rats: 980

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.

The experiments will use mostly use juvenile rainbow trout, and sometimes adult zebrafish, carp or sticklebacks, as well as selected studies on rats. These animals, and their life stages, are chosen as those most relevant to the regulatory testing that we are seeking to reduce or replace. All animals are listed as test species in the international guidance by the OECD and other bodies. At the OECD working party on manufactured nanomaterials (WPMN) data are required on herbivorous fish (e.g., carp) to compare against the trout as a carnivore, and also on rats for read-across between vertebrates species. Zebrafish are used as the preferred species for toxicity screening with fish, and the sticklebacks are also relevant as an ecologically important endemic species in the UK and Europe. It is necessary to show the results of those tests for nanomaterials in order to develop an alternative testing strategy that uses less animals.

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

For waterborne exposures, typically around 10-12 fish/tank (~30 per treatment) would be exposed via the water for up to 6 weeks. For dietary exposures, around 150 fish will typically be fed the nanomaterial of interest in experimental diets for up to six weeks, and their growth and nutritional performance measured. At the end of the experiments, animals will be humanely killed (e.g., by terminal anesthesia) and the tissues collected for bioaccumulation measurements and other aspects relating the biochemical, histological or physiological effects of the materials. Oral studies on rodents will be similarly performed to those with fish, but using around 32 animals/material in selected experiments.

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

The project intends to look mostly at physiological and sublethal effects using exposures via the food. So, the impacts are expected to be mild. With novel materials there is a risk of unintended or unexpected effects. For example, irritation of the gut during feeding studies or changes in nutrition in animals fed food containing nanomaterials (moderate). These effects may be transient (a few hours/days) or persist for the duration of an experiment (e.g., 4-6 weeks). In some experiments with very novel materials, some range finding on the lethal concentration maybe necessary using early life stages of zebrafish, adult zebrafish or juvenile trout (severe). However, computer predictions of hazard, cell culture work, and ex-vivo studies on organs will inform on reducing the amount of animal testing in the moderate and severe categories. Animals will be killed humanely at the end of each experiment.

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 project is expected to be mild in all the main studies on all types of fish or rodents. If moderate impacts are observed on fish (e.g. during dietary exposure to nanomaterials), then we would not proceed to studies on rats, so no moderate or severe effects are expected on any rodents.The proportions are indicated below.

Species	Mild	Moderate	Severe
Rainbow trout	95% or more	5%	<5%
Zebrafish	95% or more	5%	<5%
Carp	95%	5%	0%
Sticklebacks	95%	5%	0%
Rats	100%	0%	0%

The project also includes an initial range finding protocol to establish the dose for the other studies in the project, and uses a lethal aquatic toxicity test method (i.e., defined as severe) in early life stages of zebrafish, adult zebrafish, or juvenile trout only. In most cases (9 out of 10 materials) this severe protocol should not be needed and mild doses estimated from existing knowledge or experience.
Sometimes, (1:10 materials) we may find a very novel material requires range finding data. In these cases, only for this protocol where the LC50 is the end point, for both zebrafish and trout the proportions of animals in each category would be: mild (20%), moderate (30%), severe (50%). But overall in the project, the estimate for zebrafish and trout would be for both species: mild (90% or more), moderate (~5%), severe (<5%). Carp, sticklebacks and rats are not used for range finding, so their proportions of severity for the licence are as indicated in the table above.

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

- Killed

A retrospective assessment of these predicted harms will be due by 28 August 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?

It is not possible to make prediction tools or alternative models/methods without the *in vivo* data on each specific nanomaterial. The animal data is absolutely necessary for the validation of any tiered approach to testing and for the models or steps in the approach. Furthermore, to strive for changes in regulatory testing such that animals are used less often, it is necessary to show that the alternatives give an accurate prediction of *in vivo*. The vertebrate animal data is needed to show those correlations with alternative methods, such as alternative invertebrate tests, use of cell cultures, and *in chemico* or in silico approaches.

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

Alternatives to vertebrate animal testing is integral to progressing towards an internationally agreed strategy that incorporates the 3Rs, and minimises animal testing of nanomaterials. We are considering computational tools and predictions, in chemico digestibility assays to predict bioavailable fractions, and tissue/cell culture alternatives to fish and rodents.

Why were they not suitable?

The alternatives need to be proven to be suitable - the only way we can do that in a regulatory context is to show that the alternatives give an answer that is the same as the animal test, and therefore a viable alternative to the animal test. Data on the animals and the alternatives are both needed in order to change the testing strategy away from animal testing.

A retrospective assessment of replacement will be due by 28 August 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?

Based on our experience of the minimum number of animals needed to achieve the time points and doses in each of our experiments, and then estimating how many experiments might be conducted in the duration of the licence. Our statistical approach and experimental designs ensure the use of animals is minimal within each type of experiment.

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

We consider the scientific objectives of the experiment, whether or not one experiment can consider multiple objectives with careful design. Also, the minimum number of doses (treatments) to achieve the objective, and the minimum number of time points to show effects. These all consider the known variance in the data (e.g., expected size of error bars), whether data are normally distributed, and the usefulness of power analysis to define the number of replicates. Sometimes our tiered approach to testing will remove the need to use animals entirely.

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

The statistical measures outlined above, and reflective learning - considering the results of each experiment before doing the next. For example, deciding if treatments can be reduced in concentration or time, or removed from the next study.

A retrospective assessment of reduction will be due by 28 August 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 first protocol in the licence is a lethal range finding experiment with fish that will enable refinement of dosing in any subsequent experiments. Other protocols with fish will be sub-lethal exposures to substances via the water or food. The animal models will include rainbow trout or zebrafish, or other freshwater fish species, and these are selected for practical reasons relating to standardisation of regulatory testing and permitted species in those tests, not for ethical refinement per se. However, measures taken to reduce suffering will include using lower doses that are less toxic, shortening experiments where possible, and withdrawing individual animals from experiments for health reasons. It may be necessary to conduct a few oral studies with laboratory rats, but only as a last resort in the scheme of work, and will be mild or non-procedural.

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

In vivo studies are necessary to generate the data to compare against our proposed alternatives to vertebrate animal testing at the OECD. The vertebrate animal alternatives will include invertebrate species, and in order to stop or reduce the use of fish and rodents, the existing methods need to be compared against the invertebrate alternatives and other tools. Unfortunately, fish embryo tests are not sensitive to nanomaterials because the egg membranes prevent the fish being exposed, so we cannot use this model for our work.

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

Our procedures are already very well refined based on our long experience and expertise of each of the methods we use over the last 30 years. With respect to refinements in experiments with fish, we always seek sub-lethal indicators to minimize the risk of lasting harm or other effects, and review these as the data sets progress.

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

The problem is that we do not think the "best practice" guidance offered for standardised fish tests are good enough. We want to change the testing strategy considerably, so that the tests on vertebrate animals are no longer needed. Our methods are well refined and we follow the protocols already reported in our scientific papers, and considerations published in documents from animal welfare organisations such as NC3Rs in the UK, where we have also co-authored documents. Most recently in 2021, with our further refinements to the acute fish test (OECD TG203) and our international working group recommendation to move to more refined non-lethal endpoints.

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

We are actively engaged at the Organisation for Economic Cooperation and Development (OECD) and our objectives includes much greater consideration of the 3Rs in the animal testing strategies. Animal welfare experts are part of the working group(s) at the OECD, and we work together routinely, including scientific opinion on how best to include the 3Rs and also making recommendations on how to implement the 3Rs internationally, and especially at the OECD. We have also participated in technical meetings and produced technical papers on the 3Rs and fish tests, in collaboration with animal welfare organisations. We are therefore greatly involved with this agenda.

A retrospective assessment of refinement will be due by 28 August 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?

6. Influenza in ferrets

Project duration

5 years 0 months

Project purpose

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

Key words

Influenza, Vaccine, Epidemic, Pandemic, Antigenicity

Animal types	Life stages
Ferrets	adult

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?

To infect ferrets with human and animal influenza viruses for the generation of post-infection antisera for the antigenic analysis of influenza viruses.

To produce ferret hyperimmune antisera also for the antigenic analysis of influenza viruses. To characterise the replication of influenza viruses in the ferret in vivo model of influenza.

A retrospective assessment of these aims will be due by 16 August 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 it's 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?

Influenza viruses continue to threaten human and animal health. They are best controlled by vaccines and antiviral medicines. Our aim is to define the antigenic properties of new and emerging influenza viruses and to understand their replication in a small animal model.

This work leads to the generation of information about the antigenic properties of circulating and emerging viruses to guide the selection of influenza vaccines, how vaccination might be effective against these viruses, and to generate information that can be used to assess the risks associated with influenza viruses of animals infecting humans (zoonotic influenza viruses) in terms of replication and spread in mammalian models and their sensitivity to antiviral treatments.

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

Antigenic characterisation of circulating and emerging influenza viruses is key to the development of seasonal influenza vaccines, vaccines prepared for pandemic preparedness purposes, and for vaccines to be used during an influenza pandemic. The data generated as an outcome of this project, on how post-infection antisera recognise new influenza viruses are used for the biannual recommendations drawn up by the World Health Organisation (WHO) on the composition of influenza vaccines for seasonal influenza. Similarly, recommendations on the development of vaccines made for pandemic preparedness purposes include the antigenic characterisation of influenza viruses of animals (e.g. birds, pigs, dogs and horses) that might pose a risk to humans.

Data generated on how viruses replicate in mammals and cause disease, and how they spread between infected and uninfected animals will be used in risk assessments of newly emerging influenza viruses (done, for example, using the WHO The Influenza Pandemic Risk Assessment tool, see https://apps.who.int/iris/handle/10665/250130).

Assessing how vaccines or antiviral medicines protect against influenza infection in animal models is one of the starting points for assessing the likely benefits of vaccination by established and new methods, or antiviral use.

The results of these analyses will be shared with the WHO. This will be done as an ongoing process throughout the period of the project. Work will be published in peer-reviewed journals where appropriate.

Who or what will benefit from these outputs, and how?
Public health will benefit from the work.

Antigenic characterisation of circulating viruses, newly emerging and zoonotic viruses and candidate vaccine viruses for seasonal influenza vaccines and vaccines made for pandemic preparedness purposes, is essential for the development of influenza vaccine recommendations. Antisera recovered following infection and the use of these for determining the antigenic properties of new and circulating viruses and candidate vaccine viruses are the linchpin for the global influenza surveillance activity conducted through the

WHO Global Influenza Surveillance and Response System (GISRS).

The results from examining the properties of viruses of animals that infect humans, zoonotic influenza viruses, will be shared with WHO for risk assessments of the pandemic potential of such viruses. These assessments lead to the subsequent prioritization of viruses for the generation of vaccines for pandemic preparedness purposes.

The efficacy of vaccines and antiviral medicines will, in the long term, we hope lead to improvements to medical practice.

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

It is critical to characterise antigenically and genetically any newly emerging influenza virus. WHO GISRS provides a framework to ensure that viruses from around the world are analysed antigenically and genetically to build up a global picture of the changing characteristics of influenza viruses. Results are shared with other centres to develop recommendations on the composition of seasonal influenza vaccines and vaccines made for pandemic preparedness purposes. Therefore, from these analyses with ferret antisera new vaccines are developed.

Assessment of the properties of influenza viruses of animals causing zoonotic infection is a key step in influenza pandemic preparedness and this will be done, in conjunction with WHO, as new viruses emerge and their properties are examined. These risk assessments are carried out to mitigate the impact of influenza.

This work will continue throughout the project. Results from new vaccines and new antiviral medicines will be shared with WHO and other public health agencies in a timely fashion, and published in Open Access peer-reviewed journals.

Species and numbers of animals expected to be used

- Ferrets: 450

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.
Adult ferrets are to be used in this work. Ferrets have been used in research on human influenza since the human influenza virus was first isolated in ferrets in 1933. They are recognised to be the best animal model for human influenza since they are susceptible to human influenza viruses without virus adaptation, following infection ferrets show a similar course of disease to humans, and post-infection ferret antiserum reveals a similar immune response to that seen in humans.

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

Adult ferrets will be infected with influenza viruses by intranasal instillation of virus and monitored for disease signs. If disease signs are severe then antiviral treatment will be given. In addition, in some cases, treatment with antiviral medicines will be given over the course of infection to assess their potential in an aniaml model to control infection by by

standard virus or a newly emerged influenza virus. Prior vaccination of ferrets may be undertaken to assess the efficacy of a vaccine in a small animal model. Ferrets may be sampled for virus or serum antibody titres over the course of infection, or following secondary infection, or following boosting with virus antigen or vaccine. At the end of the procedure ferrets will be killed by a schedule 1 method, or exsanguinated under terminal anaesthesia, completed by killing using a Schedule 1 method.

Most experiments will last for a period of two weeks.

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

Most of the influenza viruses used only cause mild adverse effects on the ferret, exhibited for only a few days – mild adverse effects include sneezing and nasal discharge, some lethargy. For those influenza viruses that cause more severe disease, like highly pathogenic avian influenza viruses that can cause zoonotic infections and may have pandemic potential, antiviral treatment is an option to moderate the disease signs. Severe disease signs include a sustained lack of movement, a failure to eat food, a loss of response to stimuli, diarrhoea, and laboured breathing.

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)?

As indicated above, most of the viruses used cause no serious adverse effects on the ferret, ferrets might be lethargic and show mild disease signs (e.g. sneezing, nasal discharge). Some influenza viruses, e.g. H5N1 and H7N9 avian influenza viruses, can show severe disease signs. Over the past five years, such viruses have represented the very small minority of viruses used, less than 5%. The large majority of viruses (95%) cause only relatively mild infection, resulting in sneezing and nasal discharge and some loss of interest in the environment.

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

- Killed

A retrospective assessment of these predicted harms will be due by 16 August 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?

It is essential to analyse the antigenic characteristics of new and emerging human

influenza viruses in order for recommendations for seasonal influenza vaccines and for vaccines made for pandemic preparedness purposes to be developed. This is done using antisera generated to reference viruses, and newly emerging influenza virus variants. The antigenic characteristics of influenza vaccines and candidate vaccine viruses are also required to be established prior to their use in humans. The generation of antisera (usually post-infection, sometimes hyperimmune) in a timely fashion is a necessary step in these processes. These processes to determine how the immune response of an animal to a virus recognises different stains of the same virus cannot be done without the use of animals to generate antisera.

The growth properties of newly emerging animal influenza viruses able to infect humans is a necessary component of the risk assessment of such viruses. The best animal model for understanding the disease, virus tissue tropism and virus transmission is the ferret. These results from such work done in animal models complements work done in vitro and so no alternative to this in vivo work is feasible and so cannot be done without the use of animals. The work done in animals characterising new viruses complements those done with biochemical and biophysical methods on the virus done in vitro or ex vivo or in other systems.

New influenza vaccines and new influenza antivirals need to be tested in animals prior to human trials. Notably for the development of antivirals extensive work in tissue culture, in organ culture and in less sentient animal systems (e.g. embryonated eggs) is carried out prior to the work done on animals.
Because the ferret is the best animal model for influenza, examining the efficacy of new influenza vaccines and antiviral treatments in ferrets is an important step in their development prior to testing in humans.

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

Human monoclonal antibody (h-mAb) panels have been considered as an alternative to using ferret antisera for tracking the antigenic characteristics of influenza viruses. These mAbs are made from white blood cells after usually after vaccination although they can also be made after infection. The window for taking the human blood sample is small, two to four weeks after vaccination or infection.

Why were they not suitable?

Panels of human monoclonal antibodies (h-mAb) have not been able to replace the use of post- infection ferret antisera for the antigenic analysis of newly emerging influenza viruses. As stated above, such h-mAb panels would usually be made following vaccination. However, it is essential to assess the antigenic property of any newly emerging influenza virus as soon as it is recognised because it is the antigenic properties of newly emerging viruses that are the main factors that lead to new influenza vaccines. Only after making a vaccine can a panel of human monoclonal antibodies usually be made – perhaps 18 months or more after the emergence of an antigenically distinct strain that might have gone on to cause an influenza epidemic. An alternative of making monoclonal antibodies following influenza infection is very difficult to set up currently due to the unpredictable nature of the infection and influenza epidemics, making access to relevant human plasma cells and B-cells in a timely manner highly problematic.

A retrospective assessment of replacement will be due by 16 August 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 need to have produced panels of antisera against a number of influenza viruses of each influenza A sub-type (H1N1, H3N2) and influenza B lineage (B/Victoria lineage and B/Yamagata lineage) for generating antigenic data for developing recommendations for influenza vaccines for use globally. We use in the order of 10 to 12 antisera in the tests carried out throughout the year on viruses from many countries around the world. The panel of antisera is updated on a regular basis as new viruses emerge and others are displaced from circulation. In our experience 40 to 50 ferrets each year can provide suitable panels for seasonal influenza viruses in circulation. More antisera are needed to confirm the antigenic characteristics of candidate vaccine viruses (viruses with the potential to become vaccine viruses) and vaccine viruses, and antisera are also needed to characterise antigenically animal influenza viruses that infect humans for risk assessment purposes.

Those ferrets infected with animal influenza viruses that infect humans are also used to determine the replication characteristics of these new viruses, and transmission studies might be needed. These studies have been a minor component of our work over the last five years, but should such new viruses emerge we will need to study them in considerable detail for an assessment of the risk that these viruses might have pandemic potential. The numbers used will be dependent on the frequency of the emergence of unpredictable influenza zoonoses.

Ferrets used in assessing the potential of new vaccines and new antiviral medicines will be used in relatively small numbers, but reproducibility of treatments will be required to be established before further study is done.

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

We use small numbers of animals for each virus to be examined. Quite often single ferrets will be infected with single strain of viruses; here, reproducibility can be established by raising antisera against genetically similar viruses. Antisera raised against some viruses, for example vaccine viruses, are used in more tests, and can be shared with other centres for their parallel analyses. Therefore, antisera from more than one ferret are needed for some of these viruses that serve as candidate vaccine viruses and vaccine viruses, and those that serve as reference viruses for longer periods of time.

Studies on newly emerging viruses and on new vaccines or antivirals will be done on only small numbers of animals, enough to ensure reproducibility. The design of the experiments

will be done by applying the NC3R's Experimental Design Assistant, or other similar design assistants.

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

We estimate the numbers from our experience over the last decade. For some studies, for example on new animal influenza viruses that infect humans, several animals will be infected to ensure reproducible and robust results.

A retrospective assessment of reduction will be due by 16 August 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 ferret model of influenza is to be used. The ferret antibody response is the mainstay of the global surveillance of emerging human influenza viruses. The ferret is chosen because human influenza viruses are usually able to replicate in ferrets without prior adaptation, the disease signs of ferrets are usually similar to those observed in humans, and the immune response to infection is similar to a primary influenza infection of children.

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

Mature, usually adult, ferrets are used for this work. It is not possible to do this type of work with less sentient animals for the reasons outlined above: the disease signs of ferrets are usually similar to those observed in humans, and the immune response to infection is similar to a primary influenza infection of children. For example, human influenza viruses often adapt when mice are infected, and so the outcome of infection or the immunological response is caused by, and directed towards, the mouse- adapted variant and not the human virus under study. Similar problems can be seen in other animal models.

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

Infected ferrets will be monitored closely and should a ferret show disease signs it is possible to prescribe an antiviral medicine, e.g. oseltamivir, to reduce disease severity, given with food and water or by direct oral delivery or in food supplements like custard.

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

Methods of infection of ferrets and following infection are very well established and we will follow this best practice. Examples of good practise have been described in Besler et al., Am. J. Pathology 190, p11-24 (2020)

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

The host institution provides regular updates on advances in the 3Rs and news from the NC3Rs is regularly provided.

A retrospective assessment of refinement will be due by 16 August 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?

7. Nutrient sensing in the brain

Project duration

5 years 0 months

Project purpose

- Basic research

Key words

obesity, appetite, diabetes, brain, nutrient sensing

Animal types	Life stages
Mice	adult, embryo, neonate, juvenile, 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 understand how the brain controls our appetite, weight and health. In particular, we want to understand how the brain detects the different types of food that we eat and the different types of energy that we have available in the body, and uses this information to regulate appetite, weight and health.

A retrospective assessment of these aims will be due by 12 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 it's 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 is important because when brain pathways regulating appetite and energy use in the body are not working, this can cause diseases like obesity, diabetes and cardiovascular diseases like heart disease or atherosclerosis. Understanding these pathways will help develop new more efficient and safer therapies to lift the huge burden that these diseases represent (63% adults in the UK are overweight or obese, causing over 30,000 deaths each year and £6 billion of related health care cost).

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

I hope to discover pathways that the brain uses to control appetite and weight that we could target to treat obesity and metabolic diseases.

I hope to produce new databases of genes involved in how the brain detects the nutrients we eat or have available in our body. These databases will be made available to others. I hope to produce a detailed characterisation of the mechanisms through which 3 of these pathways work. In the end, I may demonstrate that targeting these pathways is an efficient strategy to treat obesity and related health complications , leading to new research to develop new anti-obesity drugs. Such findings may be of interest for the pharmaceutical industry and I will submit patent applications when relevant. Data arising from this research may support future funding applications/clinical trials .

This work is important because our understanding of how the brain controls appetite and metabolism is very limited and as a consequence, there are only a few drug options to treat obesity. Obesity is a major threat to public health and costs billions of pounds each year in healthcare treatments. Most of the available pharmacological options have limited efficiency.

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

The annual UK-wide NHS costs attributable to overweight and obesity are proposed to reach £9.7 billion by 2050. Most of this cost relates to dealing with the metabolic diseases associated with obesity, including type 2 diabetes, fatty liver disease and cardiovascular disease. This license will support research that will help generate new insights into the causes of type 2 diabetes and other common metabolic diseases associated with obesity, and to potentially reveal novel therapeutic drug targets.

In the short term, the scientific community will gain new information on the pathways and genes that we will investigate. The proposed work will increase our knowledge of how the brain works which will be useful for other researchers in neuroscience but also researchers who study nutrition and interactions between the body and the brain.

Clinical researchers may benefit from this work, in particular if we show that some of the pathways we identify can help reduce obesity.

Eventually this may benefit patients and society as a whole if we manage to decrease the burden of obesity and metabolic diseases.

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

We will disseminate all our findings, including negative results and unsuccessful

approaches, in local, national and international scientific conferences and seminars, and via publications in specialised open access peer-reviewed journals.

We coordinate with other researchers in the university and outside our institution internationally to share tissue samples and mouse models. This helps maximise the output of each animals or model we are using to prevent wastage.

We will engage with clinical researchers to initiate the translation of our work.

Species and numbers of animals expected to be used

- Mice: 12,000

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.

Our goal is to produce knowledge that can be applied to improve human health. Therefore we need to use an adult mammalian organism. Mammals have unique sophisticated pathways to regulate feeding and metabolism. We cannot use a lower life-form, e.g. fish or insects, as they regulate energy balance, and in particular the expenditure side of the equation, very differently from mammals.

Rodents allow the study of whole-body control of energy balance in a manner relevant to humans, as pathways involved in the control of appetite and body weight are largely similar between rodents and humans. Rodents allow access to several tissues critical to the control of metabolism (brain, pancreas) that are inaccessible in humans. Rodents are amenable to genetic manipulations, offering endless possibilities to characterise mechanisms underlying diseases in a specific and relevant manner.

We need to understand how an adult mammalian organism functions as a whole to obtain and use energy. This happens at the level of the “whole organism” and not simply at a cellular level. Therefore, in the majority of the cases we cannot perform experiments in a dish.

We will focus on juveniles and adult mice because before weaning, the way pups obtain and use energy is very different. Also, fat mass storage occurs typically in the adult and cannot be studied in younger animals. Last, younger animals have different nutritional needs as they are still growing.

Typically, what will be done to an animal used in your project?
Firstly, we will use established standardised breeding protocols to generate genetically altered mice.

Before performing large scale studies, we will use small groups of animals (pilot study) to confirm the details of our experiments (dose, timing), identify potential problems, confirm the experimental design, as well as implement improvements early on in the licence using a reduced number of animals.

Mice will typically be group-housed in their home cages while being supplied with a regular diet or a modified diet enriched or depleted in specific nutrients for the entire lifetime. They may be housed at different ambient temperatures ranging for 4C to 30C, typically for 4 weeks, and may be single housed typically for up to 8 weeks but sometimes for their entire lifetime. Mice may also be housed on modified flooring with a continuous pattern of holes. Mice may be food restricted overnight, typically on 4 occasions, or in some cases may be calorie-restricted typically for 4 weeks.

Mice on moderate severity protocols will typically undergo 1 surgery to inject substances into the brain, the blood or the intestine, and/or position permanent cannula for repeated injections in awake animals.

- In brain surgery (up to 2 times in the lifetime of the animal), mice will undergo general anaesthesia for up to 30min. A small hole in the skull will be performed an a cannula will be inserted temporarily for the purpose of an acute injection, or secured permanently.

- Vascular surgery (once per animal) will typically last 60 min and involves general anaesthesia, the opening of the upper body to isolate the jugular vein and carotid artery and the insertion of a permanent catheter into these blood vessels.

- Intestine surgery (once per animal, 30min per procedure) involves general anaesthesia, the exposure of the intestine after opening of the body cavity, and the insertion of a permanent cannula.

- Surgery for the insertion of a minipump or a telemetric device involves general anaesthesia and typically takes up to 5 min per procedure. Minipumps are used to deliver a substance in a continuous manner typically for 2 weeks in awake animals. Telemetric devices are used to measure body temperature and activity in a continuous manner in awake animals. Both these surgeries involve the opening of the body cavity or a small incision of the back, the insertion of the device, and closure of the cavity with sutures.

The majority of the mice will receive substances administered either into a vein, into the brain, into the intestine, into the body cavity, under the skin and/or via a tube inserted via the mouth onto the stomach dietary. The total number of substance administration through all these different routes will typically be 20 and in rare cases up to 60 over a minimum of 50 days.

In the majority of the mice, we will collect blood from either a superficial vein or via a pre-inserted catheter.

Some mice may be tested in bespoke experimental chambers that allow advanced measurements or experimental paradigms. These include:

- behavioural chambers: typically, animals will be acclimatised to these chambers and trained to perform a task of drinking or eating a predefined amount of diet or solution. This may be in a conditioning paradigm where the animals need to perform a task before accessing the diet or solution. Animals will be tested typically once a day (up to 2 times a day) and each session will last typically for 1h, typically for 8 weeks. Mice might be food restricted (80% of normal intake) in these experiments.

- calorimetry chambers: mice will be housed in a home-cage based system measuring gas consumption to determine how the body uses energy. Mice will be housed in these chambers typically for 3 days.

Typically, mice may need to have 2 scans in their lifetime to measure nutrient absorption in specific tissues or determine the amount of fat or muscle in their bodies.

At the end of the experiment, animals will be humanely killed, tissues will be collected under deep, terminal anaesthetic unconsciousness by first removing the blood by pumping a salt-containing liquid through the blood vessels, called perfusion, followed by tissue preservation in a fixative solution for follow-up analyses. We with coordinate with other groups to share animal tissues including tissues from genetically modified mouse lines and post-mortem tissues in order to further reduce overall mouse numbers.

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

Our studies will involve the following:

Blood sampling with its associated handling stress and transient discomfort.

Injections with their associated handling stress and transient discomfort.

Surgeries that will produce moderate transient discomfort. All surgical procedure will be completed under general anaesthesia so that the animal will remain in a state of sleep/unconsciousness throughout.

Some mice may develop diabetes and other metabolic complication which will result in increased drinking and urine production. If this is noted, we will monitor blood sugar levels and if they go too high the animal will be killed.

They will experience stress related to experimental procedures in bespoke environmental chambers or equipment and social isolation, producing mild transient or long-term harm.

Mice will also experience the cumulative effect of successive procedures producing mild and transient discomfort. All studies involving multiple steps will be performed in a longitudinal manner. Mice will be allowed most of the time 1 week (minimum 3 days) recovery between distinct steps and their welfare will be constantly monitored.

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)?

Mice :

- Mild 48%
- Moderate 50%
- Severe 2%

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

- Killed
- Used in other projects

A retrospective assessment of these predicted harms will be due by 12 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 control of energy balance and metabolism occurs at the level of the “whole organism” and not simply at a cellular level. Therefore, the scientific understanding of these processes requires the study of whole organisms and cannot be studied in a dish.

We want to understand how the human body uses and obtains energy and the role of the brain in these processes. Therefore, we need to use a specie where the interactions between the brain and the body are similar. Also the interactions between the brain and the body are integrated multi-organ processes that require the study of live animals and whole organisms.

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

As much as possible we will study our research questions in a dish. In particular, we have recently developed the use neuronal cell cultures that we can study in a dish. These are useful to test sensing mechanisms with a number of different assays. They enable us to test the role of candidate pathways in a dish before investigating their role in whole organisms. In addition, we have developed the use of brain tissues in a dish. In this model, we can test nutrients or hormones and measure a number of metrics relevant to brain function.

We use genetic and health data obtained from large group of human participants to guide our research in rodents.

We obtain tissues from collaborators as often as possible and share tissues as well.

Why were they not suitable?

We cannot use a lower life-form, e.g. fish or insects, because in these species, the way the body uses energy is different from mammals. Our goal is to produce data that are relevant to humans, therefore we need to use species where energy use is similar to that in humans.

A retrospective assessment of replacement will be due by 12 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?

I used data from the past 4 years of my research to estimate numbers given the expected level of funding I will have in the next 5 years.

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

In this project, we have the option to use diverse "phenotyping steps" in phenotyping protocols. Phenotyping steps are procedures where we measure how the body obtains and uses energy. They allow us to characterise the role of a pathway and the mechanisms through which this pathway eventually regulates body weight and health.The specific phenotyping steps we will use in a given study will depend on our specific experimental goals, varying from study to study. For example, we may find that one pathway modifies body weight by increasing the addiction to sugar and decreasing how the body burns fat. We may find that a pathway increases diabetes by increasing the preference to sugar and decreasing how the body uses sugar. Therefore, it is difficult to predict which steps may be used together. For that reason, we chose to propose modular phenotyping protocols, allowing us to optimise the quantity and type of information we will obtain from each study. In our experience, such modular approach will promote reduction.

In addition, we included a number of generic phenotyping steps aimed at measuring covariables that can explain variability in our main outcome measures. These steps will increase the quality of the datasets we collect and promote reduction.

To further reduce the number of animals necessary to reach statistical significance and demonstrate an effect, we:

- consult a senior biostatistician to advise us on statistics and experimental designs,

- reduce experimental variability using procedures, designs and technologies that limit variability in the measures,

- acclimatise animals to stressful procedures such as injections (using mocking injections where the animals are handled as during actual injections but do not receive any substance). This allows to familiarise the animal to the procedure, decreasing stress during the actual experiment where data are collected, and therefore decreasing

variability,

- maximise the information that can be recovered from a single animal by testing several conditions and measuring multiple variables in the same mouse. See also above paragraph on modular protocols

- use the guidelines and online tools from NC3Rs and PREPARE to design experiments

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

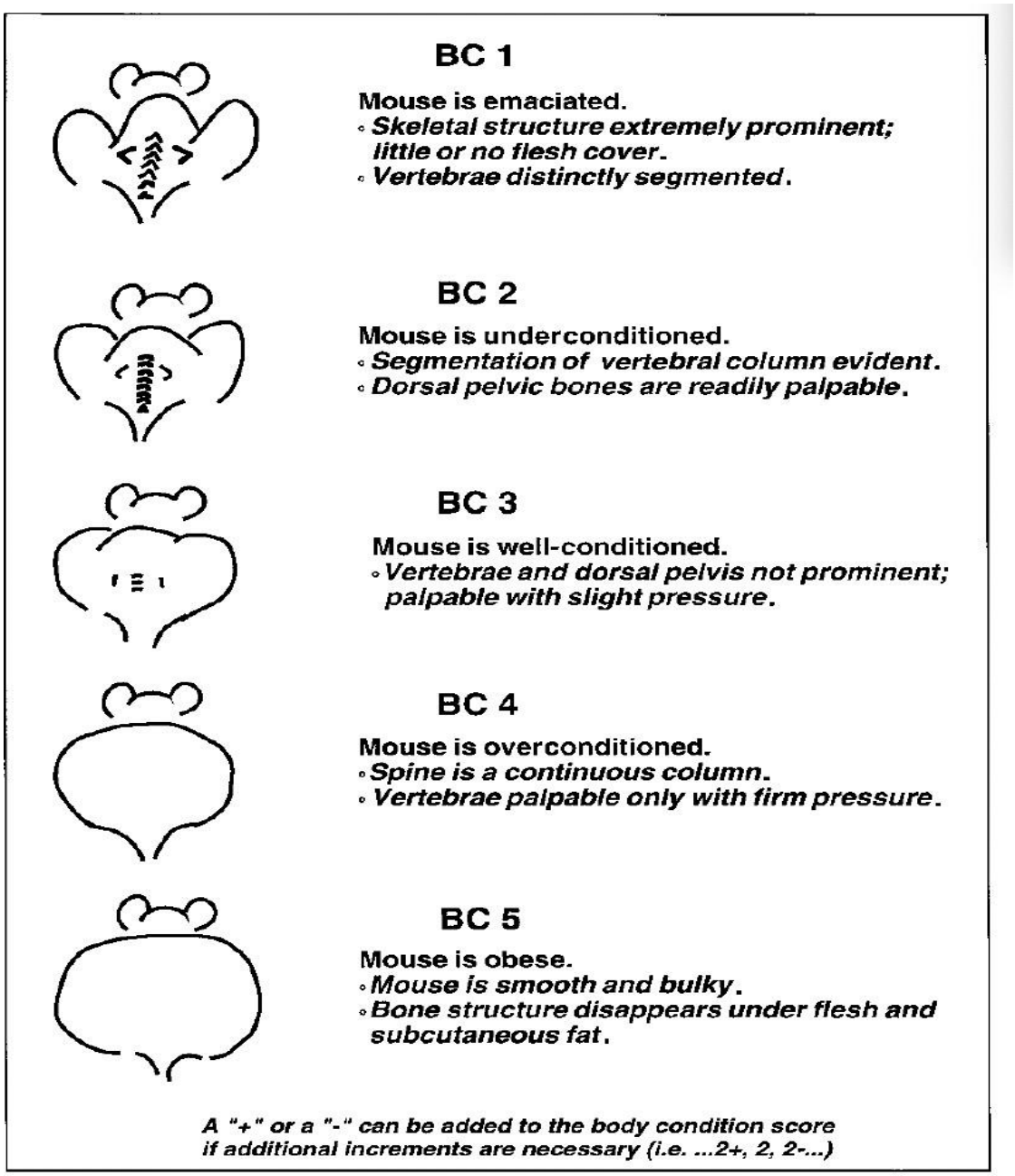
We use randomisation, blinding protocols, and crossover designs when possible. To further reduce variability, we use inbred strains, littermates, body-weight-matched, gender-matched and age-matched groups. We acclimatise animals to experimental procedures to reduce stress-induced variability. For example, for animals who will receive substances through a pre-implanted brain cannula, we habituate the animals to the injection paradigm with "mocking brain injections" during which we connect the mice to the injection system without delivering any substance. We do this on 4 consecutive days before the actual substance administration. In behavioural experiments, will acclimatise animals to modified testing conditions and train them to perform expected tasks in order to increase the quality of the data collected.

We measure production and breeding performance and ensure the minimum numbers of animals are used. Whenever possible, we maintain homozygous lines. We also reuse WT animals for embryo recipients and in the generation of vasectomised males. However, it is best to keep cre alleles hemizygote, which we use routinely. Because we use bi- or tri-allelic strains, a number of animals with unwanted genotypes are being created.

Data collection from behaving undisturbed animals (telemetry, behavioural or calorimetric chambers) significantly reduces stress-induced variability. These technologies often enable continuous data collection, a way to detect small dynamic changes. Using these technologies, each animal can be used as its own control, which negates the need to have a second cohort of control mice running alongside.

Surgical procedures also promote reduction by allowing the administration of substances in freely moving animals (undisturbed), hence reducing stress and variability in collected experimental data, therefore reducing the number of animals required to reach good statistical power. To reduce the number of animal needed in surgical procedures, we implement strategies to maintain the potency of implanted catheters or cannulas, including regular flushing, and we use of surgery bedding.

Systematic use of body weight loss as humane endpoint sometimes leads to the loss of precious experimental animals otherwise clinically fine. Therefore, we use adequate techniques to assess welfare depending on each procedure (body composition scoring, assessment of clinical signs of suffering).



A retrospective assessment of reduction will be due by 12 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.

Rodents have the enormous advantage of being readily susceptible to genetic manipulation enabling precise alteration in the function/expression of specific genes and the creation of animal models of relevant human diseases. We will use genetically altered mice or viral vectors to manipulate the activity of pathways of interest.

We use and continuously develop non-invasive technologies that enable the collection of data without killing the animals (e.g., use of a scan to determine the amount of fat in the body). For example, we have recently developed the use of telemetry to monitor body temperature in behaving animals in their normal housing environment, and we are planning to use telemetric devices to monitor blood glucose continuously.

Cannulas inserted into a blood vessels, into the brain or into the intestine, although invasive, permit the development of refined animal models with discrete manipulation of candidate pathways, which helps for the generation of data more easily interpretable. Cannulas inserted into a blood vessel also enable decreased suffering in protocols with repeated blood sampling and delivery of substance into the blood. We are developing the use of new technology that use transgenes to insert proteins into some cells which then allow to rapidly turn on and off these cell populations. These tools permit reversible and discrete pathway manipulations, therefore minimally affect the phenotype of the animals. We are also currently developing the use of programmable minipumps, which are small devices that we insert under the skin or in the body cavity and deliver drugs continuously. The programmable versions refine chronic drug delivery by enabling pulsatile delivery, better representing how the body works. To limit the adverse events related to the use of tools to determine how neurons in the brain are connected together, we are continuously integrating new tools that are less toxic to the brain.

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

We need to use animal models relevant to human diseases. In addition, the complexity of the mammalian brain is key to model central metabolic-sensing circuits, and this research question cannot be addressed in lower organisms.

Very rarely we can use terminal anaesthesia to measure how the body uses energy, but often anaesthetics inhibit the brain control of metabolism and are therefore not appropriate. In addition, when we study behaviour, terminal anaesthesia are not appropriate either.

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

To improve the quality of life of the animals we:

reduce stress by group housing where possible to keep singly housed mice to a minimum.

use environmental enrichment (EE), within what is available to us at our animal facility. In general, EE is an animal housing technique composed of increased space, physical activity, and social interactions, which in turn increases sensory, mental, motor, and social stimulation. Igloos, running wheels, saucer wheels, fun tunnels, and other objects in the housing environment provide stimulation by promoting exploration and interaction. EE can be maintained when animals are handled (e.g. handling tunnels), thus minimising stress when for example an injection is needed. In mice housed on grid flooring (typically 35% of the surface punctures with 5mm diameter holes), a plain nesting area will be provided to

mitigate harm.

use a series of non-invasive methods for characterising mice so that we can generate useful data without killing the animals, thus minimising the number of mice needed (telemetry etc..) use pain killers to lessen pain.

provide 'behavioural' training to mice undergoing specific procedures (e.g. acclimatisation to single housing in cages used to measure food intake and energy burned).

use scoring sheets to monitor the health of animals undergoing procedures.

monitor harm and potential adverse events with daily observations and provide energy-dense enrichment when appropriate.

provide additional intervention in post-surgical conditions (post-operative bedding, nesting, heating pads and mash)

provide medicated palatable substances for voluntary treatment such as flavoured jelly, paste or milk shake liquid, when necessary.

In experiments with LPS administration we will always test new batches in pilot studies, adjust doses based on strain, age and gender and typically kill the animals at 48 to 72h after a single injection.

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

Laboratory Animal Science Association (LASA) guiding principles documents of aseptic technique (https://www.lasa.co.uk/current_publications/)

ARRIVE (Animal Research: Reporting of In Vivo Experiment) guidelines for preparing papers for publication (https://www.nc3rs.org.uk/arrive-guidelines)

PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) guidelines for planning our experiments (15 topics including formulation of the study, dialogue between scientists and the animal facility, and methods) (https://www.ncbi.nlm.nih.gov/pubmed/28771074).

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

To be informed about latest advances we will primarily use the National Centre for the Replacement and Reduction of Animals in Research (NC3R) website (https://www.nc3rs.org.uk) and 3Rs tools in- house tool and external resources such as Norecopa https://norecopa.no/databases-guidelines; https://resources.jax.org/; Systematic Review Facility (SyRF). It provides an extensive library of 3Rs guidelines, resources, practical information and themed hubs. It also provides links to publications, other online resources, and video and training materials.

Implementation of the advances will be defined on a case-by-case basis and will be informed by the latest NC3R recommendations.

We also share expertise across our institute with regular internal seminars and have an external seminar series so hear from other experts on a regular basis.

We have a team of researchers at our institute who specialise in using mice in obesity and diabetes research and they regularly provide us with updates on new advances.

A retrospective assessment of refinement will be due by 12 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?

8. Immunobiology of pregnancy in the mare in health and disease

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
    - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
    - Assessment, detection, regulation or modification of physiological conditions in man, animals or plants

Key words

reproduction, miscarriage, diagnosis, placenta

Animal types	Life stages
Horses	adult, pregnant
Ponies	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.

- Uses cats, dogs or equidae

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 overarching goal of our research program is to improve fertility and reproductive health in mares through a better understanding of reproductive events such as conception and early pregnancy.

Retrospective assessment

Published: 01 August 2023

Is there a plan for this work to continue under another licence?

Yes

Did the project achieve its aims and if not, why not?

The overarching goal of this project was to improve fertility and reproductive health in mares through a better understanding of reproductive events around conception and early pregnancy. Research was performed under this project for a 6 month period (of the 5 years proposed) as the work was moved to another institution. Therefore not all of the original goals were achieved.

This project achieved its goal to significantly increase knowledge of key genes and pathways involved in placental development in mares. Immune cells were successfully isolated from the endometrium of the pregnant mares and a methodology successfully performed which assessed the genes expressed in individual immune cells. The analysis of this data is ongoing but preliminary data indicates that we have identified some unique immune populations in the uterus that express genes consistent with dampening of the immune response and other populations associated with inflammation. We have also found that these cells change in both number and the genes they express when we compare two stages in early pregnancy, before and after implantation. We have also looked at what genes are expressed in the adjacent placental tissue at the same time we measured these immune cell populations. The next step is to use computer programmes to work out which genes in the placenta play a role in changing the types of immune cells present in the uterus in early pregnancy. These experiments are also some of the first in the horse to use a technique called single cell RNAseq. Therefore the information we have gleamed also has relevance to understanding the types of immune cells present in the horse which may also be relevant to other immunological conditions including allergy, tumours and inflammatory responses.

Analysis of the data is ongoing so publications have not been generated to date but the results to date were presented as a selected oral presentation at an International conference in 2023.

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?

Despite continuous development of our knowledge in veterinary medicine, reproductive measures in mares remain unchanged over decades. Early pregnancy loss, defined as pregnancies that fail in the first two months after conception, is the greatest contributor to low reproductive outcome of mares. Knowledge on this period of pregnancy is still not well understood in horses. As a result, the diagnosis of the underlying cause is only possible in approximately 40% of cases making clinical management of the current and subsequent pregnancies challenging. Further, early pregnancy loss is associated with additional procedures for the mare and emotional and economic loss for the horses' owners, so any advances to reduce its occurrence has direct welfare benefits for the mare and the owner. An abnormal immune response to the fetus as well as underlying genetic variants key to embryonic survival and thus successful pregnancy are thought to contribute to pregnancy loss in other species, but knowledge of these processes and how they relate to pregnancy

loss is limited in mares. Research on these key processes during critical periods of equine pregnancy will therefore ultimately leading to the development of new clinical management strategies, new therapies and diagnostic testing that could be applied to prevent such losses.

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

The cause of over 60% of cases of early pregnancy losses known remains unknown. Critical events during that time of pregnancy include the development of placenta, development of the key organs such as the heart and lungs and modulation of the mother's immune system. Results of this project will significantly increase (i) (i) the knowledge of key genes and pathways involved in placental development in mares including the first description of different types of specialised cells of the placenta;uterus and their role in guiding development of the horse placenta (ii) define the characteristics of equine immune cells in the uterus and their role in guiding development of the horse placenta (iii) identifying genetic variants (changes in the genome of the fetus) that are incompatible with life(changes in the genome of the fetus) that are incompatible with life. In the long term, identification of key proteins in cells in (i) and (ii) will inform future work that looks to understand the role of such pathways in pregnancy pathologies such as early pregnancy loss. The identification of genetic variants would result in the development of genetic tests that can be used to inform mating strategies that reduce the occurrence of pregnancy loss. Hence, this data would ultimately improve both the veterinary and breeding management of the mares. In addition, given the widespread role of immune cells in other physiological and pathological processes in the horse, comprehensive knowledge on the immune cells may inform further research in equine immunology. Our recent work on genetic variants that lead to early pregnancy loss in mares indicates that there may be some overlap in these pathologies between mares and women. Therefore, we also aim to use findings in mares to explore if the key pathways are described in early pregnancy loss in women and if not, design new studies to study their role in other species.

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

The beneficiaries of the results of this project are mares, mare owners, veterinary surgeons and the wider breeding industry. Results will provide new knowledge on the immune response and early development of the placenta and fetus in the equine pregnancy. This knowledge may help to explain pathology of reproductive disorders related to that period, such as early pregnancy loss. In turn, this may target better the veterinary and breeders care over the pregnant mare and hence reduce the incidents of the early pregnancy loss in this species. Moreover, chorionic gonadotropins which are secreted by equine placenta are also produced in human pregnancy. Thus, obtained results may be beneficial also for the human reproduction. In addition, immune cells which are involved in development of the placenta and changes to the immune system that allow the mother to tolerate the developing placenta may be important in other immune related processes hence the knowledge in this field may form groundwork for research in other fields of equine medicine. Similarly, large genetic variants have recently been described as a significant cause of early pregnancy loss in mares and it is expected that smaller variants such as loss or gain of small segments of chromosomes or single nucleotide polymorphisms will similarly contribute to the condition. Identification of these genotypes allows breeders to avoid certain matings that lead to lethal embryonic genotypes. The mare would benefit from avoiding a pregnancy loss, as when a mare loses her pregnancy, she requires an increase in veterinary procedures to investigate its underlying cause and then further procedures to establish pregnancy in a subsequent month. The owners would

benefit as they are passionate about their animals and can emotionally suffer when their animals lose a pregnancy.

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

Results will be presented at international conferences and published in leading journals in the field. This project is run as a cooperation between UK and Europe so there is an opportunity to share findings both locally in the UK as well as widely in Europe and Internationally. The investigators also regularly present at CPD events and clinical meetings (for veterinary surgeons) as well as to horse breeders with this new knowledge to also be shared via these channels as and when it becomes available.

Species and numbers of animals expected to be used

- Ponies: 20
- Horses: 15

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.

The project aims to characterize pregnancy in the mare and hence the mares will be used as experimental animals. Anatomy of the placenta as well as the types of immune cells present at the maternal-fetal interface differs between species, for example mice have haemochorial placenta (only 2- 3 layers between the fetal and mothers blood supplies) and gestation lasts 21 days while mares have epitheliochorial placenta (there are 6 layers between mother and fetuses blood supplies) with pregnancy lasting 340 days. The other species that have an epitheliochorial placenta are cow and sheep, but they also have a different shaped placenta. Hence, mouse or ruminants cannot be used as a model for equine pregnancy under most situations. Moreover, this project will primarily benefit horses thus it is the species used in the project. To determine the immune mechanism of placental development, samples will be taken during important timepoints of the placenta formation that around the time of fetal cells invade in the mothers' tissue and once placental formation is completed. To identify the exact period when genetic variants can lead to lethality or a compromised fetus, conceptuses will be recovered throughout the early period between days 6 (when the blastocyst can be recovered from the uterus and day 65 corresponding to the window when pregnancies are most likely to be clinically lost) to compare normal developmental features to those features of clinical cases of early pregnancy loss.

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

Samples will be collected from clinically healthy mares over the age of 2 years. There are three sets of procedure that will be used. Protocol 1. Pregnancy will be established in mares using standard breeding procedures for artificial insemination. Under protocol 2, once pregnancy is confirmed, the embryo will be flushed out of the uterus, blood samples obtained and a biopsy of the endometrium obtained at various time points after conception (using equipment used to routinely biopsy mares in clinical practice). For a subset of mares, we will also place a probe through the cervix (non-invasive) to measure oxygen

levels. This will be removed as soon as the reading is recorded, normally after 10 minutes. In Protocol 1, some mares will have a mock pregnancy. That is mares will be monitored for ovulation then receive a hormone daily after ovulation that mimics the hormonal state of pregnancy. These control mares will also go onto P2, where they have similar procedures (biopsy of endometrium, blood sampling) except they do not have a uterine flush for recovery of an embryo. For a subset of mares, we will also place a probe through the cervix (non-invasive) to measure oxygen levels. Under protocol 3, we will use non-pregnant mares, usually over the winter, where blood samples will be collected from mares usually whilst remaining in the field to be used to isolate white blood cells for laboratory based experiments. This procedure is typically completed in under 5 minutes and the mares usual grazing is minimally disrupted. If we want the blood samples to be collected from a mare at a specific stage of her cycle, we may also administer standard hormones (used in breeding mares across the UK) prior to blood collection to mimic the hormonal environment such as progesterone of the pregnant state.

Prior to entering the project all mares will be examined by a veterinary surgeon and ultrasound examination of the uterus and ovaries will be performed to confirm their health status. They will be brought into the stocks in advance of commencement of procedures to acclimatise them to the space where the majority of procedures will be performed and a positive experience offered such as the use of food whilst in stocks. To establish pregnancy, mares will restrained in stocks and monitored by transrectal ultrasonography (similar to the management of breeding mares) and when the clinical findings indicate impending ovulation, the mares will be inseminated with commercially available cooled semen using standard veterinary procedures. As controls, some mares will either receive injections of a hormone, oxytocin (licensed and routinely used in clinical practice), or altrenogest orally in order to prolong the life of structure on the ovaries that produces the hormone of pregnancy called progesterone, in order to mimic a pregnancy. Pregnancy will be determined and monitored by transrectal ultrasound of the mare. At 6 to 65 days after establishment of the pregnancy, the mare will be sedated and biopsy samples of the endometrium (using biopsy forceps) and blood samples from the jugular vein will be collected. The conceptus will be recovered following non-invasive uterine lavage. Endometrial biopsies and blood samples will be collected from the control mares in the same days after ovulation also under sedation. After sample collection all mares will be administered a medication in order to stop progesterone production and return the mare to oestrous. In the consecutive cycle mares may be returned to P1, and will be again monitored daily with the ultrasound and at the appropriate day of the cycle, will be inseminated again or receive medications in order to act as a control. Each mare will have no more than three control cycle/embryo recoveries in any one year under P2. Before every collection of the endometrial samples and embryos, mares will be sedated. In the case of post-breeding endometritis, (a relatively common condition in the mare, that can be detected through a clinical examination of the uterus but not by a change in behaviour), will be treated with a medication to clear fluid. After all procedures in the stocks, mares will be returned to the field. In our experience, they are usually back grazing immediately after rectal examinations and after sedation, within 10-60 min of the procedure being completed.

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

The main impact will be some transient discomfort at the time of embryo recovery, although due to sedation and our previous experience this is predicted to be mild. Mares are expected to be returned to their fields and grazing very shortly after procedures and no change in behaviour has been noted over the previous 10 years performing these

procedures. Some mares may experience a transient (1-2 days) inflammation of the endometrium. This level of inflammation causes no detectable discomfort in the mares (in the majority of cases with this condition, they show normal feeding and paddock behaviour) but can impact the fertility of the mare so mares will be monitored by examination of the uterus by ultrasonography after the procedures to ensure if they do experience this transient inflammation, it is treated.

There are possible other adverse effects from some of the hormones we use, but such effects are transient (10 minutes to one hour), mild (sweating, mild abdominal discomfort) and uncommon (between 1:100 to 1:1000 mares) so are likely to not be experienced with the number of mares used in this project. Horses subjected to rectal examination can experience a tear to the rectum. This is incredibly rare though, estimated to be 1:5000 - 1:10 000 horses. Precautions are made to minimise these risks such as use of previously trained staff, training of new staff and good restraint of the mare.

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)?

All Planned procedures within the project are of mild to (low) moderate severity and will be performed in horses and ponies. Over a 5 year period, we expect to use between 25-30 mares.

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

- Rehomed
- Kept alive
- Killed

Retrospective assessment

Published: 01 August 2023

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

Ten mares were used in these studies for 31 mild procedures and 6 moderate procedures. All procedures caused no more than a transient discomfort and mares were either kept in the field for blood sampling and immediately returned to grazing or returned to the field after the effects of sedation passes. Animals immediately returned to normal grazing activity. Mild procedures included taking a very small volume of blood (10-20 ml) from the jugular vein of the mare (8 procedures), monitoring the reproductive cycle of the mare via rectal examination, administrating reproductive hormones and insemination (15 mild procedures) and flushing of the embryo from the uterus and/or taking a single small biopsy of the endometrium under sedation (8 procedures). There were no accumulative effects of the procedures noted, with mares returning to normal behaviour (grazing in the herd) after the effects of sedation passed.

Six procedures were classified as moderate. This was due to three consecutive pregnancy establishments and associated embryo recovery/biopsy within a 8 week period taken from

a single mare as outlined and expected from the proposed experiments (2 moderate) or the mare had a small volume of fluid accumulate in the uterus following the embryo flush that was detected on a follow up ultrasound of the uterus and treated with either an injection which cleared the fluid or a small flush of the uterus, which were all predicted adverse effects on the licence (3 moderate). One mare experienced some uterine bleeding following the biopsy that was detected through a routine ultrasound examination in the days following the biopsy (1 moderate). In the future, we would delay flushing the uterus for 48 hours and instead treat with oxytocin alone if indicated. In all 6 of the moderate procedures, the mares were returned to the field within 30 minutes after the procedure and any follow up treatments and grazed normally throughout and showed no behavioural changes consistent with pain.

No mares experienced rare adverse effects listed in the licence such as rectal tears or abdominal discomfort.

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?

Currently there are no in vitro models to study equine pregnancy, specifically maternal immune response to the developing fetus nor the complex development of the placenta. Immunomodulatory signals by which the conceptus influences maternal cells remain unknown in a horse thus it is not possible to design suitable in vitro models. Moreover, functioning of the maternal immune system is a part of physiological changes of many body systems which influence each other in response to pregnancy and due its complexity can't be replaced by an in vitro model. In order to confirm genotypes associated with lethality, it is important to show that they are absent in pregnancies that are viable and also characterize the associated genes and proteins impacted by the variant in normal equine conceptuses – thus requiring a whole animal.

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

Reproductive tissues obtained from the abattoir and our bank of archived tissue remaining from previous studies can supplement aspects of this work but not the delivery of all objectives. We also looked for cell lines but these are currently not developed for the placental cells or the immune cells in the horse.

Why were they not suitable?

As we have not as yet established the phenotype of the immune cells at the maternal-fetal interface, alternative non-animal models are not possible. Once we establish the phenotype of important cell populations, we could then model the interactions between the immune cells and endometrial cells using in vitro models established using ex vivo tissue from abattoir tracts and new systems such as organoids along with white blood cells isolated from peripheral blood. We did consider the use of abattoir material, and indeed that has been used for optimization of the assays but this was deemed only of use once we had a better understanding of the cell types and is unable to provide pregnancy tissues as pregnant mares are not sent to slaughter. We have used conceptuses from clinical cases of early pregnancy loss that would otherwise be discarded by the veterinary surgeon

to generate our preliminary data for genetic experiments. The next step is to verify the role of the genes we have discovered using normal conceptuses. We can use some tissues archived in previous projects but such tissues do not include all time points needed to verify the impact of the variant under investigation. Further conceptus material in early development is key to understand the expression of proteins key to early pregnancy.

Retrospective assessment

Published: 01 August 2023

What, if any, non-animal alternatives were used or explored after the project started, how effective were they and are there any lessons worth sharing with others?

As this part of the project aimed to study the genes expressed by cells when they are located within the uterine tissue, there were no other suitable models available to obtain this information. This is due to the fact that we were aiming to identify novel cell types important to pregnancy around the time of implantation. Therefore the cells we studied needed to be exposed to the signals of the uterine tissue and that tissue needed to be primed with the complex systemic hormonal environment of pregnancy. We also assessed the genes expressed by the placenta tissue which again could not be gleaned from in vitro models and computer modelling. We can though now use the extensive information obtained from these studies to generate computer models of pathways involved in early pregnancy and validate such pathways using in vitro models of the maternal-fetal interface. Currently there are no cell lines for trophoblast available in the horse, although organoids have recently been reported which are generated from ex vivo tissue that is cultured long term enabling in vitro experiments. These models will be utilized in all the follow up experiments that aim to interrogate the pathways identified in the in vivo studies completed in this current project.

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 will primarily use ponies for the experiments as they can be best acclimatised to the procedures and be easily handled. We may also use horses (primarily Thoroughbreds) for some experiments when the genetic background is of particular importance for the experiment.

We have estimated the number of animals to be used based on (i) our experimental design and the number replicates required to obtain statistically significant results or describe gene and protein expression in the equine conceptus and (ii) our knowledge of reproductive efficiency in mares including conception rates per cycle and the common reproductive conditions that might impact the fertility of mares and thus conception per cycle.

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

We did review the NC3Rs experimental design assistant but it was deemed unsuitable for these particular projects where we are utilizing ex vivo tissues for in vitro experiments. Therefore, we have focused on the design of the in vitro experiments.

Thorough review of the available literature has been performed and based on other studies with the use of scRNA sequencing the minimal number of samples and hence animals which would bring significant results was determined. Because the procedures planned within the project are of mild severity every mare will be used both as a pregnant and control animal. This approach reduces the number of mares.
For example, for our immune response experiments, samples will be collected from 4 biological replicates (4 mares). The reported minimal technical variation of the provider of scRNAseq and published studies where scRNAseq was used on human placenta supports the use of 4 replicates per time point for this experiment. Samples will be taken at 4 time points (two stages of pregnancy and then control samples for each stage) meaning a total of 16 samples. As each mare will act as its own control, we would expect each mare to contribute a control and pregnancy so would need 8 mares. Even with efficient veterinary care a mare may not become pregnant during every single reproductive cycle. In our experience and in line with published data, the chances of conception per cycle is 60% and mares can only be bred over the breeding season (spring to early autumn). Further, also some mares will enter a period of prolonged abnormal cyclicity due to dioestrus ovulations or haemorrhagic anovulatory follicles reducing fertility. Therefore, two additional mares will be monitored as part of the herd so the most fertile 8 mares can be used for the procedure at any one time. For the genetic experiments, our published experiments suggest that n=3-5 (pending on the specific gene/protein being studied) provides sufficient power to detect differences in gene expression across pregnancy, demonstrate proteins are expressed in tissues at the time point of interest. We plan to collect samples at three stages in development and therefore need 15 collections. Similar to the calculations above, we expect 10 mares to be necessary to provide the conceptus material.

The ARRIVE guidelines have also been consulted.

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

All mares used in the experiment will be clinically healthy and the pregnancies will be established according to the current veterinary protocol to maximize the probability of establishing pregnancy during one cycle. It will not reduce the total number of the animals, however, it may reduce the number of procedures performed on every mare in order to collect samples needed. We will utilize archived tissue samples where possible. We will also continue to obtain tissue samples from dead pregnant mares submitted by veterinary surgeons under owner consent. These are mares that die/ are euthanased whilst pregnant following a life terminating condition such as severe colic or fracture to a limb but otherwise have been healthy and to be carrying a clinically normal pregnancy immediately prior to the death. These will provide tissues that will be used in two ways: to obtain stages of pregnancy beyond 70 days that are not generated as part of the project licence and also if the gestational age is less than 70 days, to supplement the tissues generated as part of this licence (ie directly reducing the number of procedures we do).

Retrospective assessment

Published: 01 August 2023

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

The study assessed the characteristics of immune cells in the uterus during pregnancy. Cells were assessed at three different time points. Our analysis completed to date show that these time points were informative to address our question and indeed showed differences in what genes were expressed and the subpopulations of cells present in the uterus. We used the minimum number of replicates to get a robust answer to our research question (n=5 per group x 2 groups, n=4 for one group as the sample quality of these 4 sample was deemed very good and a 5th sample decided unnecessary) and preliminary analysis of the data indicates this was sufficient animals to detect statistical and biologically relevant differences in the cells before and after implantation. The reduction in animal number was also possible due to the high number of viable cells that could be isolated for the experiment which exceeded the number previously reported and expected. Therefore, it is feasible that group sizes of 4 could be used in the future, although it will be very dependent on the specific scientific question being addressed.

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 main methods to be used are blood sampling, establishment of pregnancy using standard veterinary procedures and flushing of the uterus and endometrial biopsies using standard veterinary procedures. The horses will be housed outdoors in paddocks under conditions very similar to companion animals and stimulation provided through the interactions both with other horses (housed in a herd) and people. The mares will be given an opportunity to acclimatize both to the stocks and people prior to initiating any procedures. This will involve hand feeding in the stocks to provide a positive experience. Conceptus recovery will be performed at early stages of pregnancy (first 2 months of an 11 months gestation). At this stage of gestation, the conceptus is easily dislodged and can be recovered via a catheter placed in through the cervix into the uterus. This non-surgical method of trophoblast recovery is the least severe method available to obtain trophoblast cells. All aspects of this procedure are also carried out in clinical practice for either establishment of pregnancy or in embryo transfer programmes and complications associated with the procedures are rare. Endometrial biopsies will be performed in stocks to minimize the risk of rectal tear. Both the embryo flush procedure and biopsy will be performed under sedation to minimise any stress experienced by the mare.

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

This project is important for equine reproductive health hence mares must be used to establish key cells and proteins involved in early development. Placentation differs notably

between species and hence immune mechanisms facilitating this process as well as maternal tolerance. For example mice have haemochorial placenta and pregnancy lasting 21 days whereas placenta in horses is epitheliochorial with pregnancy around 340 days. Thus the mouse can not be used as a model species for horses.

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

The proposed in vivo studies is the most refined available to study pregnancy in the horse, as the alternative would be to obtain trophoblast and endometrial samples surgically from pregnant mares, a procedure that would require general anaesthesia or euthanasia and a more significant impact on the animal. These less invasive procedures have been regularly performed by the investigators originally clinically in practice and over the last 15 years in research animals. Therefore, many of the refinements have already been introduced for the proposed procedures through previous experience (for example, monitoring of endometrial health independent of behavioural changes in the mare, use of reproductive hormones to minimize the number of oestrous cycles required to establish pregnancy). We propose to add the use flunixin immediately after the endometrial biopsies to ensure any potential pain or discomfort we might not detect through behavioural changes is prevented by reducing inflammation. All mares are already regularly monitored daily for their response to procedures and should any complications occur, monitoring increased further as required.

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

We will follow the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines that are intended to improve the reporting of research using animals and maximise information published as well as minimise unnecessary studies. These are followed to ensure we record appropriate information as part of the project, to audit methodologies and ensure, when publishing, we report the methodology and mare characteristics arising correctly.

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

The researchers will continue to attend lectures provided by the NC3Rs representative and also review the literature to ensure high quality reproductive care (and hence the greatest efficiency and least procedures) is achieved to overall reduce procedures. The website of the NC3R’s will be followed throughout the project duration to keep up to date with new developments. The applicant and team regularly attend and present at both national and international clinical equine reproduction conferences allowing them to remain up to date on best practices for breeding mares.

Retrospective assessment

Published: 01 August 2023

With the knowledge you have now, could the choice of animals or models used have been improved at all? How did you minimise harm to animals during the project?

The ponies used for the studies worked very well. They relaxed quickly and also could be rehomed/returned to their owners having benefited from handling over the period of the project. They were in good health. Therefore pony breeds were deemed the most suitable in this circumstance and would be recommended in the future as long as breed is not required for the experimental design. The mares used were very fertile and pregnancy rates were high which resulted in an overall reduction in the number of rectal examinations. As the research question needed to measure the characteristics of the cells in vivo and within a tissue environment, there were no other suitable models that could be used here or that could be recommended in the future for this specific research question, although in vitro models will certainly be used to interrogate the pathways identified here.

Mares were kept in fields throughout the study in a herd mimicking the normal environment in which they are kept as closely as possible. Mares were acclimatised to the stocks prior to performing any procedures reducing possible anxiety associated with procedures. A companion mare was bought into the stable area during procedures for visual and vocal contact, providing reassurance via social buffering. They were also provided with food (including carrots and apples) in the stocks (when not sedated) so they had a positive experience associated with the procedure. One mare was very nervous about moving into the stocks in the acclimatisation phase so was rehomed without any procedures performed. The weight of the mares was monitored and the diet adjusted based on the availability of grass and the weight of the mares. Mares were closely monitored after biopsies to detect any inflammation in the uterus. This worked well as the mares showed no signs of pain or distress nor any change in behaviour indicating that ultrasound evaluation was required.

9. Ensuring quality and safety of biological medicines

Project duration

5 years 0 months

Project purpose

- Translational or applied research with one of the following aims:
  - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
- Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

control testing of biologics, hormones, antibodies, adverse events, development of alternative in vitro assays

Animal types	Life stages
Mice	adult
Rats	juvenile
Alpaca	adult, juvenile
Cynomolgus macaques	adult
Rhesus macaques	adult

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.

- Uses non-human primates

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 ensure that biological medicines are both safe and effective. This will be done by testing the quality and potency of biological medicines before release onto the market, by enabling the development of accurate preclinical tests.

A retrospective assessment of these aims will be due by 26 November 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 is important to ensure that biological medicines are well characterised, safe and effective. It is also crucial to understand if and why a specific drug is inducing side effects in some patients in order to minimise them.

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

Evaluation of glycoprotein hormone products:

Biological materials used in medicine are typically very complex. Control testing such as the potency assays for glycoprotein hormones is indispensable to ensure the quality of endocrine medicines and establish reference reagents to assign correct potency to these products. The outputs will be an independent assurance of the quality of these products which is important for safety and maintaining public confidence.

Generation of antibodies for in vitro assays:

The development and production of specific antibodies is critical to investigate the biological activity, structure-function relationships, and immunotoxicological properties of biologics. This programme of work will benefit the research community, the pharmaceutical companies, clinician and patients by allowing more specific and accurate in vitro assays. The output from such research would be published in peer reviewed journals.

Collection of non-human primate blood: New biologics such as immunotherapeutics are constantly developed to treat diseases such as infections, and cancer. These types of biologics are powerful drugs designed to directly interact with the immune system and can cause severe adverse reaction in man. Non-human primate is the most recognised preclinical model used by pharmaceutical industries to evaluate immune safety. We are developing new in vitro assays using human cells to better predict potential adverse reactions. To validate these assays we need to compare the responses obtained with human cells and macaques cells. The use of non-human primate blood samples will help to develop better *in vitro* assays to improve the predictive value of pre-clinical safety testing and to diminish the future use of non-human primates in *in vivo* studies. The output from such research would be published in peer reviewed journals. This research will improve confidence in immune safety testing, facilitate innovation and improve patient safety.

Who or what will benefit from these outputs, and how?
Science will benefit from this project through a greater understanding of the mechanisms of adverse drug responses to new biological medicines. People will benefit from this project through the use of proven safe and effective biological medicines. Animals will

benefit from this project through the development and validation of safe alternative assays that do not require animal testing.

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

We work closely with health organisations to develop and establish reference reagents to assess potency of glycoprotein hormones. Typically, the output from this work will be published in peer reviewed journals to inform the scientific community of any finding.

Species and numbers of animals expected to be used

- Rhesus macaques: 4
- Cynomolgus macaques: 4
- Mice: 1510
- Rats: 3300
- Camelids: No answer provided

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.

Evaluation of glycoprotein hormone products: Juvenile rats and adult mice are used. Rodents are used for testing potency of batches of glycoprotein hormone drugs, e.g. Erythropoietin (EPO), Follicle Stimulating Hormone (FSH), Luteinising Hormone (LH) and Human Chorionic Gonadotrophin (HCG). Juvenile immature rats are needed to assess the effect of FSH, LH and HCG on the development of their reproductive organs. The testing procedures used are defined by internationally agreed guidelines and monographs, established by regulatory agencies. The in vivo assay procedures are, therefore, not original and do not form part of new investigations but follow defined protocols which form part of standard operating procedures that are regularly audited as elements of the Institute's quality system.

Generation of antibodies for in vitro assays: Adult immunocompetent animals are used. Mouse monoclonal antibodies will be generated using hybridoma technology. Mouse spleen containing antibody producing cells are fused with immortal B cells to produce hybridoma cells. Each single cell clone of hybridoma produces a specific monoclonal antibody against the antigen of interest and can be preserved to have an unlimited production source of the same quality and specificity monoclonal antibody. Alpacas are used to generate highly specific and unique monoclonal antibodies to challenging targets. Isolation of B-cells from alpacas allow the cloning of single domain antibodies (nanobodies) which present better characteristic than conventional full-size antibodies (eg, heat and pH stability, better penetration due to their small size, easy engineering for different applications).

Collection of non-human primate blood: Adult immunocompetent animals are used. Cell-based tests are used to evaluate biological medicines prior to their pre-clinical safety testing in macaques. We perform these tests to determine whether or not the response of macaque white blood cells is sufficiently similar to that of human white blood cells to make

in vivo testing in this species likely to be of value in predicting toxicity in man. A typical assay requires blood samples (around 10-20 ml) from four macaques.

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

Evaluation of glycoprotein hormone products:
Erythropoietin (EPO) testing: Administration of substances by the subcutaneous route on one occasion and Withdrawal of blood on a single occasion. Duration of 5 days.

Human Chorionic Gonadotrophin (HCG) and Luteinising Hormone (LH) testing: Administration of substances by the subcutaneous route on up to three consecutive days. Animals are killed and seminal vesicles are dissected out and weighted. Duration of 4 days.

Follicle Stimulating Hormone (FSH) testing: Administration of substances by the subcutaneous route on three occasions approximately 24 hours apart. Animals are killed and ovaries are dissected out and weighted. Duration of 4 days.

Generation of monoclonal antibodies for in vitro assays:

Alpacas: Administration of immunogen and adjuvant by the intramuscular route into a maximum of 4 sites, on up to seven occasions (primary immunisation plus up to six boosts). Withdrawal of blood from superficial vessels at any time during the study to monitor the progress of the immune response. Alpacas are rehomed or re-used after the study. Duration 3-4 months.

Mice: Administration of immunogen and adjuvant by the subcutaneous route into a maximum of 2 sites, on up to seven occasions (primary immunisation plus up to six boosts). Withdrawal of blood from superficial vessels on up to 5 occasions including, optionally, a pre-immunisation bleed. Mice are killed and spleen are dissected out to isolate antibody producing white blood cells. Duration 3-4 months.

Collection of non-human primate blood: General anaesthesia. Withdrawal of blood from a superficial vessel. Duration 1h. A single bleed will be performed on each animal at the frequency of 4 times/year. Animals will be reused a maximum of 20 times under this licence.

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

Evaluation of glycoprotein hormone products: Unless otherwise specified, the administration of substances and withdrawal of body fluids will be undertaken using a combination of volumes, routes, and frequencies that of themselves will result in no more than transient discomfort and no lasting harm.

Generation of antibodies for in vitro assays:

Alpacas: The immunogens are proteins and are not expected to produce systemic adverse effects. Typically, most animals used in this protocol are not expected to experience more than moderate discomfort. Unless otherwise specified, the administration of substances and withdrawal of body fluids will be undertaken using a combination of volumes, routes and frequencies that of themselves will result in no more than transient discomfort and no lasting harm. Rarely (less than 5% of animals), temporary lameness, granuloma formation

at an injection site, the formation of a sterile abscess at an injection site maybe observed. Although the severity limit for most animals (>95%) under this protocol will be mild, we have stated moderate to account for the possibility of an adverse response.

Mice: Typically, the majority of animals used in this protocol are not expected to experience more than transient discomfort. The immunogens are proteins and are not expected to produce systemic adverse effects. There may be some local irritation at the sites of injection. Although the severity limit for the majority of animals (>95%) under this protocol will be mild, we have stated moderate to account for the possibility of an adverse response.

Collection of non-human primate blood: Animals used in this protocol are not expected to experience more than transient discomfort. On rare occasions following withdrawal of the needle at blood sampling a haematoma may develop. This will be prevented by applying firm digital pressure at the site as the needle is withdrawn.

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)?

Evaluation of glycoprotein hormone products: mice and rats, ~100% mild, no adverse effects observed previously.

Generation of monoclonal antibodies for in vitro assays: mice, alpacas, 95% mild, 5% moderate

Collection of non-human primate blood: ~100% mild, no adverse effects observed previously.

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

- Killed
- Rehomed
- Used in other projects
- Kept alive

A retrospective assessment of these predicted harms will be due by 26 November 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?

Objective 1: Animals (mice and rats) are used for control testing of glycoprotein hormone products where it is prescribed in the European Pharmacopoeia or in product licences as a legal requirement. There is no in vitro alternative to test the potency of glycoprotein hormone products that have been recognised by regulatory agencies. These potency testing are crucial to confirm if a product is efficient and safe to be used by patients or to provide accurate controls during blood tests.

Objective 2: Animals are used to generate monoclonal (mice) and small size single domain monoclonal antibodies (alpaca). These protocols are recognised by the scientific community as efficient way to produce monoclonal antibodies against specific targets. Animals are still needed to generate monoclonal antibodies. With characterisation of different unique monoclonal antibody specificities, we will also be able to generate pools of 'oligo clonal mixtures' recombinant antibodies that can as efficiently mimic the diversity of antibodies in a polyclonal antibody reagent.

Objective 3: Non-Human Primates are used to isolate white blood cells and assess their responses to biologics. This non-invasive method (~10-20 ml blood withdrawal) will help to confirm if the response of macaque white blood cells is sufficiently similar to that of human white blood cells to make in vivo testing in this species likely to be of value in predicting toxicity in man. Non-Human Primates will not be kept in the facility only for this project. Blood donation will be coordinated between different licences using Non-Human Primates. Alternatively, Non-Human Primate white blood cells may be ordered from commercial sources if possible.

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

Objective 1: There are no in vitro alternative to test the potency of glycoprotein hormone products that have been recognised by regulatory agencies.

Objective 2: We are exploring in parallel the use of recombinant technologies such as phage display from naïve (non-immune) library generated from alpacas to isolate specific monoclonal antibodies. Once validated it will replace the immune library approaches to generate monoclonal antibodies from alpacas.

Objective 3: In vitro studies are carried out in parallel to evaluate their suitability as potential replacement of some in vivo procedures. As example, the in vitro monocyte activation test (for pro- inflammatory and pyrogenic contaminants, MAT) has been developed during previous projects and is now used as an alternative to the rabbit pyrogen test.

Why were they not suitable?
The testing procedures used are defined by internationally agreed guidelines established by regulatory agencies and pharmacopoeias. Some cell-based assays are not recognised protocols to assess potency of some biological products. Further work needs to be done for new *in vitro* assays to be recognised and to replace some *in vivo* procedures.

A retrospective assessment of replacement will be due by 26 November 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?

Group are specified in official guidance for glycoprotein hormones (60 mice or rats per test x 2 for duplicate testing, 120 animal per potency assay). Based on the number of potency testing needed during the 5-year period, 1500 mice (25 tests) and 3300 rats will be needed (55 tests).

In all other cases minimum animal numbers will be determined in consultation with the Biostatistics team and AWERB.

In general, only one alpaca and no more than 6 mice are used for monoclonal antibody production against a specific antigen.

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

With regard to the evaluation of biological medicines (protocols 1-3), the number of animals required per dose (group) and numbers of groups are specified in regulatory documents, relevant Pharmacopoeia monographs (as referred to in each protocol) and in-house SOPs or are considered the minimum required to give reliable results of satisfactory precision. Typically each test is performed using 3 doses of glycoprotein hormone product and 6 animals are used per dose. Two tests are performed, and each test is performed with 60 animals.

Steps are taken to minimise the effects of subjective bias when allocating animals to treatment (e.g. randomisation procedure) and when assessing results (operator is blind) following the ARRIVE guidelines.

Regarding the production of monoclonal antibodies, initially only one alpaca will be immunised with each immunogen or a pool of antigens (Protocol 4) and not more than 6 mice will be immunised with each immunogen, based upon historical data (Protocol 5).

In experiments requiring blood from macaques (Protocol 6), the minimum number of “donors” per experiment will be used. We have reduced the total number of "donors" by allowing re-use under other project licences at the institute.

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

We are developing phage display technology within selected projects as a means to refine and reduce the number of animals required for antibody generation. In addition we are using multiplexed immunisation of alpacas with up to 4 different antigens to reduce the number of animals used. This approach allows the cloning of recombinant antibody genes

from immunized animals (e.g. Alpaca) which can then be used to produce antibodies in unlimited amounts using heterologous gene expression methods using bacterial, yeast or mammalian cells. This unlimited supply of recombinant monoclonal antibodies means there is no requirement for repeat animal usage. In addition the alpacas are re-used or re-homed after study has been completed. Non-Human Primates are also re-used.

A retrospective assessment of reduction will be due by 26 November 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.

Testing of glycoprotein hormones (protocol 1-3) requires the use of rodents (mice and rats). The assays described are those prescribed in the European Pharmacopoeia and in product licences and so scientists are not using protocols, procedures and models that they have chosen but, rather, are using the protocols, procedures and models that they are legally required to follow if the institute is to discharge its responsibilities as Control laboratory.

The generation of monoclonal antibodies (protocols 4-5) requires the use of mice or alpaca. Adjuvants that cause low irritation such as Quil-A &Titermax gold are used. Titermax gold is for example routinely used as an adjuvant with alpacas and Quil-A with mice. Titers of antibodies in serum are quantified after each boost to determine if a further boost is required. In rare cases, if the antigen doesn't induce a strong immune response (titer low despite boosts), Freund’s adjuvant could be used to increase immune response.

Macaque white blood cells (protocol 6) are needed to assess their response to biological medicines and confirm if the in vivo testing in this species is likely to be of value in predicting toxicity in man.

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

The species used in the different protocols are the relevant species to obtain valuable scientific results.

Testing of glycoprotein hormones (protocol 1-3) requires the use of rodents (mice and rats) as described in the European Pharmacopoeia and in product licences and the use of these animals is legally required for control testing of these products.

For production of antibodies (protocols 4-5), the respective species have been established as reliable sources of monoclonal (mice and alpacas). Comparison of the response to biologics between macaque and human white blood cells requires the use of Non-Human Primates (protocol 6).

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

Animals are housed in a dedicated facility and well cared for by professional animal care staff. Additional measures such as provision of species-appropriate enrichments are used. Animals are housed in groups when appropriate and scientifically valid. Score sheets and monitoring regimes are routinely applied. Increased monitoring of the injection sites to detect potential inflammation will be done when adjuvants are used.

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

We comply with the best practice guidelines such as the European Federation of Pharmaceutical Industries Associations (EFPIA) and the European Centre for the Validation of Alternative Methods (ECVAM) for administration of substances and blood sampling (Diehl et al., Appl. Toxicol.21, 15-23, 2001).

The testing methods of glycoprotein hormones are described in European Pharmacopoeial monograph for Urofollitropin (0958) or the British Pharmacopoeial monograph for Menotrophin (9002-68-0); European Pharmacopoeial monograph for Gonadotrophin, Chorionic, 0498 and European Pharmacopoeia (8th edition) in the monograph for Erythropoietin concentrated solution (1316).

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

Scientists involved in this project are keeping up to date on new developments through participation to scientific meetings and literature search. Our Named Information Officer is advertising any relevant 3Rs communications to the scientists. Scientists are also receiving newsletters from the NC3Rs by email. We will continue our effort to develop and validate alternatives to in vivo testing and to propose them to Pharmacopoeias and product licensing bodies.

A retrospective assessment of refinement will be due by 26 November 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?

10. Cancer progression and Metastasis

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
    - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
    - Assessment, detection, regulation or modification of physiological conditions in man, animals or plants
    - Improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes

Key words

Cancer, Metastasis, Microenvironment, Therapeutic response, Therapeutic resistance

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

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?

This project aims to expand our understanding on the different steps in the growth of tumours and spread to secondary sites in the body, and on the response and resistance of tumour cells to anti- cancer agents. In particular this project will interrogate the role of the surrounding non-tumour components (e.g. immune cells, blood vessels and other cells, like fibroblasts) in these processes. Our goal is to identify novel strategies for preventing the spread of tumour cells to, and the growth in, secondary sites in the body.
A retrospective assessment of these aims will be due by 26 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 it's 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?

In the UK, >11,000 women and men die of breast cancer each year. The majority of these deaths result from the spread of the cancer to secondary sites in the body, the process known as metastasis. The spread of cancers involves a close interaction between the cancer cells and their microenvironment - the normal cells and matrix components of the body. The aim of this project is to identify novel strategies for preventing or limiting the development of metastatic disease. To achieve this we have two main goals (a) to identify how cancer cells adapt to a new environment when they spread to secondary sites and who they develop resistance to therapy, and (b) to identify novel therapeutic strategies for targeting the tumour/metastatic microenvironment to limit advanced disease.

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

The outputs from this work will mainly be scientific papers in peer-reviewed journals, presentations at national and international conferences, meetings with collaborators. Additionally, we may derive tumour cell lines to select for populations with enhanced metastatic potential that will be made available to research community.

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

Within the timeframe of the new licence (5 years), the outputs of the work will benefit other scientist working in this research field. The advances in our knowledge will aid in the development of novel therapeutic strategies for treatment of advanced cancer and planning of the "next stage projects". In particular, this project will help to determine the efficacy of targeting the interactions between tumour cells and the non-tumour-cells in their environment.

Our establishment has an unrivalled track record of discovering novel cancer treatments and biomarkers. Consequently, it is highly likely that in this project, promising novel therapeutic strategies would be identified which could be moved towards drug discovery and translational developments within 5 to 10 years.

The outputs of this work will also enable us, and others in the field, to develop better in vitro models and, leading to a reduction in the number of animals used in subsequent research.

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

Findings will be made available to other scientists through peer-reviewed publications, presentations at national and international conferences, at meetings with established and potential collaborators, and via our website and social media outputs.

Species and numbers of animals expected to be used

- Mice: 17000 mice including both genetically altered and wild type animals over a 5-year period.

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.

In this project we will use mice of all ages for breeding proposes and adult and aged mice for experimental protocols. Human and mouse share similarities in their genetic make-up. This is important as there are a number of questions that can only be addressed in a complex organism. In particular, the later stages of tumorigeneses (development of new blood vessels, travel through blood system and invasion into surrounding tissues) all involve complex interactions between the tumour and non-tumour cells that cannot be recapitulated in vitro.

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

Mice will be housed in cages with sterile bedding, food, and water. Trained competent personal with experience of using animals in research will perform all procedures. The welfare of mice entering a study is closely monitored throughout each procedure.

For murine cancers most studies will last between 10 and 120 days, however, for human cancer studies this may be longer due to the increased time before tumours start growing.

Prior to tumour cell injection, mice may be prepared by (a) insertion of hormone pellets to allow growth of tumour cells that are hormone dependent; (b) female mice may be surgically ovariectomised to mimic menopause; (c) in rare cases, mice will be subject to a bone marrow transplant to assess the contribution of bone marrow cells to the tumorigenic process; (d) as a major strand of our study is to understand how clinically relevant anti-cancer therapies affect tumour growth at secondary sites in the body, we will on occasion treat naive (i.e. non-tumour bearing mice) with a course clinically relevant chemotherapy or agents that promote fibrosis to study the effects on normal tissues and assess the ability of agents to reverse adverse tissue responses.

Mice will then receive tumour cell injections. The route of injection will depend on the organ or tissue we wish to study. For example, subcutaneous or intra-mammary fat pad for establishment of primary tumours, whereas to mimic growth in secondary organs mice will receive intravenous injection to give rise to tumours in the lung, or intraperitoneal injections for growth in the abdomen.

Finally, administration of therapies may be delayed until after the excision of the primary tumour to mimic clinically relevant therapy protocols for the control of tumour growth at secondary sites. Mice will receive the optimal drug dosing that have been assessed using (a) optimal doses and schedules derived from the literature, (b) previous studies carried out at our establishment or (c) using dose tolerability studies in tumour-bearing and non-tumour bearing mice.

In our breeding protocol, genetically altered mice will be generated by conventional breeding methods. This may include crossing different lines of transgenic mice and/or non-transgenic mice to generate mice with the appropriate genotype on the appropriate background.

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

The mice used in this project will exhibit clinical signs of cancer similar to those seen in humans such as weight loss, lethargy and pain. Where we are testing the effects of anti-cancer drugs, we need the mice to have established tumours and will therefore inevitably show some or all of these clinical signs. At all times humane endpoints will be established to ensure that the mice do not suffer any more than is absolutely necessary.

During surgical procedures aseptic techniques will be used to avoid and minimise the likelihood of wound infection, general anaesthesia coupled with peri- and post-operative analgesia will be administered to limit the transient pain/discomfort from surgical procedures.

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)?

78% of the total mice used in this project are expected to have effects of a moderate severity as they will have (a) tumour cells implanted using well refined techniques, (b) minor surgery e.g. implantation of hormone pellets under skin or removal of the primary tumour, (c) anti-cancer therapies according to known doses and frequencies.

However, a very small proportion (4%) may experience a severe severity. This is because with new test agents we need to make sure they are safe by conducting maximum tolerated dose (MTD) studies, and at times there are unforeseen effects from these new test agents.

18% of the mice in this project will have a subthreshold or mild severity as they will only experience mild techniques such as ear notching to confirm their genetic status.

Extensive animal health status monitoring procedures have been clearly defined in each protocol to end procedures in due course.

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

- Killed

A retrospective assessment of these predicted harms will be due by 26 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 spread of cancer cells from the primary tumour to distant sites such as the lungs, brain and bones is a multistep process involving (a) cancer cell invasion and remodelling of the surrounding tissue, (b) recruitment of new blood vessels, (c) escape into the circulation and transport to distant sites, and (d) the ability to productively colonise these secondary sites. Substantial amounts of information have been obtained by studying and manipulating cancer cells in the laboratory and such studies continue to be a major part of our activities. However, cancer cells cultured in the laboratory are very different from when they are in the body and there are no good culture systems to model the complex architecture of the tumour and the interaction of the cancer cells with the normal tissues of the body. Most importantly, culture systems cannot be used to adequately study the response of the tumour to drugs. For this reason we need to study these complex cellular events in a mammalian system (mouse) system.

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

In our laboratory we strive to use 2D and 3D in vitro models as replacements for animals wherever possible. In addition, our laboratory makes extensive use of in silico (e.g. interrogation of human cancer databases), in vitro, and ex vivo (e.g. assays to monitor outgrowth of dormant metastatic cells in the lungs) techniques. These non-animal alternatives are an integral and essential part of our research programme.

Why were they not suitable?

Enormous progress has been made in cancer research by exploiting tumour cell lines that can be manipulated in vitro. However, there are a number of questions in that can only be addressed using animal models. In particular, the later stages of tumorigeneses (angiogenesis, invasion and metastasis) all involve complex interactions between the tumour and stromal cells and/or the extracellular matrix that cannot be recapitulated in vitro. In addition, it has become abundantly clear that monitoring therapeutic response and resistance requires pre-clinical in vivo models. For these reasons, studies on in vivo tumour models need to be performed, in which the benefits are weighted against the likely adverse effects, and humane endpoints utilised.

A retrospective assessment of replacement will be due by 26 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?

The numbers of mice have been estimated based on our experience from previous research combined with the use of statistical tools to calculate optimal and minimum number of animals for each experiment.

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

To ensure that we are using optimum groups sizes, and hence minimum number of mice the following criteria have been used:

our current knowledge of breast cancer models

ethical consideration

involving statistical experts previous experience of using the Protocols listed in this project

we have used sperm banking for the strains that we don't anticipate using in this licence.

where the pattern of local growth and biology of tumour models is unknown a pilot study on a small group of animals will be performed to ensure the minimal number of mice will be used in subsequent studies.

where the toxicity of test agent is not known a dose tolerance study will be performed.

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

We take several measures to ensure that the minimum number of mice is used during the design of an experiment. These include:

using non-invasive imaging so that a single cohort of animals can be followed throughout the course of an experiment,

using optimum procedures to reduce the number of mice and to reduce experimental variability. For example, where possible using ultrasound guided tumour cell inoculation results in a high degree of success in generating tumour models,
ensuring that each experiment is appropriately and maximally analysed, thus reducing the need for repeat experiments,

only breeding mice that are essential for our research and maintaining lines in collaboration with other laboratories whenever possible, or sperm banking

performing a small pilot study to ensure feasibility before the full study is undertaken.

A retrospective assessment of reduction will be due by 26 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.

Wild-type and genetically altered mice will be used in this project. Propagation of primary and metastatic tumours is a routine and refined procedure in cancer research resulting in the generation of reproducible tumours in practical cohort sizes. The availability of well-studied genetically altered and wild-type mouse strains that allow the engraftment and development of tumours and growth at secondary sites of the body most closely mimic human tumour growth, tumour-host interactions, tumour angiogenesis and response to treatment. Animal suffering will be minimised by making every effort to keep the tumour models at the subclinical levels. Where possible we will use ultrasound guided tumour inoculation as a refinement as this allows us to successfully inoculate tumour cells via specific routes. Additionally, we will be using non-invasive imaging modalities to monitor tumour growth and the development of metastatic disease. . Throughout, steps will be taken to minimise the severity and animal suffering by (a) having experienced staff undertake the procedures, (b) using extensive and clearly well-defined animal health status monitoring, and (c) performing pilot studies where appropriate to ensure feasibility of experiments.

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

In this project we will use mice. Human and mice share similarities in their genetic makeup. This makes them a good model for breast cancer as human and mouse share similarities in breast cancer development as well, e.g. tumour biology, interaction between tumour and host cells, and response to therapeutics. The disadvantage of less sentient animals or animals at a more immature life stage is that they are very different from humans and can lack common genes. Additionally, the physiological structures of most organs are very different to humans.

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

Mice will be housed in cages with sterile bedding, food, and water. Trained competent personal with experience of using mice in cancer research and who are familiar with the effects of anti-cancer drugs on rodents will perform all procedures. Studies will be designed to use the minimum number of mice. The welfare of mice entering a study is closely monitored throughout each procedure. We will use timely remedies to prevent or reduce the extend of unnecessary pain, suffering, distress, or lasting harm. Anaesthesia and analgesia will be used to minimise stress and suffering during surgical procedures. The procedures chosen are always considered to be the least severe ones that would produce satisfactory results. In addition to monitoring tumour growth and metastasis, we routinely perform detailed analysis on post-mortem tumour and non-tumour tissue e.g. to

monitor stromal cell activation/infiltration, immune cell distribution, tissue fibrosis and cellular senescence. In some cases, we derive tumour cell lines to select for populations with enhanced metastatic potential. At the end of an experiment, mice will be humanely killed. This detailed and comprehensive analysis will provide us with the most effective way to conduct these studies in terms of animal use and research cost.

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

We follow NC3Rs resources for practical guidelines and ARRIVE guidelines to report our animal research to ensure that enough detail is reported.

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

We maintain the highest levels of care and welfare. All our activities are covered by standard operating procedures. Our BSU produces quarterly newsletter that will keep us informed of any new information relevant to animal research, including 3Rs. Our laboratory manager attends our establishment's BSU user meetings as a representative of our team. The minutes of BSU user meetings which includes minutes from the Named Persons meetings and Technician Discussion Forums is shared with the team. Every team member also receives NC3Rs newsletter and publications which will inform them of any new information. All team members attend national or international conferences to stay informed of advances in the field. The principles of 3Rs is implemented in our animal work.

A retrospective assessment of refinement will be due by 26 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?

11. Understanding gene function in cardiovascular disease

Project duration

5 years 0 months

Project purpose

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

Key words

cardiovascular disease, heart failure, myocardial infarction, comorbidities, therapy

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

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?

This project will identify genes involved in cardiovascular disease and will determine their role in both the healthy and diseased cardiovascular system. In particular, there is a need to understand the genes involved in the development of heart failure because current treatments are not effective.

A retrospective assessment of these aims will be due by 04 November 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 it's 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?

Worldwide more people die from cardiovascular diseases than any other cause. Currently approximately a third of all deaths are a result of cardiovascular disease, with heart failure being one of the commonest causes of illness and death. The risk of developing heart failure is greater as people age. This means that the prevalence of heart failure is increasing in developed countries such as the UK because people are living longer. Current treatments for heart failure are not very effective which means that 5 years after diagnosis less than half of patients will survive.

Heart failure is the end stage of a number of conditions including high blood pressure, heart attacks and obesity. Nearly 1 million people in the UK are living with heart failure and 200,000 new cases are identified each year.

There is a clinical need to improve our understanding of the genes and mechanisms underlying cardiovascular disease, in particular heart failure and its associated conditions and causes in order to identify new treatments.

This project will generate new data showing the role of a number of genes in the development of heart failure and associated diseases and conditions in order to address the ultimate aim of developing more effective treatments. By characterising the fundamental role of genes in the healthy and diseased cardiovascular system this project provides an essential link between basic scientific research and future medical treatment because once we know the identity of the proteins and pathways responsible it may be possible to develop treatment options to either reverse/ enhance their detrimental/beneficial effects.

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

The major output expected from this programme of work is an increased understanding of the mechanisms underlying the development of heart failure. The study will have both scientific and clinical/translational impacts informing the development of more effective treatments for heart failure and associated diseases.

We will generate new information about the roles of our genes and pathways of interest in cardiovascular health and disease and generate large datasets from experiments where changes in protein abundance and gene expression are investigated. We will understand more about how these genes are regulated and we will identify modulators of these genes/pathways.

We will provide proof of concept that pharmacological inhibition of a number of our genes of interest reduce the development of adverse cardiac remodelling and heart failure. These studies will provide the key foundations for further translational studies leading to the use of such inhibitors for clinical purposes against cardiovascular disease.
The new data we generate will be shared with the scientific community via published papers, conferences, workshops and open access data repositories.

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

In the short term, the project will help us to better understand the processes that lead to cardiovascular remodelling. This knowledge will help other researchers who are working in the cardiovascular field along with those with a research interest in our genes and pathways of interest.

Industry will benefit directly as it is interested in defining new targets for the treatment of heart failure. Within the term of this project we expect to identify a number potential targets against cardiovascular remodelling and heart failure.

Clinicians and patients will benefit indirectly from this work because the more we understand the mechanisms underlying the development and progression of cardiovascular diseases, such as heart failure, heart attacks and high blood pressure, the greater the potential to develop effective treatment strategies.

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

Our current genetically altered mouse strains, along with those that we will generate in this project, will likely to be of interest to other researchers, so we would be open to collaboration and the sharing of materials to maximise the impact of our work.

We will maximise access to our outputs by publishing in open access journals. Data sets, including those with negative outcomes, will be made available to other researchers via the institution's data repository and other public databases.

We will share good practice in terms of surgical techniques, disease model development and *in vivo* analyses. We already collaborate with numerous research groups, both at our own institution and elsewhere, so we envisage that the outputs of the current work will feed into further refinement of techniques and research partnerships.

Species and numbers of animals expected to be used

- Mice: 12500

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.

The project will use mice, predominantly with genetic modifications in our genes of interest. We can therefore study the impact of altered gene function in the whole animal, allowing us to model the complex disease processes that occurs in patients with cardiovascular disease.

The mouse is a highly relevant animal model for understanding disease processes in the cardiovascular system. In comparison to humans the mouse heart has the same gross and cellular structure; the pressure and volume characteristics closely resemble the human situation; and the vascular system is similar in terms of structure, function and its response to changes in blood pressure.

In order to generate mice for use in experiments, we will have to maintain breeding colonies of genetically modified mice and will use adult mice in the subsequent experiments.

Overall, the mouse is an excellent model from which to determine crucial information regarding pathophysiology, and hence future treatment strategies which are needed to make progress in the field.

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

Typically, mice will be bred to carry a genetic alteration followed by subsequent study of their cardiovascular function in (1) health or (2) disease.

A series of methods similar to those used to assess human heart and vessel (cardiovascular) function eg blood pressure measurement, an electrocardiogram (ECG) and cardiac ultrasound will be used to analyse cardiovascular structure and function in mouse lines carrying genetic alterations. These analyses may be carried out at a single time point, or at multiple time points to analyse changes over time.

Cardiovascular disease will be induced in the mice using a number of different surgical approaches. Mice will undergo surgery under anaesthesia followed by a period of monitoring to ensure full recovery from the surgical procedure. We will also use radiation treatment to induce cardiovascular disease to study why lung cancer patients treated with radiation therapy are at higher risk of developing cardiovascular disease. Using the series of tests described above, the effect of the disease/radiation on the heart and vessels will be assessed at a single time point, or at multiple time points to analyse changes as the disease progresses.

In some of these experiments we will assess the impact of an intervention. For example, a mouse may receive a potential therapeutic drug by injection or by oral gavage. In other experiments we may feed mice a modified diet to mimic the high fat and high sugar diets that can lead to obesity and cardiovascular disease in humans. Blood samples may be taken to analyse markers of cardiac damage or to measure glucose and insulin levels; this may require the mice to be fasted for up to 8 hours.

At the end of each study described above, tissue will be collected for cellular, molecular, histological and *in vitro* analyses.

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

The genetic modifications are not expected to cause any overt effects, so the mice are not expected to suffer from any adverse effects prior to entering an experimental procedure. However, in <10% of the mice we will induce the genetic alteration by treatment with tamoxifen which may lead to some transient weight loss.
No adverse effects are expected as a result of multiple cardiovascular assessments, conducted whilst the animal is conscious or under recovery or non-recovery anaesthesia.

We expect the majority of animals to make a full recovery from surgical interventions conducted under anaesthesia. Post-operative pain will be prevented by administering analgesics. Some animals may not recover from anaesthesia as a result of surgical complications, and as such will feel no pain or distress.

After recovery from surgery to induce myocardial infarction, an animal is at risk of sudden death from rupture of the artery, lethal arrhythmia or cardiac rupture. These sudden deaths can occur in animals without external signs of ill health, up to 7 days post-surgery. These deaths are sudden, will likely cause minimal suffering, and death is effectively instantaneous.

The methods we use to induce cardiovascular disease may lead to the development of acute or chronic heart failure. However, in our experience, the length of time that mice are subjected to the cardiovascular stress leads to left ventricular remodelling and dysfunction, which does not progress to heart failure and therefore the vast majority of the mice will not display any clinical symptoms. Animals will be humanely killed if clinical signs of heart failure are observed. Radiation therapy may cause localised irritation/damage to the skin at the site targeted with radiation, the effects are temporary and self healing.

No adverse effects are expected by withdrawing food for up to 8 hours and water will always be provided. Whilst we do not anticipate any adverse effects from the drugs we will deliver it is known that oral gavage causes transient discomfort and will only be used when there is no other suitable route.

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 expected severity level associated with the breeding and maintenance of the genetically altered mice and assessment of their cardiovascular related phenotype is mild. A small proportion of animals will be aged up to 30 months and are likely to experience a moderate severity.

Where animals undergo a surgical intervention with recovery from anaesthesia we expect the majority to be within the moderate severity category. Some mice subjected to ligation of the coronary artery to induce myocardial infarction may experience sudden death, which is effectively instantaneous; however, a short period of pain cannot be ruled out. These animals will be classed as severe. Where mice undergo radiation therapy we expect the majority to be within the mild category.

In this project we approximate mice to fall into the following severity categories: 50% mild; 45% moderate; 5% severe.

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

- Killed

A retrospective assessment of these predicted harms will be due by 04 November 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?

Cardiovascular disease leads to complex changes in physiological function in both the cardiovascular system and in the body as a whole. This complex interaction is difficult to predict and given that the changes are not yet fully understood they cannot be effectively reproduced using isolated cells or computer modelling alone.

The cardiovascular system responds to factors carried in the blood and from cell to cell, as well as to the physical forces imposed by the beating heart and blood pressure. In combination with gene modification these factors will affect the disease process.

Cardiovascular pathological remodelling is a complex process involving different cell types in the heart including cardiomyocytes, fibroblasts, endothelial and inflammatory cells. Many processes, such as hypertrophy, fibrosis, inflammation and apoptosis, are known to contribute to the adverse remodelling. Whilst studies using cellular models may provide information on pathological processes in individual cell types, it would be impossible to understand the complex cellular interactions involved in cardiovascular remodelling at the organ level, without the use of animal models. The mouse provides the best available species to model cardiovascular remodelling and failure, as its cardiovascular system shares significant structural and physiological similarities with humans.

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

We have used isolated and cultured cells; cardiomyocytes derived from stem cells generated from human tissues; and tissue from patients with heart failure to perform many of the experiments that underpin this project and we will continue to do so where appropriate. These studies mean that we are better informed to design our animal-based experiments.

Why were they not suitable?

Isolated cells are useful for providing proof of principle and for testing drugs or understanding at the molecular level how signalling processes occur.

There has been a recent increase in the use of stem cells as an experimental model. There is a lack of human cardiovascular tissue available for research, which has led to the development of techniques to induce cardiomyocytes (heart muscle cells) from human stem cells. These cells have many of the characteristics of human cardiomyocytes and thus we are using them as a model system in which to characterise the effect of gene modification on hypoxia and hypertrophy and to understand the signalling pathways involved.

These *in vitro* systems can complement and enhance our research involving animals but will not act as a replacement for experiments, which require the understanding of gene function within the context of the whole organ and the whole body, especially in disease states.

A retrospective assessment of replacement will be due by 04 November 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?

For each step of the project we have calculated the number of animals required to complete each experiment. This calculation is based on our own experience of similar experiments and/or from experiments reported in the scientific literature. We are very experienced at understanding the variability typically encountered in the experiments we will perform and we will use the minimum number of animals required to determine whether there is a difference between experimental groups.

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

We have designed our experiments guided by the NC3R's Experimental Design Assistant so that we are able to gain the most information from each individual animal.

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

The majority of animals used in this project will come from our own breeding colonies of genetically altered mice. In maintaining these colonies we will employ efficient breeding strategies to ensure that the number of excess animals is kept to a minimum. Colony size will be reviewed regularly to ensure that breeding matches the anticipated demand for experimental animals.
We constantly monitor whether to continue with a particular objective or not. Thus, if an experimental outcome indicates that there is no value in continuing, that aspect of the work will cease.

Where relevant we will also conduct cell culture experiments to complement and inform our animal- based research. For example, to understand a potential gene or pathway prior to investigating their effect in the intact animal or disease model.

A retrospective assessment of reduction will be due by 04 November 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.

To minimise the harm to the mice used in these studies we have selected and refined approaches and techniques which cause the least pain, distress and lasting harm whilst being able to meet our objectives.

The effect of any genetic modification on the health of the animal is unpredictable, as is the effect of a procedure on the mouse, so all animals are inspected at least once a day. The genetically altered strains we are currently studying do not have observable phenotypes that impact on behaviour.

All surgical techniques are carried out under general anaesthetic to ensure that the animal does not feel any pain, and any post-surgical pain is treated with the use of analgesics. Any animal in which pain is uncontrolled, or which has significant surgical complications, or whose general health deteriorates, will be humanely killed. We have found optimal ages and weights for particular surgical procedures and we keep within these parameters.

Physiological analyses (eg ultrasound, imaging, ECG) are performed under general anaesthetic, in the majority of cases the mouse is under terminal anaesthetic from which it does not recover. ECG and blood pressure may also be monitored in conscious animals; this is a quick procedure which is not stressful and does not require anaesthesia.

Any stress caused by administration of pharmacological agents is momentary as the injection is given. Where applicable mini-osmotic pumps will be used to administer hypertrophic, hypertensive and other pharmacological agents. Although their use initially involves minor surgery, the technique in our experience leads to highly reproducible and consistent results requiring fewer animals per experimental group. The use of osmotic pumps also reduces handling and stress in animals.

Coronary artery ligation for induction of myocardial infarction is a severe protocol. This can lead to death if the mice develop acute heart failure or if the heart ruptures but these are minimized by careful placement of ligatures, refined surgical techniques and post-operative care.

Radiation therapy will be restricted to the area of the chest where the heart is located. Other tissues will be shielded and the radiotherapy will be guided by imaging to allow precise targeting.

All personnel involved in conducting animal experiments are fully trained in the theory and technical aspects of procedures.

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

While non-mammalian models are available, such as *drosophila* and zebrafish, there are key differences in their cardiovascular physiology compared to humans (they do not possess a four chambered heart and have a different circulatory system). Whilst we do not use these model organisms directly in our own work we keep abreast of current findings using this models through discussions with colleagues and through the published scientific literature.

We are not aware of any non-mammalian animal models of heart failure and its associate diseases.

In many of our experiments we are studying a disease process which develops over time (days – weeks). This precludes us from completing all aspects of our experiments in terminally anaesthetised animals.

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

Over the course of the previous licence we have made a number of refinements to minimise harm to the animals used.

Refinements to anaesthesia and surgical approaches

Work as team during surgery. This has led to a reduction in surgical time and improved recovery. Refined use of pre-op and post-operative analgesia.

We have found optimal ages and weights for particular surgical procedures and we keep within these parameters.

We have shown that two commonly used C57Bl/6 mouse sub-strains have different responses to cardiac stress and that these differences should be taken into consideration when designing an experiment

Timing of surgery: all surgery is conducted in the morning and rarely at the end of the week to allow frequent monitoring during the normal working week. This ensures that more research staff and animal unit staff are available for advice during the surgical recovery period.

We limit the number of surgical procedures conducted per session, dependent on complexity of the surgical approach.

Some strains of mice are more sensitive to ischaemic damage and sudden death following ligation of the coronary artery. In these cases we take two approaches: (1) The position of coronary artery ligation (LAD) is adjusted. A lower position is chosen to induce mild myocardial infarction for the strains sensitive to ischemic damage. (2) Male mice are replaced with females as they have a milder response to cardiac stress.

Monitoring

Detailed study plans are drawn up for each experiment and named persons consulted. This allows us to readily monitor and question the benefit of each mouse added to the study.

Regular appraisal of surgical outcomes.

Continually refine the monitoring documentation to aid in assessing mouse welfare

Study of aged mice: Refined monitoring and husbandry practices will be used when studying old mice to ensure that they continue to feed well and maintain good body condition.

We will continue to review our procedures and make refinements wherever possible to minimise the welfare costs incurred.

Use of both sexes in studies
Until very recently it has been accepted practice within the cardiovascular research field that male mice are used in experiments to model cardiovascular disease.

Arguments for this practice have focused on the perception of increased variability in response from females; the protective effect of estrogens; historic use of males; and reduction in animal numbers, as conducting experiments in both sexes would lead to increased use of animals.

There is strong evidence of sex differences in cardiovascular morbidity and mortality in humans and in mice. However, rather than this being a justification to limit our studies to male mice only in order to reduce variability, it is important that we study both sexes to understand underlying disease mechanisms and identify new potential targets for treatment for cardiovascular diseases that affect both men and women.

Assuming there is no rationale for studying one sex, we will use equal numbers of males and females (eg n=10, 5xM/5xF) and report in the literature as such. This approach will also ensure that we are avoiding unnecessarily breeding female mice that are not used for experiments.

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

Procedures will be undertaken in accordance with institutional standard operating procedures (SOPs) and guidelines.

The approach to surgical procedures will be further informed by the Laboratory Animal Science Association's (LASA) Guiding Principles for Preparing for and Undertaking Aseptic Surgery (https://www.lasa.co.uk/wp-content/uploads/2018/05/Aseptic-Surgery.pdf).

Blood sampling will conform to guidance published on by NC3Rs (https://nc3rs.org.uk/blood-sampling- mouse).

During the planning phase of our experiments we will refer to the PREPARE: guidelines for planning animal research and testing (DOI: 10.1177/00236772177248).

When publishing the outcome of our work we will adhere to the ARRIVE guidelines (https://arriveguidelines.org/).

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

We seek to stay informed of 3Rs advances through innovations published in the literature, discussions with colleagues at our own and other institutions, regular updates provided by named individuals at our own institution and the local NC3Rs Regional Programme Manager and through NC3Rs webpages.

A retrospective assessment of refinement will be due by 04 November 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?

12. Mechanisms of Immunoregulation

Project duration

5 years 0 months

Project purpose

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

Key words

Immunology, Inflammatory Diseases, Infectious Diseases, Imaging, Vaccination

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

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?

This project aims to understand how immune cells communicate and decide whether to turn the immune system 'on' or 'off'. This is applied in disease models where switching the immune response off could lead to improvement or cure (e.g. rheumatoid arthritis) or where switching the immune system on could lead to disease prevention (e.g. vaccination or malaria infection).

A retrospective assessment of these aims will be due by 18 November 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 is important because understanding how and where immune cells communicate will allow targeted discovery of new drugs that could control the immune system to treat inflammatory and infectious diseases or improve vaccination. It will also allow us to deliver drugs to the right tissue and at the right time to achieve more effective immunological intervention. This will be tested in animal models of arthritis and malaria. These are significant human diseases in their own right, causing massive global mortality (malaria caused 625000 deaths in 2021) and morbidity (arthritis affects over 430,000 people in the UK alone).

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

Work carried out in this project will identify the important cellular and molecular interactions that lead to inflammatory and infectious diseases. These studies will show when and where and what immune cell interactions drive immune or inflammatory responses. This information is of fundamental scientific importance, leading to scientific presentations and publications. However it will also reveal new targets for drug development. These studies are extended into models of diseases with significant human health impact, opening opportunities to translate this work.

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

In the short term, the outputs from these studies will help the biomedical community to understand how to control the immune system more effectively. This will help to design new and better drugs as well as modifying and applying existing drugs more rationally.

In the longer term, this will bring benefit to patients through the development of new drugs to treat inflammatory and infectious disease areas where existing drug treatment is partially or totally ineffective. This research will also contribute to the development of new vaccine approaches to improve protection against infectious diseases.

The economic benefits of this work are mostly realised through collaboration with a range of industrial partners (pharmaceutical and biotech) on mechanism of action studies of existing drugs. However, we are increasingly interested in translating research from our lab to develop new drug targets.

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

We will work to enhance the visibility of our research by ensuring that as much of our data as possible is published, either in primary scientific articles or via methodological reports and reviews. We will also publish unsuccessful or 'negative' results using preprint servers (such as Biorxiv.org) – these are not peer reviewed but allow this work to disseminate in the scientific community. We will continue to share our data via presentations and posters at scientific conferences. We will also routinely provide detailed methods and training in methodological approaches to colleagues nationally and internationally.

Species and numbers of animals expected to be used

- Mice: - 20000

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.

The work in this licence will be performed in adult and juvenile mice.

Mice have a very well characterised immune system and most of our understanding of immune function in humans has been extrapolated from mice. For this reason, mice are the most widely used species for immune characterisation. This also means that we can interpret data from our research in the context of the existing body of research on humans and other animals.

A second major factor for studying the immune system in mice is the unique range of genetically modified animals available. This includes mice expressing fluorescent (coloured) markers to identify where and when cells participate in immune responses as well as mice with defined deletions in their immune system to help understand the role of molecules and cells of the immune system. This allows investigation of exactly how, where and when a particular molecule or cell is involved in an immunological mechanism, that isn't possible in other species.

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

Typically, our experiments will intervene during the induction of an immune response, which typically takes three to five days. During this period, we would inject drugs or other chemicals daily, or use genetically modified mice, to enhance or suppress the immune response. We would then observe the effects of these interventions on immune cell behaviour by taking blood samples, removing tissues post mortem or by directly imaging tissues after surgical preparation. Alternatively, we would investigate the outcomes of these interventions on the immune response to infections (mouse malaria models) or autoimmune disease (mouse models of arthritis). These models have an immune induction phase, followed by a disease phase, so some studies will require immune intervention in one or both of these phases.

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

The immune interventions (drugs, genetic modification) should not have any adverse effects and the imaging experiments are performed on anaesthetised mice and last less than 6 hours before mice are euthanised.

Strong immunisation protocols are required for studies of immunity and also in arthritis models. These can cause local inflammation at the site of injection which resolves over one to two weeks.

Mouse models of malaria will cause anaemia and weight loss 6-10 days after infection and can lead to death. After this period, mouse condition recovers.

Arthritis models cause joint inflammation and mice demonstrate reluctance to move and weight loss over a period of two to three weeks.

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)?

Over all of the proposed procedures these categories are - Mild - 70%
Moderate - 29%

Severe - 1%

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

- Killed

A retrospective assessment of these predicted harms will be due by 18 November 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?

Immune cell interactions are complex and strongly influenced by the tissue where they occur (e.g. lymph nodes, joints, spleen). While studies in tissue culture can recapitulate some aspects of these (see below) they currently cannot fully replicate this complexity. This is particularly true in inflammatory and infectious diseases, where immune cells play an important role in the development of disease by their effects on tissue cells. To understand the mechanisms of immune regulation in disease therefore requires studies of immune cell interactions with intact tissues.

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

As a result of our previous animal research, we have developed a tissue culture model alternative to identify immunoregulators and immunostimulators. While this model represents an improvement on existing replacement models and a step forward in reducing animal use in the field, it does not take into account or allow analysis of immune cell interactions with intact tissues.

Why were they not suitable?

As mentioned above, the tissue culture model we developed does not allow analysis of immune cell interactions with intact tissues in inflammatory or infectious diseases. For the field to move forward, we need to perform further mechanism of action studies, which in turn will develop improved tissue culture systems to replace animal models.

A retrospective assessment of replacement will be due by 18 November 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?

A balance between keeping animal numbers to a minimum and achieving statistical validity in experiments has to be achieved. Previous extensive experience has allowed us to ensure that animal usage in our studies is optimised and each experiment is carefully designed to ensure the minimum use of animals necessary to achieve our scientific aims.

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

Each protocol is based on extensive previous experience and has been optimised to ensure minimal suffering for the animals involved. Before starting an experiment, we use power calculations and randomisation approaches where appropriate. These allow us to avoid using excess animals, while allowing us to identify differences (e.g. in response to an immune intervention). In designing new experiments, we take advice on statistical approaches and experimental design eg. via colleagues in Biostatistics and/or the NC3Rs Experimental Design Assistant (EDA).

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

Where required, we will perform pilot experiments e.g. when using new interventions or approaches. These use fewer mice and fewer groups as their aim is to determine appropriate use/dose or effect size (how big an impact does an immune intervention have) rather than address a specific scientific question.

We have developed protocols using whole body imaging systems that allow us to monitor disease progression in mice while under anaesthesia. Repeated imaging of the same animal can be carried out not only improving the quality of our data but also reducing the number of animals required for time course studies.

Cryopreservation is also available to us to avoid unnecessary breeding.

A retrospective assessment of reduction will be due by 18 November 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.

Visualising Immune modulation – Through our previous studies and those of colleagues we know that the key immune decisions/interactions occur within 24 – 72 hours after immunisation. This allows refinement of these studies, focussing on relatively short, and mild procedures, that produce a wealth of imaging and immunological data.

Immune modulation in malaria - Most of our proposed work will use infection with a strain of malaria (*P. chabaudi*) that is relatively less severe, while being more appropriate to understand immune cell interactions underlying immunity and disease. In a minority of studies (10%), we aim to understand immune regulation in severe disease models (*P. berghei*).

Immune modulation in arthritis – All mouse models of arthritis cause distress to varying degrees depending on model. We developed a refined model of arthritis (OVA induced arthritis) that allows improved analysis of key, early steps in the development of autoimmunity (break of self tolerance). This model also has reduced severity compared with other models of arthritis (e.g. collagen induced arthritis).

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

The major aim of our project is to understand how immune modulation affects the way immune cells communicate. We use adult and juvenile mice, as younger mice do not have fully developed immune systems. Mice are most appropriate animal to use for our studies as their immune systems are similar to humans and we have a great deal of knowledge about immune responses in mice, helping us to put our data in the context of a wider field and accelerate new understanding.

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

The models used in this licence are well characterised and mice on procedures are monitored when we expect them to show signs of ill health. Based on experience with the malaria model, this occurs around 6-8 days after infection. With the arthritis model, disease persists for around 14-28 days after induction of arthritis. We monitor the condition of mice through this phase by weighing and by observing their behaviour and appearance. We euthanise mice if they lose 20% of their starting weight or show unexpected clinical signs.

In the arthritis model, there is a well-recognised articular inflammation score (Rodent Protection Tests Working Party (Lab. Animals 1994 28: 13-18), that also contributes to management of disease severity. In addition to the weight controls above, mice are euthanised if footpad swelling or the total articular inflammation score exceeds set levels (see protocols). Application of analgesia (pain relief) is limited due to anti-inflammatory effects (Inflammopharmacology. 2015; 23(4): 131–150). Use of these drugs will be discussed with the NVS where appropriate.

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

We follow guidance from NC3Rs and the ARRIVE guidelines.

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

Up to date guidance will be monitored via the NC3Rs website (http://www.nc3rs.org.uk/) and through updates from our Animal Welfare and Ethical Review Board and Culture of Care subgroup.

We keep up to date on relevant scientific literature around immunomodulation and imaging in mice. Should there be relevant and feasible modifications to the current methods that would enable us to reduce the numbers of animals we use, we would first test whether these altered our main readouts of disease and immune response, and if our main findings could be replicated, adapt these modifications.

A retrospective assessment of refinement will be due by 18 November 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?

13. Lysosomes in health and disease

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
  - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - Assessment, detection, regulation or modification of physiological conditions in man, animals or plants

Key words

Lysosome, Lysosomal Storage Disorders, Neurodegenerative diseases, Rare diseases, Therapy

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

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?

To study how lysosomal function regulates multiple aspects of mammalian health and to study how lysosomal dysfunction leads to rare and common human diseases, with a particular focus on neurodegeneration and infection and immunity.

A retrospective assessment of these aims will be due by 19 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 it's 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?

Lysosomal storage diseases are a family of over 70 rare inherited metabolic disorders that collectively affect 1 in 5000 live births. The lysosome is the cellular organelle responsible for breaking down macromolecules and recycling metabolites. The lysosome is also a signalling organelle and forms contact sites with other organelles, including mitochondria and the endoplasmic reticulum (ER). Most of the genes that are mutated in lysosomal diseases encode lysosomal enzymes or lysosomal membrane proteins. Substrates build up in the lysosome because of these faulty genes leading to “storage”.

Lysosomal storage diseases are progressive disorders and invariably result in premature death, often in childhood. Over 70% of these diseases affect the brain and typically have a neurodegenerative clinical course.

In this programme of research, we aim to better understand the pathogenic mechanisms driving these severe human diseases, identify novel clinical intervention points and trial therapeutic strategies in mouse models prior to conducting clinical trials.

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

We will generate fundamental knowledge on the roles of lysosomes in health and disease leading to scientific publications. We will also identify therapeutic strategies for treating diseases that result from lysosomal dysfunction and progress these therapies to clinical trials in patients. The outputs will therefore be adding to the scientific literature and improving human health.

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

The scientific and clinical communities that studies lysosomes and lysosomal diseases will be a beneficiary of this research as will the patients who suffer from rare and common diseases resulting from lysosomal dysfunction.

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

We collaborate widely within the field, are leaders on translation of our basic research findings and we work closely with scientists, clinicians, patient advocacy groups and regulators.

Species and numbers of animals expected to be used

- Mice: 68,500

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.

We will conduct all our studies on mice as they are the lowest organism that recapitulates the human diseases we study. Most of our research is from weaning to adulthood. We also use mice in our research that have mutations or are engineered to have changes in lysosomal function, with a particular interest in the genes involved in modulating sphingolipid metabolism in the lysosome and in other cellular compartments.

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

Typically, mice will have behavioural analysis performed throughout their life span with or without the administration of a potential therapeutic agent. Tissues will be harvested over the life span of the mice to correlate biochemical parameters with clinical signs in the mice with or without a therapeutic intervention. We may also cross mutant/transgenic mice to generate new models or test new mechanistic hypotheses about disease pathways. In a small number of studies, we may challenge mice with infectious agents or derivatives of microbial origin.

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

The mouse models we study typically have a progressive neurodegenerative clinical course and so develop abnormal behaviours (e.g., altered patterns of walking). The mice typically have an asymptomatic period then develop early, mid and late-stage clinical signs as they age. Life spans vary by disease model and background strain. Typically, the mice live for several months.

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)?

80% Mild

10% Moderate

10% Severe

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

- Killed

A retrospective assessment of these predicted harms will be due by 19 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?

We study diseased cells derived from patients for many of our cell biological studies determining which metabolic pathways are affected in these diseases. We can trial treatments in these cellular models to study biochemical efficacy and to provide the initial proof of concept. However, to study complex degenerative processes in the central nervous system (CNS) and to model treatment outcomes we must study the intact organism as these are complex multi-morbidity diseases that affect the CNS but also peripheral organs. The innate immune system is also triggered and so to study this we again need an intact mammalian system to model what is occurring in patients.

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

We have access to two main sources of patient derived cells, blood samples that we analyse mononuclear cells from. We can transform B cells from these blood samples to form immortal cell lines. Skin fibroblasts are often derived from lysosomal disease patients and can be kept in culture and studied for several weeks. These are from clinical centres or from cell repositories. From fibroblasts induced pluripotent stem cells (iPSCs) can be generated and differentiated into multiple cell lineages providing more clinically relevant cell models we can study.

Why were they not suitable?

They are suitable for modelling cellular processes, but they are not sufficient to inform us about whole organs pathophysiology and the interplay between different organ systems in these diseases. They are essential for providing whole animal proof of concept for trialling therapies prior to clinical trials.

A retrospective assessment of replacement will be due by 19 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?

The numbers estimated in this project come from our previous experimental design, and also the most refined breeding methods, which have shown reduced Mendelian ratios in some of our genetically modified lines.

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

Application of best practice from the following resources.

NC3R's Experimental Design Assistant (www.nc3rs.org.uk/experimental-design-assistant-eda) PERPARE guidelines – (https://norecopa.no/prepare)
We conduct our experiments so that they comply with the ARRIVE guidelines (www.nc3rs.org.uk/arrive- guidelines) when we prepare to publish.

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

Optimisation of breeding strategies, we have a mouse data base we keep real life data that helps inform optimal breeding strategy.

We use shared control groups between concurrent studies when possible.

A retrospective assessment of reduction will be due by 19 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.

We use genetically altered animals that mimic the human lysosomal storage disorders that our work is based upon. Like the human diseases the mice show a progressive clinical course. The symptoms we see such as decreased mobility, are relieved by modifying the provision of food and water to the animals and we provide additional nesting material to ensure we avoid hypothermia.
Why can't you use animals that are less sentient?

The mouse is the lowest sentient mammal to study lysosomal disorders as all other mammalian models are in companion animal species such as cats and dogs or in large farm animal species such as cattle and sheep. The mouse models we study have supported translation of effective disease modifying therapies to the clinic and so are validated models. The storage of lipids progresses over time and so we cannot work on very early stages of development as the pathology takes time to manifest itself.

There are invertebrate models of some of the diseases, but the behavioural deficits are

limited to e.g., climbing defects and the biochemistry of the sphingolipids in the fly are not the same as in mammalian species so mice are the optimal models for these reasons.

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

We increase monitoring towards the humane endpoint, and we familiarise mice with behavioural equipment before gathering data. We use the best anaesthetic methods available through regular dialogue with veterinary colleagues.

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

We follow local AWERB guidelines; ARRIVE guidelines of the NC3Rs; guidance on Animal Testing and Research from the Home Office; Good research practice guidelines from LASA and RSPCA guidelines and PREPARE guidelines.

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

By regular review of our practices, implementation of best practice cascaded to us from the Home Office, veterinary staff and animal care and welfare officer and staying up to date on new advances in therapies and animal models in this field. We regularly attend NC3Rs events.

A retrospective assessment of refinement will be due by 19 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?

14. Control of Bacterial Products Used in Medicine

Project duration

5 years 0 months

Project purpose

- Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

Vaccine, Immunisation, Medicine, Quality, Safety

Animal types	Life stages
Mice	adult, juvenile
Guinea pigs	adult

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?

Immunological medicinal products, such as vaccines and therapeutic antitoxins, are tested by an independent laboratory before being released onto the market. These tests, some involving animals, help to ensure the quality of these products

A retrospective assessment of these aims will be due by 18 November 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 is important to provide an appropriate level of assurance that batches of immunological medicinal product will be safe and effective for use in humans. A high level of assurance is particularly important for vaccines. If public confidence in vaccine safety/effectiveness falls, vaccine uptake and coverage may be reduced and diseases that were previously well controlled by immunisation can re- emerge. The number of doses in a single vaccine batch may be in the hundreds of thousands and, in many cases, the target population for a vaccine is healthy infants and children where the tolerance for adverse effects is much lower than for other medicinal products. Consequently, the regulation of these biological medicines is rigorous and necessitates the use of animals for some of the tests that are performed by the independent regulatory laboratory

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

This project provides independent assurance that biological medicines are safe and effective for supply to patients

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

The principal benefit from this project is the assurance obtained that batches of immunological medicinal product are likely to be safe and effective for use in humans. A high level of assurance is particularly important for vaccines. If public confidence in vaccine safety/effectiveness falls, vaccine uptake and coverage may be reduced and diseases that were previously well controlled by immunisation can re-emerge. In many cases, the target population is healthy infants and children and the tolerance for adverse effects from prophylactic medicinal products such as vaccines is much lower than for other medicinal products. Consequently, the regulation of these biological medicines is rigorous and necessitates the use of animals for some of the tests that are performed by the independent regulatory laboratory.

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

In addition to the independent assurance for supply of safe and effective biological medicines, the expertise that we maintain for this work also allows us to identify limitations with existing methodology and work collaboratively with regulatory partners to revise regulatory guidelines and include refined or alternative methods where appropriate. This work also facilitates our own development of non-animal methods

Species and numbers of animals expected to be used

- Mice: 5084
- Guinea pigs: 408

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.

The protocols in this project will use mice and guinea pigs. The protocols in this project and therefore the choice of animal are based on established methods described in Pharmacopoeia monographs or other regulatory guidelines (such as WHO guidelines). Adult animals are used for all protocols in this project with the exception of the potency test and safety test for whole cell pertussis vaccine, both of which require juvenile mice. Juvenile mice are used for the potency test (Kendrick test) because adult mice will have a more developed skull that is harder to penetrate during the intracerebral challenge step. Juvenile mice are used for the safety test (mouse weight gain test) to help with the read out of the assay - the weight range of mice to use in the test is specified in WHO recommendations for whole cell pertussis vaccine and is based on the fact that juvenile mice will be actively growing and will be gaining weight during the course of the test as part of their natural development.

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

Protocol 1 Tetanus Vaccine Potency (Challenge): mice are immunised and then challenged 4 weeks later by injection with tetanus toxin and observed over 4 days for signs of local paralysis

Protocol 2 Diphtheria and Tetanus Vaccine Potency (Serology): guinea pigs are immunised and blood is taken under terminal anaesthesia at the end of the test (usually 35 days following immunisation)

Protocol 3 Whole Cell Pertussis Vaccine Potency (Kendrick Test): mice are immunised and then challenged 15 days later with a strain of the disease-causing bacteria that is injected intracerebrally, and then observed over a 14 day period for clinical signs of pertussis infection

Protocol 4 Whole Cell Pertussis Vaccine Toxicity (Mouse Weight Gain Test): mice are injected with the vaccine sample and then observed over a seven day period with weight measurements taken on 4 separate occasions

Protocol 5 Acellular Pertussis Vaccine Immunogenicity: mice are immunised with vaccine on up to two occasions, 21 days apart, and blood is taken under terminal anaesthesia at the end of the test (usually 35 days following the last injection)

Protocol 6 Diphtheria Antitoxin Assay: guinea pigs are injected at multiple (up to 6) sites on shaved flanks with a mixture of diphtheria toxin and antitoxin. Animals are observed for two days and the degree of erythema at the injection site is recorded

Protocol 7 Botulinum Antitoxin Assay (non-lethal): mice are injected with a mixture of botulinum toxin and antitoxin and observed over 2 days for signs of localised flaccid paralysis characterised by abdominal ptosis (displacement of abdominal organs)

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

Protocol 1 Tetanus Vaccine Potency (Challenge): mice that are incompletely protected (i.e. those receiving the lowest dose of vaccine) and/or those receiving the higher doses of toxin may experience increasing spastic paralysis due to tetanus intoxication The estimated duration of these effects is 1 to 3 days

Protocol 2 Diphtheria and Tetanus Vaccine Potency (Serology): this protocol has a mild severity limit and the guinea pigs used are not expected to experience any adverse effects

Protocol 3 Whole Cell Pertussis Vaccine Potency (Kendrick Test): following immunisation, some animals may show signs attributed to mild to moderate pain or discomfort, including partial pilo- erection, orbital tightening, lethargy and abdominal contraction and/or distension. Most effects will typically pass off within a few hours, but some may persist, usually to a lesser degree, for up to 24 hours. A very small number of mice (2%) may suffer severe brain trauma due to the intracerebral inoculation, which is characterised by head tilt and circling. The estimated duration of these effects is up to 18 h. Following challenge, approximately 55% of animals will experience adverse effects as a result of overwhelming cerebral infection that include weight loss, pilo-erection, loss of muscle control, convulsive motions and laboured breathing, which may lead to death. Some animals will be expected to show varying degrees of effects for up to 12 days but may fully recover or survive to the end of the test

Protocol 4 Whole Cell Pertussis Vaccine Toxicity (Mouse Weight Gain Test): following immunisation, some animals may show signs attributed to mild to moderate pain or discomfort, including partial pilo- erection, orbital tightening, lethargy and abdominal contraction. Most effects will typically pass off within a few hours, but some may persist, usually to a lesser degree, for up to 24 hours. Weight loss of up to 10% is normally observed during the first 24 hours and animals start to regain weight after 24 hours and have regained or exceeded their start weight by day 3

Protocol 5 Acellular Pertussis Vaccine Immunogenicity: this protocol has a mild severity limit and the mice used are not expected to experience any adverse effects

Protocol 6 Diphtheria Antitoxin Assay: the guinea pigs will develop erythema at one or more of the injection sites and, rarely, some animals may develop localised necrotic lesions (dry, non-exudative necrotic scabs) at the site of injection. These effects will be seen for up to 48h

Protocol 7 Botulinum Antitoxin Assay (non-lethal): mice will develop a varying degree of abdominal ptosis with localised muscular paralysis. This does not significantly affect mobility, activity or general health. These effects will be seen for up to 48h

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)?

Mice: approximately 40% mild, 35% moderate, 25% severe

Guinea pigs: approximately 88% mild, 12% moderate

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

- Killed

A retrospective assessment of these predicted harms will be due by 18 November 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 tests used in this project are performed according to methods described in regulatory documents such as European Pharmacopoeia monographs and WHO recommendations, or are based on methods included in product licence dossiers. In all cases, no suitable validated non-animal alternatives are currently available. Where validated alternative test methods exist, they have been introduced and this has resulted in a removal of some protocols from this project licence that were included in the previous project licence. Efforts are continuing to develop and validate alternatives to some of the methods that are retained in this project licence.

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

None, for the reasons stated above

Why were they not suitable?

There are currently no non-animal alternatives for the tests included in this project licence that have been validated and implemented for regulatory use.

A retrospective assessment of replacement will be due by 18 November 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?

The protocols included in this project are based on established methods that are described in regulatory guidelines and monographs. The estimate for total number of animals used is based on predicted volumes of batches of biological medicine that will require testing over the 5 year period.

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

Where possible, for tests that require reference and/or control groups, multiple test samples will be included in the same assay to maximise the use of these reference/control groups and ultimately reduce the total number of animals needed during the project.

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

For the tests used in this project, the number of dose groups and size of each group is based on well- established methods that are described in regulatory monographs and guidelines.

A retrospective assessment of reduction will be due by 18 November 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.

Animal models of vaccine potency, antitoxin potency and vaccine toxicity are used in this project. Two vaccine potency models are challenge tests that require challenge with toxin or virulent bacteria following immunisation: the tetanus vaccine potency test (protocol 1) and the whole cell pertussis potency test (protocol 3). There is currently no refined alternative available for the whole cell pertussis potency test although there are efforts underway to develop a serological assay that would remove the requirement for challenge and reduce the severity limit. However, this is not yet at a stage where it can be implemented for regulatory use. For tetanus vaccine potency, a refined method is available and is included in this project (protocol 2) - where possible, the refined protocol will be used but regulatory guidelines state that comparison of potency estimates with the challenge test is required before it can be used. For products tested infrequently and with a small number of batches, such validation would increase the number of animals required in the short term.

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

The majority of protocols used in this project require the use of mice and are based on well-established methods that are described in regulatory guidelines and monographs. Guinea pigs, rather than mice, are used in two protocols for the following reasons: the

assay for diphtheria antitoxin potency cannot be done in mice because that species is insensitive to diphtheria toxin; the serological assay for diphtheria and tetanus vaccine potency can be done in mice in theory, but the very large differences in the level of immune response to the difference vaccine components means that additional vaccine dilutions would be needed (and therefore additional animals) to cover the dose response range for both vaccine components. The assay in guinea pigs can be done with the same dilutions of vaccine for both components, reducing the number of groups and animals to the minimum required to generate valid potency estimates.

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

For all protocols, animals are habituated prior to commencement of procedures, typically for 7-10 days. Inoculation volumes are based on those described in the regulatory guidelines or monographs and do not exceed those recommended by LASA. We strive for continual improvement to animal housing, husbandry and handling aimed at minimising stress in animals and encouraging natural behaviours.
Animals undergo thorough pre- and during-study checks. Frequent monitoring by experienced staff, including out-of-hours checks for some procedures help to ensure that welfare costs are minimised wherever possible and analgesics will be administered where needed and where their effect will not interfere with the scientific outcome of the study.

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

We are guided by the regulatory guidelines and monographs that describe the methods included in this project. Guidance is also available through the UK Home Office including newsletters via the ASRU. Best practice guidance from LASA and FELASA will also be followed and minimum standards of accommodation and care for laboratory animals outlined in Annex III of Directive 2010/63/EU will be met or exceeded for all work performed under this project.

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

Scientists at the Establishment are members of, or advisors to, International expert groups that elaborate and revise regulatory guidelines for vaccines and therefore have a high level of awareness of 3Rs developments that relate to this project. This information is disseminated within the organisation to other colleagues. In addition, the Establishment's AWERB circulates up to date information on the 3Rs through the Named Information Officer (NIO). I am directly involved in the development of refined in vivo methods or alternative non-animal methods as part of my role and will work closely with the Establishment NACWO to implement refinements where they are identified.

A retrospective assessment of refinement will be due by 18 November 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?

15. Production of High Antibody Equine Plasma

Project duration

5 years 0 months

Project purpose

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

Key words

Immunity, transfusion medicine, septic disease, therapy,foal welfare

Animal types	Life stages
Horses	adult

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.

- Uses cats, dogs or equidae

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?

To produce high antibody equine plasma of sufficient high quality to be safe and efficacious to transfuse into foals.

A retrospective assessment of these aims will be due by 04 September 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 it's 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?

Primarily, neonatal foals can be transfused with equine plasma to prevent septic disease and therefore prevent the pain and suffering that would cause. Secondly a proportion of the herd of donor horses would otherwise be put to sleep as they would not have any other purpose they could fulfil.

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

The outputs will be the provision of high antibody plasma to transfer into foals with Failure of Passive Transfer of Maternal immunity

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

New born foals will be saved from having septic disease and the pain and suffering which it causes. It will also reduce the antibiotic usage in such foals and the plasma is environmentally friendly.

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

Attending equine veterinary meetings, web site, newsletters to equine vets and personal communications.

Species and numbers of animals expected to be used

- Horses: Only adult horses are to be used of which about 45 will be needed over the five years

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.

Adult horses are used as the source of the plasma as the product is to be used in foals to transfer immunity. Adult horses have a well developed mature immune system. There are no synthetic alternatives to fulfil this purpose. It is not acceptable to transfuse plasma intravenously from one species into a different species

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

Each adult horse is vaccinated with vaccines to boost its immunity with non-specific antibodies and specific antibodies against known septic diseases of foals. The horse is blood sampled to assess that the response has been good and then the horse is brought into a procedure room where it is catheterised and connected to a blood processing machine to collect its plasma containing those antibodies. This process may last up to four hours at which time it is offered food to eat and the process can be repeated at 3-5 week intervals.

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

This activity is no different to human plasma donation and is considered mild in terms of severity and therefore very rarely is any significant or long lasting adverse effect expected. A small number of instances of local reaction to vaccines or blood sampling is possible all of which are mild and have very little adverse impact on the welfare of the horse. The horses will be kept at the end of the project either for re-use in the same project again or re-homed.

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 severity of the process is described as- mild for all the animals.

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

- Kept alive
- Used in other projects

A retrospective assessment of these predicted harms will be due by 04 September 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?

Due to the structure of the equine placenta antibodies do not cross into the foal during pregnancy and it therefore relies on passive transfer of immunity in the first few hours after birth from its first ingestion of colostrum. This passive transfer of immunity does not always occur successfully and such foals are much more susceptible to septic disease in the first few weeks of life. It is well documented in the veterinary literature that equine plasma from suitable donors administered intravenously can remedy this situation.

In addition, in certain situations, specific septic disease becomes established on stud farms and in the absence of vaccines or alternatives it has been recognised that equine plasma containing specific antibodies to the causal infection administered to foals at or soon after birth contributes to the significant decrease in the incidence and severity of such disease.

It is not possible to produce this in other species for transfusion into foals due to incompatibility, nor is there any synthetic alternative. In summary it improves the welfare of foals and reduces the economic loss.

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

There are none

Why were they not suitable?

There is no source of horse immune proteins and antibodies other than other horses.

A retrospective assessment of replacement will be due by 04 September 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?

This is estimated from our past history over 29 years. Horses are kept until very old if possible but eventually there is natural wastage from illness and debility as occurs in any horse population.

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

This project work is not experimental and consists of establishing and maintaining a herd of suitable donor horses in manageable groups free to graze but with adequate accessible housing with supplementary food all the year round. The horses are vaccinated, blood sampled and their plasma harvested in compliance with strictly controlled parameters to ensure welfare and safety of the animal. Their health and welfare is assured by daily health checks and the employment of best practice routine veterinary preventive medicine procedures. They are inspected by the Named Veterinary Surgeon in compliance with current regulatory requirements and their continued welfare assured by additional veterinary attention as required. Incremental gains are achieved in maximising donor horse health to make the most of production at each donation, monitoring systems are utilised to avoid wasted procedures. Together these steps reduce the number of procedures required and also the number of horses overall.

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

The number of animals is optimised by predicting what is required for the next foaling season, having at least 10% more horses than theoretically required. These are managed to the highest standards of welfare, feeding and management to maximise plasma quality and yields of plasma. This means that a single animal can reduce the chance of two less capable donor horses being used in the project.

A retrospective assessment of reduction will be due by 04 September 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 procedures used to harvest plasma closely mimics plasma collection from humans and thus in terms of severity it is accepted as being so mild that suffering is not considered to occur. During plasma harvesting food is always available and in all cases the donor horse feeds at will during the plasma harvesting process.

It is anticipated that up to 45 horses will be used for this project. Records from previous projects indicate there are no adverse effects from harvesting plasma under the established protocols with the animals being managed under natural conditions for the whole of their natural lives and kept in an exemplary culture of care.

Detailed records of each pheresing procedure are maintained along with detailed individual horse health records which are used to manage each animal prior to and during each pheresis.

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

Only adult horses are used and a breed which is inherently calm and quiet demeanour and must be alive and in good health to be used to generate the antibodies and produce the plasma.

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

The horses are managed at close quarters every morning and afternoon including weekends. All procedures are conducted in the mornings with a particularly close eye being kept on horses every afternoon when they have been used in the morning.

All procedures adopt current best practice.

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

This is not an experiment. It is the current best practice method of collecting plasma from the horses in as short a time as possible.

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

Good record keeping of each individual with look back procedures to minimise unnecessary blood sampling and vaccination. Maximising yields over time reduces duration of process and reduces number of processes.

Publications are consulted on all matters veterinary and horse., as well as welfare highlights

A retrospective assessment of refinement will be due by 04 September 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?

16. Molecular mechanisms of blood vessel development in brain tumours.

Project duration

5 years 0 months

Project purpose

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

Key words

brain, cancer, angiogenesis, biology, therapy

Animal types	Life stages
Mice	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?

To understand the in vivo role of regulators of blood vessel development (angiogenesis) and how they influence brain tumour growth, progression and response to therapy.

A retrospective assessment of these aims will be due by 16 November 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 it's 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?

Aggressive brain tumours have poor outcomes with devastating effects for patients and their families. Available therapies (radiotherapy and chemotherapy) lead to small patient survival of approximately 15 months. In order to improve treatment options, we need to understand better brain tumour biology. Brain tumours are highly dependent on blood supply for growth and progression. Hence, manipulating molecules involved in blood vessel development would impact on tumour growth, progression and response to treatment. In this project, we investigate the *in vivo* role of regulators of blood vessel development, identified using cell culture organotypic assays that recapitulate to some extent the in vivo process of blood vessel formation. Using in vivo models, we now investigate how DOCK4 and regulators influence vascularisation, growth, progression, and response to therapy of brain tumours.
The work will improve our understanding of the molecular mechanisms of blood vessel development in brain tumours, and the impact of the vasculature on tumour progression and response to treatment. Importantly, it may present new therapeutic targets to modulate blood vessel development and improve available therapies.

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

Aggressive brain tumours are untreatable and have devastating outcomes for patients and their families. This presents an unmet clinical need that requires better understanding of the tumour biology, identification of new targets, and pre-clinical testing of new inhibitors. The work will advance our basic understanding of brain tumour biology with respect to the brain tumour blood vessel development and its influence on tumour growth, progression, and resistance to therapy.

There will be significant knowledge and publications generated through this programme of work with regards to how blood vessels develop in brain tumours and on the role of the Dock4 signalling pathway in this process. Published studies have shown that Dock4 is a potential therapeutic target in cardiovascular disease and neurological disorders. This work has the potential to validate Dock4 and signalling partners as targets in brain tumour angiogenesis which will in turn lead to development of therapeutics to target the Dock4 signalling pathway in tumour blood vessels improving the outcome of standard therapy.

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

In the short term the outputs will be used by researchers to help advance our scientific knowledge in the fields of angiogenesis and brain tumour biology. This is a field of outmost importance given the critical role of the tumour vasculature in regulating tumour growth and providing the sole route of delivery of therapeutic agents.

In the longer term, understanding the differences between physiological and pathological blood vessel development in the brain has the potential to improve anti-angiogenic therapies, so that they become more effective for brain tumours. We expect that this will benefit cancer sufferers, clinicians and the pharmaceutical industry.

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

Data obtained will be disseminated through attendance of scientific conferences, and publications. Scientific conferences provide opportunity for collaboration including sharing of samples and data obtained from animal work. We will ensure that tumour samples and any datasets, for example arising from RNA sequencing analyses, will be widely available to the scientific community.

In terms of longer term therapeutic benefits, working at one of the largest teaching hospitals in Europe, St James's University Hospital, gives us the opportunity to steer our research towards patient benefit alongside advancing academic understanding. To achieve this, we will sustain excellence in our research throughout this programme of work, and will continue to engage in collaborative and multidisciplinary research with our University and external collaborators. There is significant interest in developing strategies to target the Dock4 pathway by academic drug development units, with whom we are actively engaging.

Species and numbers of animals expected to be used

- Mice: 3000

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.

Previous work using cell culture models revealed a promising group of proteins that stimulate the growth of blood vessels that support brain tumour growth. Within this group there are potential therapeutic targets for brain tumours. In this programme of work we will investigate the role of these proteins *in vivo*. We will delete genes of interest using knockout technology and ask whether this affects blood vessel development and brain tumour growth. We have chosen to work with mice as model system because knockout technology is best applied in mice. Moreover, there are numerous models of human cancer that have been characterised in detail and used successfully in mice to elucidate the function of proteins of interest. We will focus our investigations on glioblastoma and breast cancer brain metastases, some of the most aggressive brain tumours in adults. For this reason we have chosen adult mice for our studies.

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

Mice with genetic deletion will be bred and tumours will be implanted subcutaneously or intracranially.

Prior to tumour implantation it may be necessary to induce a genetic deletion by injection of an appropriate substance.
Tumour growth will be monitored non-invasively using callipers and bioluminescence imaging which may require subcutaneous or intraperitoneal injection of an appropriate agent.

Mice will be treated by standard therapy (eg. radiation therapy) to examine whether the efficacy of the treatment improves with genetic deletion.

Blood samples may be taken.

Mice may receive tracers intravenously to mark the vasculature and determine its function.

9. The mice will be killed by terminal perfusion when tumour growth reaches the specified end-points, and tumours will be excised for analysis.

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

Animals will experience temporary discomfort from subcutaneous tumour implantation. The animals will bear the implanted tumours for approximately 3 weeks and will be killed before tumours reach the maximum size specified under the protocol, so that they will not experience adverse effects due to overt tumour growth.

Animals may experience temporary discomfort or pain from surgery for intracranial implantation. Analgesics will be administered to alleviate the pain. The mice will receive radiation therapy which has no adverse effects but delivery requires use of inhalation anaesthetic which causes temporary discomfort. Non-invasive imaging also requires use of inhalation anaesthetic. In order to limit discomfort from repeated rounds of anaesthesia the maximum number of imaging and irradiation sessions will be specified under each protocol to ensure that discomfort experienced will be transient.

The animals will bear tumours intracranially for 4 to 6 weeks. Tumour growth over this time causes adverse effects ranging from slight under-grooming to strong undergrooming and reduced mobility. A scoring system is used to monitor the well being of the animals and to kill the mice before those symptoms progress further to lethargy, disorientation and distress as result of overt tumour growth. Starting from 2 weeks after intracranial implantation animals are monitored twice daily for development of symptoms and any mice that show strong under-grooming and develop reduced mobility (score 2 symptoms) will be killed immediately.

Up to 20% of mice may receive tracers while experiencing those symptoms. The tracers will be allowed to circulate for up to 5 hours during which time the mice will be monitored closely. Any animals that progress to score 3 symptoms will be killed immediately by schedule 1 method prior to the tracer circulation period.

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)?

80% of mice with intracranial implantation of brain tumours will experience moderate severity with end- point being the development of score 2 symptoms (moderate protocol). Up to 20% of mice may receive tracers upon development of score 2 symptoms (severe protocol). Mice will be monitored every hour for up to 5 hrs while the tracer circulates, and any animals that progress to score 3 symptoms will be killed immediately by schedule 1 method, prior to the tracer circulation period.

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

- Killed

A retrospective assessment of these predicted harms will be due by 16 November 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?

Blood vessel development and tumour growth are complex processes that depend on interaction of cancer cells with different cell types within the tumour microenvironment, and for this reason they are difficult to recapitulate *in vitro*. Therefore, once molecules potentially involved in these processes have been identified in cell culture assays it is necessary to assess their relevance *in vivo*. Gene knockout technology in conjunction with mouse models of human cancer can unequivocally determine the function of molecules of interest during tumour development and progression *in vivo*.

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

We have considered introducing cancer cells and tumoroids in organotypic angiogenesis assays *in vitro* as those assays are employed routinely in our laboratory in the context of a different project.

Why were they not suitable?

The *in vitro* organotypic angiogenesis assay lacks fluid flow and for this reason changes in blood vessel function and their impact on cancer cells cannot be assessed. The blood brain barrier cannot be modelled in this assay as yet and it is not possible to introduce all the different cell types that influence blood vessel development, and the interaction of blood vessels with cancer cells in tumours *in vivo*.

A retrospective assessment of replacement will be due by 16 November 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?

Over 5 years we will breed up to 3000 mice in total to maintain the mouse lines and to assess the impact of genetic modification on the development of blood vessels in brain tumours.

In tumour experiments we typically employ 8 animals per group for growth curves. At the endpoint we analyse the same mice for tumour/ brain blood vessel characteristics. On some occasions additional 4 animals will be included for analysis at an earlier timepoint of tumour and blood vessel development. As each experiment incorporates 4 groups typically (presence or absence of gene of interest and presence or absence of therapy) with average number of animals 10 per group ultimately we will employ 40 animals per experiment. As we are planning to carry out 7-8 tumour experiments each year, therefore we will use approximately 300 experimental animals per year which equates to 1500 animals over 5 years.

In order to maintain the mouse lines, perform the aforementioned analysis and generate animals with appropriate genetic constitution (genotype) for tumour experiments we will breed a total of 3000 mice over 5 years.

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

Group sizes for growth rates (8 animals) were determined with the help of an in-house statistician based on pilot studies. After determining growth rates the tumour samples are collected at the endpoint for multiple analyses of bood vessel and tumour development thus maximising the data obtained without the nedd of more experimental animals. Additional mice (4 animals) may be required to investigate blood vessel development at earlier stages of tumour growth. Multiple readouts are obtained from all tumour samples, thus reducing the overall number of amimals and experiments required for characterisation of the effects ofgenetic deletion.

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

Small animal numbers (3-4) are used in pilot experiments to assess the phenotype of blood vessels in mice with genetic deletion. Experiments to determine growth rates and perform in depth analyses are set up when positive data are obtained from the pilot studies.

Efficient breeding schedules are used to obtain animals of appropriate genetic constitution for experiments which ensures that minimum number of animals are bred over the course of the study.

A retrospective assessment of reduction will be due by 16 November 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.

Genetically modified animals. Breeding causes no harm to the animals. Animals produced under this protocol are not expected to exhibit any harmful traits. Any animals that develop a harmful trait will be killed by a schedule 1 method.

Subcutaneous implantation of cancer cells. The injected tumour cell volume is minimal and minimal number of cancer cells are injected for tumour development so that animals experience only transient discomfort from the injection. The scientific objectives require that tumours grow to a large size for development of mature blood vessels supporting tumour growth. However the tumours are not allowed to develop beyond a limit (end point) that would compromise the wellbeing of the animals. Tumour growth is measured non-invasively using callipers.

Intracranial implantation of cancer cells. A minimal tumour cell volume and cell number is injected which causes temporary discomfort. The needle used for injection is very thin, and stereotactic coordinates are used to inject cancer cells accurately into the striatum. Animals experience temporary discomfort from surgery and anaesthesia however no adverse effects like neurological symptoms, stroke or severe bleeding following injection into these brain regions have been reported or observed in experiments. As some animals may develop adverse effects for a limited time period as the tumour grows to a clinically relevant size, tumour growth is monitored very closely so that animals are killed humanely at specific end points that limit any suffering.

Delivery of fractionated irradiation. Whole brain irradiation (WBR) in rodents may lead to late structural and functional alterations (cognitive impairment) after prolonged treatment and/or high radiation doses (>30 Gy). After a prolonged WBR (5 Gy per fraction; total: 40 Gy delivered over 4 weeks) rats showed transient decrease in vascular density 10 weeks after treatment associated with late delayed radiation cognitive impairment (Brown et al., 2005, Radiation Research 164:662-8). Mice were found to develop transient loss of balance indicating reversible neurological symptoms 1 month after a single dose of 60 Gy and 3-4 months after a single dose of 30-45 Gy. (Chiang et al., 1993, Radiotherapy and Oncology 29:60-68; Chiang et al., 1993, Radiother Oncol 27:229-36). The dose typically delivered in this study is 15 Gy over 3 days. We have not observed or identified in the literature adverse effects at this irradiation dose. Potential adverse effects will be mitigated with close monitoring after delivery of the correct irradiation dose (1-5 Gy per fraction). Any animals that develop symptoms beyond the limits specified within the protocol will be killed humanely to limit any suffering.

Administration of substances. Tamoxifen may cause adverse effects in mice. Most common is anorexia and weight loss. Rarely hepatic toxicity, hepatocellular carcinoma and

death can occur but these usually occur with longer term treatment which is not used for induction of gene expression. Administration of agent for induction of genetic deletion will be at the published recommended dosage (75mg/kg body weight delivered intraperitoneally for 5 consecutive days) for which we have not observed adverse effects on the animals. Tracers are inert causing no adverse effects and are administered to mark blood vessels prior to killing of the animal. Animals that may have exhibited signs of adverse effects due to intracranial tumour growth will be monitored closely during tracer circulation and any animals that develop symptoms beyond the limits specified within the protocol will be killed immediately prior to the tracer circulation period.

Intraperitoneal injections. Repeated intraperitoneal injections may cause soreness and distress for the animals. Discomfort can be reduced by injecting fluid that is at body temperature.The maximum number of injections that will be administered will be limited to 3 per week for upto 2 weeks, or once or twice daily for upto 1 week. Typically, induction of genetic deletion will be through intraperitoneal injection of tamoxifen, which will require 5 injections 24 hrs apart. Those will be performed by the same experience researcher alternating the side injected between right and left, and using a new needle for each animal since this will reduce discomfort and risk of any injection-site infection.

General anesthesia. Long-term exposure to anesthesia can cause impairment of well-being, particularly in the immediate postanesthetic period with common symptoms being nausea and dizziness while chronic effects have also been reported. However in our imaging protocols the length of anesthesia is limited to maximum of 30 mins and frequency to 4 per week with no more than 16 in the lifetime of the animal.

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

We have considered a tumour model in zebrafish which are believed to be less sentient. This model has proved appropriate for monitoring blood vessel growth in tumours as the zebrafish blood vessels are similar to human blood vessels. However the model is still under development and there aren't enough tools to investigate in depth the mechanisms of vessel growth. Furthermore, in zebrafish it is not possible to perform gene deletion specifically in blood vessels which are focus of our investigation. Finally it is not possible to apply radiotherapy treatment which is the standard of care for brain tumours, in zebrafish.

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

Intracranial implantation of cancer cells. A minimal tumour cell volume and cell number is injected to limit discomfort. The needle used for injection is very thin, and stereotactic coordinates are used to inject cancer cells accurately into the striatum and analgesics are administered. As some animals may develop adverse effects for a limited time period as the tumour grows to a clinically relevant size, tumour growth is monitored very closely and clinical symptoms recorded daily so that animals are killed humanely at specific humane end points that limit any suffering. Specific signs of discomfort in animals are recognised by experienced staff who monitor the animals daily and with increased frequency as tumours develop. Animals are killed humanely when tumours reach maximum growth specified in each protocol, determined to minimise discomfort and limit animal suffering. Animals starting to exhibit specific adverse effects specified in each protocol are killed by a humane method.

Delivery of fractionated irradiation. As we apply the correct dose of fractionated irradiation with 1-5 Gy per dose for 3 consecutive days (total fractionated dose: 15 Gy) as described in (McAbee et al., 2019 Cancer Res. 6032-6043), we do not observe adverse effects resulting from our experiments. However, if animals show signs of lethargy after irradiation, they will be monitored closely (3 times per day) and if the symptoms persist the animals will be killed humanely to limit any suffering.

Administration of tamoxifen. Administration of tamoxifen for induction of genetic deletion will be at the published recommended dosage (75mg/kg body weight) for which we have observed no adverse effects on the animals. However, if any animals show any signs of their wellbeing being compromised, they will be monitored closely and if the symptoms persist they will be killed humanely to limit any suffering.

Intraperitoneal injections. Those will be performed by the same experienced researcher alternating the side injected between right and left, and using a new needle for each animal since this will reduce discomfort and risk of any injection-site infection.

General anesthesia. The number of procedures that require general anesthesia will be limited both in frequency and within the lifetime of the animal, as specified under the protocol details. Postoperatively, the animals are monitored as they are allowed to recover, and any animals that show any signs of their wellbeing being compromised, they will be monitored closely and if necessary killed humanely to limit any suffering.

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

Methods are based on UKCCR guidelines for the welfare and use of animals in cancer research (2010).

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

Relevant information may be obtained from the National Centre for the Replacement Refinement & Reduction of animals in research (NC3Rs) which provides information on scientific and technological advances that reduce, replace and refine the use of animals in research.

A retrospective assessment of refinement will be due by 16 November 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?

17. Safety and efficacy of a microRNA-based therapy for canine epilepsy

Project duration

5 years 0 months

Project purpose

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

Key words

Epilepsy, MicroRNA, Therapy, Seizures, Dog

Animal types	Life stages
Client owned epileptic dogs refractory to treatment	adult

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.

- Uses cats, dogs or equidae

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?

Test the effects of a novel therapy (Ant-134) in dogs with drug-resistant epilepsy:

Confirm the safety of this treatment in dogs with naturally occurring epilepsy.

Obtain evidence that this treatment is effective in controlling seizures in dogs with naturally occurring epilepsy.

A retrospective assessment of these aims will be due by 07 August 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 it's 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?

Epilepsy is a chronic disease of the brain that affects around 50 million people worldwide, making it one of the most common neurological diseases globally. It is also the most common neurological condition affecting dogs. It is characterized by recurrent seizures, which are brief episodes of involuntary movement that may involve part of the body or the entire body and are sometimes accompanied by loss of consciousness and control of bowel or bladder function. The risk of premature death in people with epilepsy is up to three times higher than for the general population. Unfortunately, it is estimated that 25-30% of epileptic humans and dogs do not respond to conventional antiepileptic treatment, affecting their quality of life. Hence there is a need to move beyond current concepts to identify mechanisms that could represent new therapeutic targets for improving seizure control.

Current treatments for epilepsy work mainly by dampening brain excitability. They fail to control seizures in many patients and must be taken repeatedly. We recently identified a completely new class of drug target for epilepsy called microRNA. These molecules coordinate signalling processes inside brain cells. We developed an inhibitor of one type of microRNA (called: Ant-134) that when injected into mice and rats almost totally abolishes seizures. We now want to take an important step towards translation by testing this therapy in dogs with drug-resistant epilepsy.

The new treatment that we want to test has the potential of providing long-lasting seizure control after a single injection and may be even cure some forms of human and canine epilepsy. This could represent an alternative treatment for many epileptic patients with the potential of significantly improving their quality of life and that of their carers.

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

The outputs from this project will include:

Evidence that this treatment is effective in naturally occurring epilepsy: Many times, mouse and rat findings do not translate to the clinic. For this reason, we want to show that Ant-134 is also effective in canine epilepsy as it will be more relevant to human epilepsy.

Scientific publication: Publication of our findings in an open peer-reviewed journal to inform the scientific community.

Novel therapy for epilepsy: Data on safety and potential effectiveness in naturally occurring epilepsy will be fundamental for bringing this new therapy into veterinary and human clinical trials.

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

Epilepsy is a chronic disease of the brain that affects around 50 million people worldwide,

making it one of the most common neurological diseases globally. The risk of premature death in people with epilepsy is up to three times higher than for the general population. It is estimated that 25-30% of epileptic humans do not respond to conventional antiepileptic treatment, affecting their quality of life. Hence there is a need to move beyond current concepts to identify mechanisms that could represent new therapeutic targets for improving seizure control.

Canine epilepsy is the most common medical neurologic disease of dogs, with an estimated prevalence of 0.6-0.75% in the general dog population, and up to 18% in some specific dog breeds. It has been associated with physical, cognitive and neurobehavioural comorbidities affecting the quality of life dogs and their owners. Currently, pharmacotherapy with seizure suppressing drugs represents the main therapy in veterinary medicine. Unfortunately, chronic administration of anti-epileptic drugs is commonly associated with significant side effects and as it is the case for humans with epilepsy, up to 30% of epileptic dogs treated with two or more anti-epileptic drugs continue to experience seizures with a less than 50% reduction in seizure frequency. Many of these dogs are euthanized because of the severity of seizures or because of severe side effects from the treatment.

The new treatment that we want to test has the potential of providing long-lasting seizure control after a single injection and may be even cure some forms of epilepsy. This could represent an alternative treatment for many epileptic patients with the potential of significantly improving their quality of life and that of their carers.

Dogs with drug-resistant epilepsy will be the main ones to benefit from the outputs of the current study, as well as the scientific community and clinicians treating epilepsy. This could also benefit patients with other neurological conditions as this will be the first study using this novel therapy to block microRNAs in the brain.

The study will aim to show if this new therapy is safe and effective in epileptic dogs. Moving forward, this will help potential development of human and veterinary clinical trials to use this drug in the clinic.

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

To maximize the outputs of this work we will publicise our findings using internet, social media, and public presentations. We will also publish any results in an open access peer-reviewed journal to make them available to anyone interested.

We are already in contact with a pharmaceutical company that will produce the Ant-134 for the present study. This company has expressed interest in the results of this study and in developing this potential treatment for eventual use in epileptic humans refractory to treatment with our collaborators that hold a patent.

Species and numbers of animals expected to be used

- Other dogs: No answer provided

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.

This study aims to assess safety and potential effectiveness of Ant-134 in a naturally occurring animal model of epilepsy. We decided to use canine epilepsy due to similarities with human epilepsy and the urgent need for new treatment strategies to improve the welfare of epileptic dogs. We want to develop a treatment for epileptic dogs refractory to current available treatment options. If successful, this could be investigated further for its use in epileptic humans.

We will be using adult (middle age) pet dogs with drug-resistant idiopathic epilepsy. Canine idiopathic epilepsy is a naturally occurring disease in which current therapies are ineffective in around 30% of dogs. We decided to use middle-age dogs (1-6 years of age) for two reasons: 1. To keep a more similar population and strengthen experimental design; 2. To avoid using older dogs that are more likely to have associated age-related comorbidities.

It is important to mention that Ant-134 has already been tested in mice and rats of different ages (early developmental stages, adult and elderly) too, with no adverse effects observed.

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

Animals in this project will have a sample of cerebrospinal fluid (fluid that surrounds the brain and spinal cord) taken with a needle and after that, Ant-134 or placebo will be injected into this space under general anaesthesia. These are routine procedures in veterinary neurology practice, pain and discomfort after the procedure is minimal and adequate analgesia will be provided. Animals will also have blood and urine samples taken at seven time points and needles connected to cables will be placed under the skin for 20-30 minutes to record the brain electrical activity (electroencephalogram) under sedation at three time points as routinely performed in veterinary practice for monitoring of epileptic dogs. Finally, previously reported non-invasive practical cognitive tests will be performed at five time points. In between time points all dogs will be at home with the owner that will keep a seizure diary. Dogs will have a minimum of 6 visits to the hospital over a 6 months period.

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

Cerebrospinal fluid collection (fluid that surrounds brain and spinal cord) and injection of drugs into this space are routinely performed in veterinary neurology practice. The procedure involves a short anaesthesia of around 30 minutes and animals recover quickly and are given appropriate pain relief. Complications are very rare and include bleeding, infection, damage to the spinal cord, seizures, and allergic reaction to injected substance.

No adverse effects of Ant-134 have been observed in rodents and similar medications injected into the cerebrospinal fluid have been given to humans. However, Ant-134 has not been tested before in dogs so we cannot exclude that they develop side effects. All dogs will be monitored by experienced veterinary staff after the procedure for a minimum of 2 days and will only be discharged if a veterinarian considers it is safe to do so. Once at home, the owners will be able to contact the hospital, if they have any concerns and recheck examinations or emergency consults will be schedule if needed. Any side effect will be assessed by a veterinarian and treated appropriately.

The rest of the procedures including blood sampling, urine sampling, sedation,

electroencephalogram (recording brain electrical activity with needles under the skin) and cognitive tests carry minimal or no risks.

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)?

Most dogs undergoing collection of cerebrospinal fluid and injection of drugs into this space experience minimal pain after the procedure, and this is well controlled with analgesics. We estimate that the highest severity for animals undergoing the procedure will be moderate but the majority of animals will only experience an actual severity of mild.

All other procedures (blood sampling, urine sampling, electroencephalogram, sedation and congntive tests) are mild severity for a transient period during sampling or recording.

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

- Kept alive

A retrospective assessment of these predicted harms will be due by 07 August 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 use of live animals is necessary as it is not possible to model the complex nature of naturally occurring epilepsy with cells in the laboratory. Ant-134 has already been proven to be safe and effective in multiple rodent models of epilepsy and this will be the next step to develop a treatment for epileptic dogs. It may also provide information that could potentially benefit humans with epilepsy.

It is important to consider that by using a pet population of epileptic dogs (which one hopes will directly benefit from this novel treatment), there's no need to use laboratory-bred dogs for this program of work. It is also not possible to induce this specific type of epilepsy in laboratory bred dogs.

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

Using human brain tissue slices and brain organoids our collaborators have shown that Ant-134 knocks down miR-134 in-vitro. This strengthens the pre-clinical evidence of Ant-134.

Why were they not suitable?

While laboratory procedures with cells can give some insights into safety and mechanism of action of Ant-134, they do not adequately model the complexity of the brain and more importantly the spontaneous and randomly occurring seizures that are seen in naturally occurring epilepsy.

A retrospective assessment of replacement will be due by 07 August 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?

The numbers have been estimated using previously published veterinary clinical trials in the field of epilepsy.

Epilepsy is the most common neurological condition in dogs and up to 30% of dogs are refractory to current treatments. There is strong interst from owners of epilpetic dogs to find novel treatments that help improving their quality of life.

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

Based on discussion with experts in animal epilepsy research, experts in human epilepsy clinical trials, a statistician with extensive experience in clinical trials, and the support network provided by our funder, we have defined inclusion criterion based on type and frequency of seizure, as well as considered age, sex, and current antiepileptic treatment for randomization. This experimental design will maximize the chances of finding differences with a smaller group of animals.

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

While the majority of the team will be blinded to the treatments received by the animals, an unblinded member of the team will perform regular interim analyses that will help us adjust the number of animals based on the actual data that is being collected during the study. This approach will avoid including more animals when it is not necessary.

Finally, to try to minimize the risk of adverse reactions, we will follow recommendations for phase I clinical trials in humans and apply a similar approach to the 3+3 design. A first cohort of 3 dogs will be treated, the number of significant adverse reaction one month after treatment will dictate if the next cohort of 3 dogs will be treated with the same dose or a

lower dose.

A retrospective assessment of reduction will be due by 07 August 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 use of dogs with naturally occurring epilepsy means that we will not use any experimental method to induce the disease. This is an important step in refinement. Dogs with naturally occurring drug-resistant epilepsy already exist, have few therapeutic options and many of them end up being euthanized. In the present study we aim to provide evidence of safety and efficacy of a novel disease modifying treatment that could help dogs and humans with epilepsy. So, there could be a direct benefit to dogs and their owners.

The cerebrospinal fluid collection and injection of Ant-134 will be performed under general anaesthesia, with advanced monitoring equipment used in veterinary clinical practice and by experienced veterinarians. All dogs will remain in hospital until the attending veterinarian deems it is safe for them to be discharged. Pain killers will be administered after the procedure.

All other procedures (blood sampling, urine sampling, sedation, electroencephalogram, and behavioural testing) are minimally invasive and routinely performed in epileptic dogs to monitor and adjust routine antiepileptic treatment, so these dogs are used to have them done.

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

The aim of this study is to demonstrate safety and efficacy in an animal model with naturally occurring epilepsy. We must use dogs as our aim is to find a new therapy that can help epileptic dogs and provide supporting evidence for phase I human clinical trials.

There are multiple advantages of using epileptic dogs as a comparative model in the development of new therapies: naturally occurring epilepsy is frequent, they share a similar lifestyle to humans, they have more complex brains than rodents, they undergo medical investigations like humans, and they are treated with other anti-epileptic drugs as humans.

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

Animal suffering will be minimized by using experienced veterinarians and veterinary nurses for all procedures. In addition, Ant-134 has been trialled in multiple rodent models with no significant adverse reactions reported. We will use the same protocols that we follow when performing these procedures in veterinary clinical practice. This includes pain management during the procedure and post-procedure. We also have protocols for continued monitoring and scoring of pain, vitals, patient level of awarness and seizures.

Dogs will be discharged to the owners care and have a normal life as soon as judge safe by a veterinary surgeon. This will give them the best welfare conditions and they will just come back for assessments at specific time points. In case of any concerns, they will have access to 24 hours emergency care at the hospital. Before any dog is included in the study, all owners will have to sign a consent form, explaining the risks and potential complications for all the tests and procedure.

A safe starting dose will be determined for each dog according to their weight following published recommendations. We will treat the first 3 dogs with a lower dose to reduce the chances of significant adverse reactions and monitor them closely before using the starting dose.

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

We have followed published best practice as laid out in PREPARE (published by Norecopa, a Norwegian platform for the advancement of the 3Rs) and will follow ARRIVE (published by the UK's NC3Rs) guidelines when reporting any results to the scientific community or other groups (e.g. epilepsy groups). We also used human clinical trial guidelines.

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

I will attend local AWERB meetings and review online resources such as NC3Rs and Norecopa.
A retrospective assessment of refinement will be due by 07 August 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?

18. Studying ageing-related processes and associated blood cancers in turquoise killifish

Project duration

5 years 0 months

Project purpose

- Basic research

Key words

ageing, stem cells, killifish, cancer, ageing-associated disease

Animal types	Life stages
Nothobranchius furzeri	adult, juvenile, embryo, neonate, aged

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?

We want to investigate the complex, multi-factorial relationships between ageing and blood cancers in the fastest-ageing vertebrate model amenable to laboratory usage, the turquoise killifish. To this end, we will generate genetic killifish models to study ageing and leukaemia, characterize the age-related changes in blood-related cells, and test ageing-delaying treatments or extracellular microenvironments for their effects on preventing or delaying the development of leukaemia.

A retrospective assessment of these aims will be due by 22 September 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 it's 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?

Ageing remains a fundamental, unresolved question in biology whose current, real-world importance stems from the ongoing changes in the demography of human populations. The proportion of older people is increasing at an alarming rate. Since age is the main risk factor for the predominant killer and debilitating diseases including cancer, cardiovascular disease and neurodegeneration, this demographic shift is incurring rising personal, medical, social and economic costs that need to be urgently addressed. Age is by far the biggest risk factor for cancer, but the relationship between carcinogenesis and ageing is poorly understood and an often overlooked aspect in oncology. The occurrence of most types of cancers strongly increases in older individuals.

The study of specific disease mechanisms has long been the focus of cancer research. But there is a growing realization of the importance to also studying the normal ageing process itself as a vital part of the problem, and exploring ways to slow its effects. A better understanding of the link between old age and complex diseases, such as cancer, will help us to delay the onset of disease and extend the healthy lifespan. Experimental examination and manipulation of ageing-related processes can only be done in animal models. There is a need for a vertebrate model that allows us to study cancer-related processes during ageing within manageable timeframes.

The short-lived turquoise killifish is such a model: it allows for experimental manipulation of ageing and provides exciting potential for substantial advancements in our understanding of associated diseases. Specific objectives are to generate genetic killifish lines to study ageing, to characterize age-related changes in blood cells and the effects of ageing-delaying treatments on the development of cancer, and to test the roles of an ageing extracellular microenvironment on cancer development. These experiments will allow us to study specific effects of altered ageing and lifespan on the development of blood-related cells and cancers emerging from these cells. The project will thus provide unique insights into the complex, poorly understood links between ageing and carcinogenesis. By developing a new experimental paradigm that makes the study of ageing and cancer feasible within realistic timeframes, this project will provide a valuable basis for future analyses of disease-related processes as a function of age. Ultimately, this research may discover novel, unique strategies for disease prevention or treatment.

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

This project will provide much-needed groundwork to develop and validate killifish as a new model animal to analyse the poorly understood link between ageing and complex diseases such as cancer. We will establish valuable tools and resources for future research with killifish, including the characterization of ageing-associated changes in blood cells as well as genetically modified fish lines to investigate processes relevant for blood cancer and other ageing-related processes. The new information gained from this research will provide unique insights into the contribution of ageing- associated factors, and treatments that delay ageing, in the development of disease. This research may inform on possible measures to extend a healthy lifespan not only in killifish but also in humans. We will publish this information in peer-reviewed scientific journals.

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

This project is fundamental research by its nature, and the immediate impacts from our work relate to scientific and knowledge advancement and the development of skills and capability. Beneficiaries will be scientists interested in adopting the killifish model system or in testing ageing-related factors in other organisms, including humans. They will benefit from our analyses and characterization of disease in this new model fish as well as from the genetically modified fish lines that enable us to study the development of blood cancers during ageing. Our research will also provide valuable clues about possible treatments that delay ageing and the onset of ageing-associated diseases. For example, we will screen for small molecules/drugs to alleviate both ageing and disease phenotypes. In the longer term, beyond the term of this PPL, the outcomes and eventual applications of this research may benefit the wider public, most notably older patients with ageing-associated diseases and their carers. Our research may also benefit the private sector who could recruit highly skilled scientists trained through this project and with experience in a new animal model system. In the longer term, after completion of this project, the private and medical sectors may benefit from the experimental data and resources which we will make readily available. They might also benefit by exploiting fresh drugs or drug targets emerging from our analyses of ageing-associated processes and pathways.

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

We will freely share the new insights and resources with the killifish community and beyond by actively participating in relevant scientific meetings (e.g., bi-annual international killifish symposium; conferences on ageing-associated diseases) and by publishing new findings in open-access scientific journals (e.g., eLife, Open Biology, PLoS Medicine). We will also disseminate and publish negative results because these can be particularly helpful for the young killifish community to prevent duplication of unproductive efforts. We will use our available contacts should the research lead to opportunities for exploitation and application, e.g. for potential new drug targets and patents. Meetings with clinicians and other stakeholders will identify outputs with medical potential and possible partners. We will also seize opportunities to attend workshops and forums to foster links between entrepreneurs and scientists. We have already established contacts to help translate basic findings into medical applications. The most immediate outcome with respect to impact beyond academia will be in public engagement, which we recognize as an important responsibility of scientists and which requires communication and learning in both directions. We have experience and established links that will facilitate outreach and effective communication of our research outputs. For example, we have manned a stand at the Royal Society Summer Science Exhibition on ‘Healthy Ageing’ and have hosted eight pupils for the in2science UK scheme, which provides opportunities to work alongside practising scientists for students from disadvantaged backgrounds, completing Science AS levels in deprived schools.

Species and numbers of animals expected to be used

- Other fish: No answer provided

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.

Turquoise killifish live only for 4-6 months and show many similarities to human ageing. This exceptionally short-lived fish allows us to study the relationship between ageing and associated diseases, such as cancer, within a reasonable timeframe and without the need to maintain animals for several years until they are old. Accordingly, much of our research will focus on older fish, although other life stages, such as embryos or juveniles, will also be used where possible (e.g. for drug screens or to study diapause as an ageing model).

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

We will genetically modify some killifish with specific mutations that imitate disease in humans. We will examine and collect small blood samples from fish under anaesthesia to test for ageing-related blood disease processes. We will also treat some animals with different drugs or diets which are suspected to prolong lifespan and delay the development of ageing-associated diseases.

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

Some of the proposed procedures may lead to discomfort in fish owing to ageing- or disease-related effects, which may include slow swimming, weight loss, and tumour development.

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 great majority of the proposed procedures are expected to have at most mild to moderate effects on the fish, while some procedures might have severe effects on a small proportion of fish. Overall, we expect that around 30% of the fish will encounter a modest adverse effect over the course of their lives, with less than 1% encountering severe suffering.

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 22 September 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?

Ageing and associated late-onset diseases are complex, multi-factorial processes, and their relationship to each other requires the study of whole live animals. We want to develop models that allow us to investigate the development and biology of ageing-associated diseases in killifish. We will use these models to establish treatments (drugs, diet) that can prevent or delay the onset of diseases such as blood cancers. Killifish are essential to this work because they allow us to analyze blood cells and their maturation, and to investigate ageing-related factors contributing to the development of cancer and other diseases.

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

We have consulted the FRAME and Altweb online resources to assess potential alternative strategies such as cell lines. There is no alternative to whole animal models to study systemic processes like ageing. However, we are experimenting with killifish embryonic cell lines and will expand this work to blood cell lines to complement and replace some experiments requiring adult fish. We are continuing to use yeast as a simple unicellular model organism to study basic principles of cellular ageing which are conserved in humans. Killifish embryos can enter a lengthy dormant stage, called diapause, where ageing is entirely suspended. This stage could be highly informative to study biological processes important to maintain health. We, therefore, focus much of our work on these dormant embryos before the onset of independent feeding, which partially replaces the need to work with adult fish.

We are currently experimenting with killifish cultured embryonic cells. We will build on this work by establishing killifish blood cell lines to possibly reduce some experiments requiring fish. We will also attempt to reduce animal numbers by employing transplantation methods, instead of genetically modified fish, to test the effects of different treatments on disease.

Why were they not suitable?

Less sentient animals, such as yeast, worms and flies, lack the organ structures, immune system and adult stem cell populations that are prone to disease development in humans. While a variety of non- animal experimental systems exist for the study of cancer, there is no substitute for animal models that can recapitulate the complex changes in physiology, metabolism and immunity that define ageing.

Cultured cells or tissues are unsuitable for this study because of insufficient material (primary cells) or the presence of many mutations (cell lines) which leads to unreliable results. Most importantly, the cultured cells do not allow the study of the complex and inter-dependent ageing-related processes occurring in whole organisms.

This project could also not be carried out in patients with blood cancers of different ages because the inevitable variations in genetics and environment would confound any insights, besides the ethical impracticability to study the development of cancer as a function of age in humans.

A retrospective assessment of replacement will be due by 22 September 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 estimated the required number of killifish based on 1) our current experience of breeding animals required to maintain a killifish line for this short-lived species; 2) knowledge of efficiency for genetic modification from published literature and talking to killifish collaborators; 3) the four genetically modified lines we propose to generate; and 4) the number of animals needed to establish statistical significance for the quantitative experiments assuming moderate to strong effects, taking into account the need for similar experiments with zebrafish.

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

To minimise the number of fish required throughout the project, all-female fish (and most males) will be used for breeding, and all-male fish (and most females) will be used for experiments. Moreover, killifish embryos can also be stored in diapause for over one year, until required for experiments; we will take advantage of this handy feature to substantially reduce the number of fish needed at any given time. For quantitative experiments (effects of drugs, diet, and transplantations), we will only consider medium- or high-level effects, e.g., a larger than 10% increase in median lifespan extension or larger than 10% decrease in the onset of disease. Limitation to such large effect sizes will substantially reduce the number of animals required to establish an effect. Statistical advice is being provided by a local biomedical statistician.

Killifish produce fewer embryos per female than zebrafish. Moreover, killifish need more space per fish than zebrafish, with adult males requiring a separate tank each. Thus, the facility costs per fish are much higher than for zebrafish. So economic considerations provide an additional incentive, besides animal welfare, to minimize the number and maximise the use of fish. Typically, researchers working with killifish, therefore, keep much smaller animal numbers than for zebrafish.

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

We are currently experimenting with cultured embryonic killifish cells. We will build on this work by establishing killifish blood cell lines to possibly reduce some experiments requiring fish. We will also attempt to reduce animal numbers by employing transplantation methods, instead of genetically modified fish, to test the effects of different treatments on

cancer. In this way, we may further reduce the need to generate and maintain genetically modified fish that may or may not develop cancer, which renders it more difficult to assess the effects of drugs or diet.

With our local killifish collaborator, we will mutually make killifish available to each other, which helps to reduce the numbers of fish used by increasing flexibility and economy of scale. Substantial efforts are invested in maintaining all adult stocks in peak breeding condition, which is especially important for this short-lived species. This ensures that we can keep the minimum number of fish required for embryo production. Naturally, all fish lines will be well documented on a database with monthly assessment and quarterly stocktaking performed by all personal licence holders. Together, these procedures will help to ensure that we only maintain those fish required for our immediate experimental research. In addition, some projects may be more active than others at certain times, and we will regularly assess the need for maintaining lines and where possible store embryos that are not actively used.

Killifish provides a more rapid model in which to interrogate the evolution of blood diseases associated with old age, and the biological effects of ageing on the stem cells that give rise to cancer. Although further development of the emerging killifish model system will require animal use, we anticipate that moving specific age-related cancer studies to killifish will reduce the overall numbers of animals (zebrafish or mice) used in ageing research by reducing the need to maintain zebrafish or mice for years in order to study ageing.

A retrospective assessment of reduction will be due by 22 September 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.

We will use the turquoise killifish as a unique model to study ageing and associated diseases. This killifish ages rapidly and only lives for 4-6 months, allowing us to follow the effects of disease and possible treatments with minimally invasive protocols. Killifish embryos are transparent during development, permitting us to visualize effects on blood development prior to the onset of hatching. Moreover, killifish embryos feature a dormant stage (diapause) during which ageing is suspended; this state will allow us to study processes relevant to ageing in embryos without the need for hatching. In order to study the development of disease, we will generate specific modified fish lines. These fish lines will allow us to effectively study ageing-associated diseases, and their prevention by drugs or diet, without the need to raise a huge number of fish to look for the few that naturally

might spontaneously develop disease. Animals will be treated with different factors with the aim to prolong healthy life and delay the onset of disease, so we are not interested in studying any external factors that cause suffering. For all experiments, we will ensure that any harmful effects on fish are minimized by strictly following the humane endpoints as defined in the protocols to prevent any strong distress, pain, harm, or other sufferings.

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

Less sentient animals, such as yeast, worms or flies, lack the organ structures and adult stem cell populations that are prone to cancer development in humans. Moreover, these animals do not feature all the complex, multi-factorial processes that determine ageing and associated late-onset diseases in fish and mammals. To study the effect of ageing on disease, we must investigate old animals.

Killifish are less sentient animals than mice and age much faster, and individuals only need to be maintained for months rather than years. While in some instances mice are preferred because they more closely model human disease, this is not the case for blood diseases or neurodegeneration, for which fish provide ideal models that closely reflect the diseases seen in humans. The establishment and validation of human disease models in killifish will continue to reduce the number of mammalian models needed to validate biological findings or drugs.

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

Harm to animals will be minimized by ensuring, where possible, that embryos prior to hatching will be analyzed (e.g. to study processes related to diapause) without the need to proceed to hatching. Any procedures that may result in suffering will be performed under anaesthesia. Where possible, we will treat embryos, rather than free-swimming fish, with drugs to look for beneficial effects on health later in life. This will allow us to limit any toxicity and severity of drugs by completing the exposure prior to hatching, thus minimizing potential suffering. Furthermore, we will check embryos for any toxic effects during development and ensure that these embryos are sacrificed before hatching. Where available, we will cross-reference records of drugs that have been documented to be toxic to zebrafish embryos and omit the use of these drugs for killifish. For drugs, we will carefully establish and apply the minimal doses required for a biological effect but preventing any harmful side effects.

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

We will follow the ARRIVE guidelines for the design, analysis and reporting of research using animals, which provide a helpful checklist for issues that need to be considered. These guidelines have recently been revised. We will also follow the PREPARE guidelines which provide further considerations regarding harm-benefit assessment, quarantine and health monitoring, and the use of humane endpoints, among others.

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

Besides papers and scientific meetings on animal research, the NC3Rs website is a valuable and rich source of information (https://www.nc3rs.org.uk/). Moreover, a new

NC3Rs officer is now associated with our university and will be on-site for advice two days per week.

A retrospective assessment of refinement will be due by 22 September 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?

19. Clinical veterinary studies of naturally occurring disease in animals (III)

Project duration

5 years 0 months

Project purpose

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

Key words

clinical trial, veterinary, spontaneous disease

Animal types	Life stages
Other dogs -Doberman	adult

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.

- Uses cats, dogs or equidae

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?

To permit the ethical conduct of phase II to IV clinical trials of new drugs, devices and techniques in client owned animals attending a veterinary setting.

A retrospective assessment of these aims will be due by 06 November 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 it's 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?

The benefit of this project licence is rapid progress of the development of novel therapies/techniques in the management of disease in client owned animals. There are a wide range of unmet veterinary needs, requiring new drugs/devices to be developed as approved veterinary medicinal products and new diagnostic techniques. This licence seeks to improve the treatment of spontaneous disease in client owned animals.

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

The primary data outputs will be clinical, clinicopathological, histopathological, diagnostic imaging data to support or refute development and use of new drugs, devices or diagnostic techniques for the management of spontaneously occurring disease in client owned animals. Data will relate primarily to clinical efficacy, safety and underlying biological mechanisms and will:

enable an objective decision to be made regarding whether or not to progress a novel therapy or technique through further stages of product development

provide an understanding of any potential adverse effects and allow for appropriate contraindications and precautions in any subsequent clinical trials

increase the number of novel safe and effective treatments and diagnostic tests available for a range of conditions affecting companion animals.

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

The benefit of this Project Licence is rapid progress of the development of novel therapies/techniques in the management of disease in client owned animals attending a veterinary setting. There are a wide range of unmet veterinary needs, requiring new drugs/devices to be developed as approved veterinary medicinal products and new diagnostic techniques. This Licence seeks to improve the treatment of spontaneous disease in animals.

Individual animals recruited to the studies will have their disease well defined and throughout the studies their progress will be closely monitored, resulting in a direct benefit to every animal recruited.

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

Data will be reported and published for use by:

Pharmaceutical companies/Sponsors to progress development of novel therapies/techniques including licencing for new indications,

Veterinary surgeons to promote evidence-based medicine in clinical practice
Additional benefits include the collection and storage of blood, urine and tissue samples for animals with specific diseases. Analysis of the samples could lead to the identification of potential new biomarkers of disease and facilitate genomic and metabolomic profiling for mechanisms of disease and identification of new therapeutic targets. These secondary

benefits will be used by veterinary researchers to further our understanding and treatment of veterinary diseases.

Species and numbers of animals expected to be used

- Other dogs: No answer provided

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.

Client-owned animals used in these studies will be selected based on the spontaneous disease being targeted for treatment. Age, sex / breed will be set to reduce variability between animals and ensure the level of disease is standardized where possible across the study.

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

Client-owned animals that have been referred to the veterinary clinic with appropriate spontaneous naturally occurring disease will be enrolled on to studies. The owner will sign an animal consent form to allow their pet to be assessed for enrolment on the study.

The animal will generally experience normal veterinary work-up and interventions for the treatment of their disease. Then a new therapy, supplement or device may be administered in conjunction with the gold standard treatment, with the aim of improving animal recovery/ outcomes. Only those studies approved by the Veterinary Medicines Directorate will allow a new therapeutic treatment to replace current licensed therapies.

Typically, the regulated procedures performed under this PPL will be the extra sampling required to show efficacy of the product or device being tested eg a series of blood samples collected for monitoring drug levels or a follow-up imaging session performed under general anaesthesia to assess the effectiveness of a new product.

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

The impacts of the regulated procedures on these client-owned animals are typically expected to be mild and transient when performing blood sampling or fluid sampling procedures only.

Those protocols involving injection of novel substances (with or without general anaesthesia), biopsy procedures or imaging are classed as moderate. Animals will be monitored closely by veterinary professionals during recovery and are only discharged from veterinary care when they are fully recovered from the procedure and any anaesthesia. Possible, but unexpected, adverse effects include; anaesthesia recovery complications, allergic response to injection, pain or infection which will most likely be observed and treated before the animal is discharged from the veterinary clinic.

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)?

All animals completing a study should experience the same severity based on the sampling protocols and expected adverse events under this PPL we would expect the following:

Dogs >90% Mild <10% Moderate

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

- Rehomed

A retrospective assessment of these predicted harms will be due by 06 November 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 primary purpose of this Licence is to improve the health of client-owned animals with spontaneously occurring diseases. Effectiveness of a new treatments require robust testing under controlled conditions in the target animal showing symptoms of the relevant disease prior to acceptance/ approval as a new veterinary treatment.

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

Effectiveness of a new treatments require robust testing under controlled conditions in the target animal showing symptoms of the relevant disease prior to acceptance/ approval as a new veterinary treatment. Whilst non-animal alternatives may be used during product development this cannot replace the use of animals for efficacy testing in the target animal.

Why were they not suitable?

There is a need to perform controlled veterinary clinical trials for novel therapies / techniques for treatment of animals in the target animal.

A retrospective assessment of replacement will be due by 06 November 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?

Each study is individually assessed and predicted animal numbers indicated on the relevant project protocol.

Review of historic veterinary clinical data can predict outcomes of spontaneous disease standard therapies when planning new studies.

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

All clinical efficacy studies will be assessed by a statistician to ensure the minimum number of animals based on standard power analysis with Bayesian considerations and will make use of bias avoidance methods, adaptive study design and interim analyses to facilitate sound decisions on whether to stop or continue treatments or terminate the study. Cross-over designs will be considered to reduce total number of animals required with a suitable wash-out period.

These studies are uniformly looking for marked or “clinically significant” effects: the number of animals required to show a large effect are smaller than if the study end-points were more subtle.

Variability will be reduced by studying animals with specific disease and excluding animals with co- morbidities where possible.

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

Review of historic veterinary clinical cases will be used to predict outcomes of spontaneous disease with standard therapies when planning each study.

Ensuring a good study plan and data capture forms are prepared will ensure optimal data is gathered for each animal to contribute to the study.

Some blood and tissue archives are available as a resource for researchers.

A retrospective assessment of reduction will be due by 06 November 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.

Client owned animals with defined specific spontaneous diseases will be used in these studies with the primary aim of conducting clinical trials of drugs/devices/techniques of these disease in these species. An alternative is therefore not an option.

A thorough review of all known adverse events of all drugs/devices/techniques to be tested will be conducted prior to commencement of studies to facilitate specific close monitoring for any expected adverse events.

If the specific target disease progresses despite study procedures, or other co-morbidities occur that are likely to compromise the study, then animals may be withdrawn from ASPA and returned to the care of the Owner and Veterinary Surgeon so that an appropriate treatment plan can be agreed under the Veterinary Surgeons Act.

All animals will remain under the care of their owners while enrolled on the study: their emotional and welfare needs will be taken care of in their own home. Regulated procedures will take place at the Veterinary Clinic to the highest standards of welfare and patient care and supported by 24/7 dedicated veterinary professional support.

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

There is a need to perform clinical trials of novel treatments / techniques in animals of the same status for which the product/ techniques is intended.

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

This PPL is performed within a world class veterinary teaching hospital where veterinary and veterinary technical support staff are required to keep up to date with CPD to advance their knowledge. Advice will be sought from these people when designing the study plan.

Any relevant post procedure care and monitoring will be performed by veterinary professionals until the animal is deemed fit to be released from the Act by the attending veterinary surgeon.

Where appropriate sedation, and/or local or general anaesthesia will be used to minimise stress or pain during procedures.

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

Veterinary surgeons and veterinary nurses involved in these studies are all required to perform regular CPD to maintain the highest standards to maintain their professional registration.

Best veterinary practice will be employed at all times.

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

NC3R website will be used as a source of information of advances in 3Rs, as well as review of the regular updates received from the designated establishment. Any advance considered appropriate in this PPL will be incorporated into the in-vivo experiments where possible.

Regular updates from the NC3R website are circulated by the establishment's AWERB.

A retrospective assessment of refinement will be due by 06 November 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?

20. Schistosomiasis life cycles to provide Schistosoma samples as a biomedical resource

Project duration

5 years 0 months

Project purpose

- Basic research

Key words

Schistosomiasis, bilharzia, life cycle, resource

Animal types	Life stages
Mice	adult
Hamsters (Syrian) (Mesocricetus auratus)	adult

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?

To generate *Schistosoma* material by maintaining the life cycles of authentic biomedically and veterinary important *Schistosoma* species as a resource for the global schistosomiasis research community. A centralised resource will reduce the requirement for other researchers to set up/maintain *Schistosoma* life cycles, including the mammalian stages, and ultimately reduce the overall numbers of small rodents that are used for schistosomiasis research.

A retrospective assessment of these aims will be due by 10 September 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 it's 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?

Schistosomiasis (bilharzia) is a parasitic disease affecting over 200 million people globally, most in sub-Saharan Africa with impoverished communities most at risk. Additionally, domestic livestock and wildlife are burdened by the disease resulting in economic and biodiversity loss, with the true impact large unknown. In terms of parasitic diseases it is second only to Malaria in terms of its human health impact.

Schistosomiasis, is caused by schistosomes – flatworms – transmitted by freshwater snails, with adults worms living in the blood vessels surrounding the intestines or urogenital tract. The disease, caused by egg depositions and/or migration in/through tissues and organs, results in chronic ill-health and death, and is especially prevalent among children in rural communities. There are no vaccines to prevent infection and only a single drug, praziquantel, is available for treatment, with the development of drug resistance an increasing concern. Additionally, a lack of knowledge surrounding the biology of the parasites, together with inadequate diagnostics to detect infection continue to hamper control and elimination efforts in many endemic countries. The limited accessibility and diversity of live material, maintained in laboratories, severely hampers research advancements towards understanding, controlling and potentially eliminating these parasites together with their impact on human and animal health.

This project, is to create a centralised biological resource, for the global research communities, of live Schistosoma material to initiate, enable, support and promote fundamental research on schistosomiasis, ultimately reducing / stopping the disease burden in endemic countries. A unique feature of this resource is the provision of not only generic Schistosoma strains (that have either been maintained in the laboratory for decades, that do not originate from sub-Saharan Africa, and that do not represent the endemic natural diversity of the parasite), but also a diverse species/strain range with more authentic endemic origins. Our project will generate and provide access to all Schistosoma life cycle stages, through this central biomedical resource, to the global research community to enable and enhance priority schistosomiasis research. This biomedical resource is funded through the Wellcome Trust and has received 39 letters of support, from the research communities detailing the need and the research opportunities that this resource will provide. By centralising such a resource this project will not only provide unique material that is quality controlled but also reduce the use and animals in multiple sites. Additionally, a key component of this project will be to develop methodologies for in vitro culturing methodologies with an aim to reduce refine and possibly replace the use of animal for future research.

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

Knowledge generation. The project will generate new information related to biomedical and veterinary important Schistosoma species, their life-history traits and also the ways of maintaining them in laboratory passage. We will also generate new information related to methods for cryopreservation, in vitro culturing of life cycle stages and other methods to reduce the numbers of animals needed to keep the life cycles going. Indirectly, through the users of the resource, the project will generate research related information. This will

range from research on therapeutics and vaccine targets, genetics and genomics, diagnostics, host-parasites interactions and parasite life-history traits.

Publications. We expect to publish protocols and SOP's as an open access resource. Directly related to the project we will publish peer-reviewed publications related to life cycle maintenance and the methodologies related to reduction, refinement and reduction for the use of animals. Indirectly related to the project will be peer-reviewed publications from users of the resource that will relate to specific research areas involved in understanding, controlling and treating schistosomiasis.

Products. We will have established the life cycles of key Schistosoma species and strains not available from any other resource. We will have optimised and developed methods and best practices for the reduction of the use of small rodents in schistosomiasis experimental research. We will have generated previously unavailable "bespoke" material from the various stages of the parasite life cycle for biomedical research. Specifically, the project will generate material (antigen) for use in the diagnosis of schistosomiasis in returning travellers and tourists.

Research. We will have supported key research areas including; improved understanding of Schistosoma biology, infection and transmission; vaccine and therapeutic candidates; diagnostic biomarkers and assays (human, animal and snail).

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

Short term

Provision of parasite material, enabling further experimental research that needs access to live material and or specific species and strains (snails and schistosomes.

Characterisation of the Schistosome life cycle and optimisation of protocols to ensure predictable outputs of each life cycle stage

Cercariae will be cryopreserved allowing them to remain viable without the animal infection. This will extend the life cycle timepoints and so reducing the number of animals in the long term. This will also allow the life cycle to be stopped and restarted as needed.

Eggs will be maintained in culture medium for several days post extraction from the animals. This will extend the life cycle timepoints and so reducing the number of animals in the long term.

Mid-term

Characterisation of new (field) species of *Schistosoma* which will help researchers to understand the biology of this important parasite and elucidate human host-parasite relationships.

Provide a reliable and standardised resource for the research community in order to support Schistosome research. It is hoped that more research can be facilitated if there is a known source of live material.

Adult worms will be kept in *in vitro* systems to keep them alive without the need for the animal host. However, no current methods have created adults capable of producing

viable eggs *in vitro*, but this is will be a key research area of this project with the potential to cut out or reduce the animal use.

Long term

The replacement or considerable reduction of the use of animals in experimental schistosomiasis research.

Methodologies, scientific knowledge and therapeutics (diagnostics, treatment and vaccines) that will be able to be used to control and potential eliminate schistosomiasis as a public health problem in the most marginalised communities living in endemic zones.

We will perform pilot experiments on transplantation of sporocysts between snails. If successful, this will allow maintenance of the schistosome isolates within the snail host for much longer periods of time. This will extend the life cycle timepoints and so reducing the number of animals in the long term. This will also allow the life cycle to be stopped and restarted as needed.

All these measures aim to maximise the time that the parasite is maintained outside of the mammalian host which will ultimately reduce the number of passages required through the mammalian host.

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

Collaborators and Resource users. This project's main objective is to generate an Open Access biomedical resource. Interactions with collaborators and users forms the major part of the work and provides the opportunity for knowledge exchange and the generation of new research questions and methodologies. We expect the outputs from this project to reach the global schistosomiasis community. The outputs generated as part of the proposed research will be managed and where appropriate shared openly with the wider research community.

Samples and Collection Data. Outputs will come in the form of samples and materials from this project, associated data and publications/reports. This will be available to interested parties, subject to appropriate GDPR requirements.

Genetic Data. Genetic data, arising from the samples (schistosomes and snails), will be accessioned within and made readily available through Open Access online databases NCBI GenBank (http://NCBI.nlm.nih.gov), WormBase (https://wormbase.org/), VectorBase (https://www.vectorbase.org). All parasite and vector genetic data will be made freely available upon publication or/immediately if publication is not intended.

Publications and reports. All publications arising from this research will be published in open access journals and will also be made freely available through online research links such as Research Gate and through personal web pages. Prior to peer review, research protocols, datasets and collections will be published on the Wellcome Open Research platform (https://wellcomeopenresearch.org/), enabling immediate dissemination of the outputs to the wider research community. All publications will be available via appropriate OpenAccess publication repositories.

Policy. The group is linked to policy makers and influential organisations e.g. through the World Health Organisation (WHO) and Global Schistosomiasis Alliance (GSA). A project partner is in the process of applying for renewal of its WHO collaborating centre status,

and members of the team are involved in GSA working groups. Relevant outputs will be disseminated through these, and other relevant channels.

Public Engagement. This application will provide further opportunities for cross institutional public engagement activities. Public engagement will also be fulfilled by updated institutional and personal webpages.

Species and numbers of animals expected to be used

- Mice: 725
- Hamsters (Syrian) (Mesocricetus auratus): 205

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.

Small rodents have been shown to be a good hosts for *Schistosoma*. Other animals have been tested in the past including gerbils, rats, rabbits and primates, however mice and hamsters are the most efficient host in an experimental system. Mice will be used for the majority of the species we aim to maintain as they are the best host with limited harms when appropriate parasite doses or patency times are adhered too. Hamsters are needed to propagate the species *S. haematobium* as this species is not highly compatible with mice. Hamsters have been shown to have minimal related adverse effects with these infections.

It is important to point out that schistosomiasis is a chronic disease with pathology and related adverse effects seen over time. The longer the persistence of infection the greater the immune response to the presence of eggs and the more pathology observed.

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

Rodent Infections. Anaesthetised animals will be infected with a known number of larval schistosome parasites (cercariae) via natural skin penetration which is pain free. Exposure time is for up to 40 mins.

Schistosome Maturation. The Schistosoma larval stages circulate in the blood system and over time develop into adult worms (~5-10 mm long) which copulate, mature and reside in the mesenteric veins of the intestine and the hepatic portal vein. Once mature (see Table 1) they produce eggs which are either eliminated via the animal's faeces or are deposited in the intestinal wall, liver and spleen. The pathology of infection is related to egg deposition in the tissues and organs and this can be predicted by infecting with known number of parasites and timing of adult worm maturation.

Collection of parasite material *post mortem*. Parasite material (adult worms and eggs) will be collected post mortem. These will be collected as a biomedical resource for schistosomiasis research. Extracted eggs will be hatched into miracidia and these will be used to carry on the life cycle. Adult worms will be retrieved, at post mortem, by perfusion and dissection. Eggs will be harvested from the liver tissue post mortem. Adult worms will either be retrieved prior to patency (see Table 1) or 2-4 weeks post patency. Eggs will be

harvested 2-4 weeks after patency which is dependent on the number of Schistosoma cercariae used in the infection and on the species involved.

Collection of other samples and other procedures. Occasionally during the infection and / or at post mortem blood samples will be collected for research purposes. This will be done via the tail. Additionally, animals may be treated with the approved anti-schistosomiasis drug, Praziquantel, via oral gavage for test for drug efficacy in specific parasite lines. This is the same drug that is used for human infections.

Table 1. Individual Schistosoma species pre-patent periods – time from infection to adult worm maturation which leads to the onset of egg laying. The Schistosoma eggs are the cause of any adverse effects. No adverse effects are related to the adult worms [1]

Schistosoma species	Pre-patent period (weeks)	Rodent Host
S. mansoni	5	Mouse
S. haematobium	8	Hamster
S. guineensis	6	Mouse
S. mattheei	6	Mouse
S. bovis	6	Mouse
S. curassoni	6	Mouse
S. margrebowiei	5	Mouse
S. rodhaini	4	Mouse

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

During the pre-patent infection stages (before adult worm maturation, pairing and egg laying), see table 1 above and table 2 below, mice and hamsters can harbour heavy infections with no observable signs of pathology or disease. When infections proceed beyond patency (see Table 1),eggs are produced by the mature adult worms and either pass through the gut wall causing a degree of haemorrhaging, or are trapped in the gut or liver causing granulomatous inflammation and fibrosis. Pathology is directly related to the number of eggs passing through or being deposited in the tissues. This is directly related to the number of adult worm pairs and the time post patency. For example the higher the number of adult worm pairs and the longer the infection duration post patency the more pathology is observed.
Following heavy, prolonged infections lethargy, hunching, piloerection, anaemia, (bloody) diarrhoea, dehydration and/or coma maybe observed. At the onset of such adverse effects animals will be euthanised and the parasite material harvested post mortem. Infection intensities will be controlled so as to minimise any adverse effects. This is will done using controlled infections, using known numbers of male and female cercariae, together with set time points beyond patency that animals will be euthanised.

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)?

Severity will be minimised with animals euthanised before the onset of adverse affects and any clinical signs (lethargy, hunching and piloerection) will be acted on within 24 hours of observance. When using established strains (Protocol 1) we do not expect moderate signs in more than 20% of infected animals. When establishing new *Schistosoma* strains (Protocol 2), where exact pre-patency timelines may vary or need to be established, or related pathology is unknown, a few infected animals may show signs of infection such as hunching or lethargy. We do not expect these moderate signs in more than 50% of infected mice (or 1% of infected hamsters). These events will be acted upon within 24 hours. Mice will not be kept longer than 10 weeks post infection and hamsters 12 weeks – to mitigate the onset of potentially more severe side effects due to the build up of eggs in the tissues.

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

- Killed

A retrospective assessment of these predicted harms will be due by 10 September 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?

Schistosomes are obligate parasites, using mammals (including humans) as their definitive host, where sexual reproduction occurs, and snails, where asexual amplification of the infective larval stage occurs. The parasite has two non-feeding, free-swimming larval stages that infect snails (miracidia) and mammals (cercariae). Animals (small rodents) are needed as surrogate definitive hosts to allow development of the adult stage of the parasite and production of eggs so that the lifecycle, for experimental research and antigen production, can be maintained in the laboratory. No successful *in vitro* methods exist for the completion of the life cycle. The mammalian host environment is currently vital for adult worm maturation, egg laying and viable egg excretion.

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

We have considered *in vitro* culture of adult schistosomes.

Why were they not suitable?

Considerable progress on schistosome *in vitro* culture has been made since the 1980s, largely based on the work of Basch [2]. However, these methods have not delivered a culture protocol that supports full sexual maturation of schistosomes and delivery of viable eggs to continue the lifecycle [3]. This is a developing area, and a new experimental protocol for *Schistosoma japonicum* shows promise in delivering viable eggs, albeit at

reduced output [4], but we are not yet at a stage where we have reliable and consistent methods for growth, maturation and reproduction of schistosomes sufficient for lifecycle maintenance.

Testing of *in vitro* culture methods will be carried out as part of the programme funded through our resource grant. As a true replacement for animal use, in vitro culture of adult schistosomes begins from the cercaria larval stage shed from snails and will not require additional animal infections.

A retrospective assessment of replacement will be due by 10 September 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?

The numbers of animals to be used has been based on known patency data from previous life cycles. We will be able to anticipate usage based on requests from collaborators. Animal use will depend of resource demand by users.

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

We maximised the time between infections and patencies and used the minimum number of animals needs for adequate egg return.

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

Traditionally *Schistosoma* infections of laboratory animals have involved exposure to a known quantity of infective cercariae, however the sex of those cercariae are usually unknown (they can only be sexed using molecular methods). This can lead to sex biases in the animals and even animals being infected with only males or females. This eventually leads to a less efficient life cycle. Using sex markers [5, 6] for cercariae, we will infect animals with specific quantities of male and female cercariae to enable efficient use of the animals and a more productive life cycle. This reduces the animal numbers used and prevents wastage of animals that are non-productive due to sex biased or single sex infection.

Additionally, there is a trend towards better infection rates / survival of male cercariae in animal infections (male sex biases). To gain a more even mix we will pilot infection ratios (females : males), with a high ratio of females to males to gain an optimal number of worm

pairs that provides the best egg output, without causing adverse effects, to improve efficiency of the life cycle and further reductions in animal use. Worms that remain unpaired (males and females) do not produce eggs and so do not cause any adverse effects on the animals.

A retrospective assessment of reduction will be due by 10 September 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.

Mice are good hosts for most schistosome species, supporting the complete life cycle. For some species (e.g. *Schistosoma haematobium*), mice are unsuitable hosts and hamsters are used instead. There is no multiplication of adult parasite worms in the host and the pathology of infection is caused by parasite eggs as they become trapped in host tissues, particularly the liver. Therefore, pathology depends on the number of worms infecting the host and the duration of infection. To continue the life cycle, the host animal must be killed and eggs prepared from the liver, where the majority of them become trapped. This can be carried out after a minimum of 6-7 weeks post infection for *Schistosoma mansoni*, which allows sexual maturation of the parasites, and eggs to mature sufficiently to produce miracidia when exposed to fresh water.

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

Less sentient animals cannot be used to maintain the adult stages of the schistosome life cycle, as schistosomes are obligate parasites of mammals and will not infect non-mammals. It is not possible to maintain schistosomes in animals under terminal anaesthesia as the parasite matures for many weeks within the host, migrating through the circulatory system as it develops.

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

We are expecting to introduce new species and isolates of schistosomes, whose rate of development may vary somewhat from long-term lab-adapted strains. Clinical scoring sheets will be piloted to refine end points.

Laboratory-adapted strains tend to develop quickly because of the selection imposed by terminating the infection as early as possible to reduce severity. As eggs are prepared

from animals, we will record the condition of the liver and estimate the number of eggs/miracidia recovered compared with a long-term laboratory isolate so that end points can be matched by modifying the infection time or the number of cercariae used.

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

As well as peer-reviewed publications (in application Bibliography below) all experimental procedures will be conducted following local best practice SOPs based on LASA guidelines and include the NC3R decision tree.

PREPARE guidelines also assist in the planning of animal experiments and ARRIVE guidelines assist in the reporting of animal experiments. Conducting research under these conditions is a requirement for UK and international funding bodies as well as for peer reviewed publication.

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

An NC3Rs regional scientist is available for consultation. A Named Information Officer (NIO) informs all licensees of developments in Animal Scientific research, including studies on animal husbandry, new handling techniques and any information on advances in the 3Rs. Training is also undertaken by licensees, e.g. NC3Rs workshop "Improving the quality of research applications.", to keep abreast of best practice when undertaking research involving animals.

A retrospective assessment of refinement will be due by 10 September 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?

21. Mechanisms and therapies for neurological and neuromuscular diseases

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
    - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
- Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

Therapy, Antisense oligonucleotides, Neuromuscular, Drug delivery

Animal types	Life stages
Mice	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 understand the genetic and pathological mechanisms that are the basis for neurological and neuromuscular diseases and to develop therapies that address these causes.

A retrospective assessment of these aims will be due by 24 September 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 it's 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?

A cure or effective treatment has yet to be developed for many, often rare, neurological and neuromuscular diseases. Only by understanding the mechanisms of disease, often through the use of relevant animal models, can we understand and evaluate an effective strategy for their treatment. Development of an effective treatment also requires optimisation of drug delivery, especially for complex technologies that address the genetic causes of these diseases.

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

This program of work will contribute to the wider scientific knowledge of the mechanisms of disease in neurological and neuromuscular disorders and how we may seek to treat them. Specifically, we will publish these findings in recognised, peer-reviewed scientific journals and present these findings to scientific and patient-based conferences. We also intend that this work will lead to new intellectual property that would allow for the development of novel therapies for these diseases.

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

In the first two years of the program we plan to investigate the potential of a number of disease-relevant therapeutic drugs that are a continuation of previous work undertaken in our group. This work will evaluate the therapeutic applicability of these drugs that would hopefully lead to patient clinical trials in the longer term. Additionally, throughout the life of the program, we are working to improve and optimise existing drug modalities, or to address an unmet need, for neurological and neuromuscular disorders.

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

As one of the main focus of our work is to improve the delivery of drugs, we will work with collaborators to be able to apply the technology developed in this program, to their relevant disease of interest. This work will also be published widely in peer reviewed journals and presented at scientific meetings to disseminate the success and failures of this area of research.

Species and numbers of animals expected to be used

- Mice: 35,000

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.

In this project we will be using mice as our animal model. Primarily, wewill be using young mice from 1 weeks to 20 weeks of age as we will be using early drug treatment intervention to attempt to improve their functional outcome in specific mouse models of neuromuscular and neurological disease. We will use genetically altered mouse models that contain a mutation that recapitulates the related human disease phenotype so as to allow us to directly assess our drug therapies in a relevant model of disease pathology. As these human diseases have significantly reduce quality of life and often cause premature death, some of these mouse models will have a corresponding adverse clinical phenotype.

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

Mice in this study will be bred to contain a disease relevant mutation. These mice will then be injected either systemically or by direct injection to muscle or brain, with drugs to address the disease pathology, typically over a period of one to eight weeks. Following this treatment, we will assess the benefit of the drug through assessment of muscle and heart function, as well as other measures of clinical relevance such as markers of protein restoration and drug toxicity. A typical mouse will undergo between two to four procedures for each experiment.

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

As our focus is on neuromuscular diseases, mice will be bred that have a muscular dystrophic, myotonic, atrophic or neurological phenotype that will show disease progression as they age. Mice will not be kept past 15 months of age. Animals may also exhibit some lethargy and weight loss following drug treatment but this is typically transient and mice return to normal behaviour within an hour.

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)?

Mice: 25% Subthreshold, 50% Mild, 25% Moderate, Severe < 1%

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

- Killed

A retrospective assessment of these predicted harms will be due by 24 September 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 goal of our work is to develop new drugs for the treatment of currently untreatable neuromuscular diseases, and therefore we are required to understand drug mechanism of action and effectiveness in the context of the whole body system. Additionally, as we want to develop these novel drugs for patients, we need to use animal models to be able to assess and understand the safety profile of any new drug prior to consideration for patient clinical trials.

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

Cell culture systems, including cell model systems derived from patients, have an important role in understanding the mechanism of drug action which allows us to refine the design of new therapies and guide future drug development as well as providing early insight into safety.

Why were they not suitable?

While many drugs are found to be active and safe in cultured cell models, the complex nature of animal physiological systems, especially the nervous system and including structures such as the blood brain barrier between the circulating blood and the brain itself, complicates the delivery of the drugs to the appropriate target organs and cells. Therefore without the information resulting from well-designed animal experiments we would not be able to enhance the efficacy and understand the safety of new drug therapies, a critical requirement for any new drug to be brought to the clinic and ultimately to benefit patients. However it should be noted that we utilise cell culture systems for preliminary testing of novel drugs where possible, so as to ensure that any animal experiments are only performed on drugs that have been validated in vitro prior to use in animals.

A retrospective assessment of replacement will be due by 24 September 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?

The number of animals estimated to be used in this program of work is based on our extensive experience of drug development of similar compounds in previous programs of work. In principle, we perform pilot studies of novel drugs to understand the size of the

drug effect and use this information to design experiments to have sufficient animal numbers to get robust, statistically significant data that will tell us whether our drug is better or worse than comparator drugs in improving either biological or functional disease relevant outcomes.

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

For each new animal study, we use the NC3R's Experimental Design Assistant to guide us in the use of the minimum number of animals to demonstrate drug related benefit, as well as providing appropriate statistical analysis. We also conduct our experiments so as to comply with the ARRIVE guidelines for when we will publish our data in peer-reviewed scientific journals.

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

We follow best practice guidelines for breeding and colony management of our genetically altered colonies. For novel drug therapies, following validation in vitro prior, we perform pilot studies to determine the statistically appropriate number of mice needed to assess that drug, and apply that optimising the number of animals in use in the project.

A retrospective assessment of reduction will be due by 24 September 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.

In this project we will primarily use genetically altered mice which model relevant diseases both physically and biochemically. These models are necessary to allow us to test new therapies so as to hopefully benefit patients with these diseases. Where possible we administer these drugs throughout the whole body in as non-invasive manner as possible to reduce pain and suffering to animals. Similarly for measures of drug efficacy, we primarily utilise behavioural and functional tests that measure the animals natural behaviour and have been refined so as to not cause lasting harm.

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

Adult animals are needed to recapitulate the disease phenotype so as to allow us to assess the functional benefit of our drug therapies. As many of our studies are dependent on assessing clinical improvement, it is necessary for us to be able to test the effects of the drug on live animals.

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

Any mice experiencing adverse effects will be regularly monitored until return to normal clinical behaviour. Post-operative analgesia will be administered to and continually provided post-surgery until mice exhibit normal behaviour.

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

Our group regularly refer to publications from the Laboratory Animal Science Association (https://www.lasa.co.uk) and the NC3Rs (www.nc3rs.org.uk) website to guide in best practice. We also can refer to our NVS and NACWO as a secondary source of guidance to keep up to date with best practices.

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

We are regularly updated with advances in the 3Rs through the nc3rs website as well as regular internal communication by our regional NC3R manager.

A retrospective assessment of refinement will be due by 24 September 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?

22. Neuroprotection and neurorepair strategies in traumatic spinal cord injury

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
    - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
- Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

Spinal cord injury, Neuroprotection, Spinal circuit repair, Cell replacement, Neurorehabilitation

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

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?

This project aims to identify and develop new strategies for the treatment of acute and chronic injuries of the spinal cord. Traumatic spinal cord injury (SCI) triggers a complex pathophysiological cascade following the primary injury, leading to further damage to the spinal cord and bleak prospects for repair, but many elements of this cascade can be targeted with neuroprotective agents to prevent or minimize further damage, and

strategies can be harnessed to promote repair in the subacute and chronic phase after injury.

A retrospective assessment of these aims will be due by 18 November 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?

From the middle of the twentieth century, the adoption of new standards of care has greatly expanded life expectancy and maintained the health of people living with chronic paralysis and other consequences of SCI, and then the use of sustained and specialised rehabilitation has helped restore function to varying degrees. However, despite the very significant progress made by research over the past 20 years, there is no available treatment for protecting the spinal cord against the major loss of tissue that is the consequence of the spread of the injury beyond the zone of the initial impact and the loss of further function that results from it and, so far, no consensus has emerged as to the best strategy to use for spinal cord repair in individual patients. These remain among the largest unmet needs in neurology and trauma medicine, and therefore research efforts in this area are still needed.

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

We aim to develop a variety of treatments for use at different phases following injury, acutely, subacutely, and in the chronic phase, to provide neuroprotection against protracted loss of tissue and support regenerative processes. The main expected outputs are:

the identification of key compounds for neuroprotection and repair of the spinal cord;

the identification of combinatorial treatment modalities (including, for example, combined use of pre-repair compounds and rehabilitation procedures) for protecting against the long-term changes seen in the spinal cord after injury and for the support of regeneration;

the identification of strategies that would be effective in newly-injured patients or these already living with a chronic injury, from spinal cord injury in young adults to injury in middle-age;

a better understanding of the mechanisms of action for optimization of the identified optimal strategies.

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

In the short-term, our research will inform the scientific community working on SCI of new potential approaches for neuroprotection and repair. But the main aim of our approach is

its translation for the benefit of the patients. SCI affects a significant number of patients worldwide and is associated not only with dramatic consequences for the concerned individual but also with major health and social care costs (over 20% of people discharged from hospital and specialised units following injury do not go back to their own homes). Two and a half million people are affected worldwide, and in the United States alone the number of patients currently living with an SCI is over 250,000, with an annual incidence of approximately 40 cases per million (an estimated 12,500 spinal cord injuries every year, 500,000 worldwide; in the UK, one person is paralyzed every 8 hours).

The acute management of SCI is mainly focused on medical interventions to decompress the spinal cord to prevent further damage, realign the spine and fix the bones in place to stabilise the spinal column. At present, there is no specific pharmacological treatment to limit the consequences of the secondary injury that continues to propagate within the tissue in the aftermath of injury – at around 0.9 mm per hour. There is thus a large unmet clinical need for agents that would protect the injured spinal cord from the acute phase into the chronic phase of the injury.

A preparation that could be administered by the emergency team in the first hour after trauma, before or during transfer to the hospital, would represent a major medical innovation and would lead to much- improved prospects for patients, particularly if followed by interventions aimed at supporting long-term protection of the spinal cord and its repair. Such integrative and multifaceted approach aims to improve the autonomy of the patients and thus their quality of life, with the long-term goal of reducing the size of the population of individuals living with a disabling SCI. It is realistic to envisage that the clinical translation of some of the approaches we propose to test in animal models could start within the duration of this project.

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

We have ongoing local and international collaborations with world-renowned experts in SCI research, lipids and lipidomics, and medicinal chemistry, and established programmes of work on neuroprotection and repair with various pharmaceutical companies involved in the development of innovative approaches to acute injury and neurodegeneration. Our partners have been involved in the development of new chemical forms of fatty acids for improving their stability and delivery to the central nervous system (CNS), and this will allow us to expand our work on the potential of fatty acids as neuroprotectants. Others have also developed new peptides with neuroprotective and pro-regenerative properties that we have started testing in our laboratory. Therefore, we have a network that will help us disseminate widely the knowledge generated by our research. Being part of such a network, which includes clinical collaborators, will also help us attract funding and will facilitate clinical translation of our experimental research on neurotrauma.

Species and numbers of animals expected to be used

- Mice: 1,300 (animal tissue extracted for in vitro work); 2,800 (in vivo work)
- Rats: 300 (animal tissue extracted for in vitro work); 1,700 (in vivo work)

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.

We will use rats and mice in the experimental models of SCI described in this project proposal. Although much remains to be understood concerning how different aspects of their pathology relates to human pathology, rodent models are the most widely used to study SCI –most of the literature published so far in neurotrauma research has been generated in these species (92% of all studies, as of 2017). The anatomy of their nervous system is well understood and has similarities with the human nervous system, well-established functional analysis techniques are available to study their recovery following injury, and few other models are translationally as relevant and robust –many therapeutic interventions have progressed towards becoming candidate treatments for SCI based on rodent studies, and some of these are currently being assessed in clinical trials. Mice have the added advantage that they can be modified genetically (although genetically modified rats have also recently become available), with the potential of providing information on key molecular determinants of both the injury mechanisms and resulting pathogenesis, and possible drug targets. Rats are usually preferred to mouse models for their larger size for many surgical interventions and device implantations, and also because they often develop large cystic cavities at the site of injury, similarly to the human pathology. However, for translational purposes, the mice offer the opportunity of confirming the efficacy of tested strategies in a second species, which is a key factor in successful translation.

Because SCIs disproportionately affect the young population and result in a life expectancy that is still below that of the general population, young adult and middle-aged rats and mice will be used in this project.

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

In a typical experiment, the animals' baseline neurological status, reaching and grasping function, and/or gait would be assessed up to three weeks before undergoing surgery for SCI, after which the animals might be injected an active compound in the tail vein or through other parenteral routes – single bolus or repeated injections, or start another form of treatment (e.g., a dietary intervention). Treatment could be started either immediately after injury, in the subacute phase (i.e., over the first 1-3 weeks after injury), or once a state of chronic injury has been reached. Post-injury, the animals' neurological status and behavioural recovery would be tested regularly under a specific schedule (daily, every 2-3 days, weekly, monthly) that would depend on the task and duration of the experiment. The duration of the experiment would vary depending on the question being investigated – e,g, from 1 month to investigate new compounds' efficacy for neuroprotection, up to 6-9 months to investigate their efficacy for repair in the chronic phase and safety of long-term treatment. Some experiments may require that animals have their spinal cord re-exposed, from 4 weeks post-injury or later, for setting in place a treatment for repair by local administration or a biomaterial scaffold.

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

Animals may be at risk of developing pain during the acute phase that follows surgery (post-operative recovery period), which will be managed by providing appropriate preemptive analgesic relief. They may also have temporary difficulty reaching the food hopper or the drinking spout, and thus appropriate adjustments will be made in the way food and water are provided. All animals undergoing SCI will also show some degree of temporary weight loss despite getting proper calorie intake, along with paralysis or paresis

of the corresponding limbs with changes in muscle tonicity, presence of muscle atrophy, and compensatory gait alteration in the intact limbs. The degree and prognosis of weight loss, motor dysfunction, and development of abnormal behaviour will vary depending on the severity and site of injury in the spinal cord. For the most severe injuries, animals typically start regaining weight between 8 to 14 days after injury. Some degree of spontaneous functional improvement is expected within 2 weeks from injury in the form of sweeping movements of the limbs up to recovery of plantar stepping for some animals, but in most severe cases individual animals may show prolonged limb paralysis. Neuropathic pain may develop in some injury models from 3 to 4 weeks post-injury and may lead to scratching or self-harming behaviour. These abnormal behaviours may recede within a week but might persist in the most severe cases. Animals will be closely monitored and, if they exhibit any early signs of self-harming with damage progressing beyond toenails, they will be immediately humanely killed. Analgesics may be administered to these animals, which will be killed if there is any indication of progression of the autotomy behaviour beyond the toenails.

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)?

Mice: Mild, 10%; severe, 50%

Rats: Mild, 5%; severe, 25%

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

- Killed

A retrospective assessment of these predicted harms will be due by 18 November 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?

Trauma in the CNS triggers complex local and systemic events that cannot be mimicked entirely in vitro. Therefore, there are no suitable in vitro alternatives for the study of cellular and axonal damage and the regeneration of neurons because such models can never reproduce the whole context of the injury. Nonetheless, where appropriate, we will test new compounds in pilot experiments that will use primary neuronal cultures (e.g., dorsal root ganglion neurons), oligodendrocyte cultures, or mixed (oligodendrocytes and neurons) cultures derived from mice or rats, with tissue obtained using Schedule 1 procedures.

Primary cell cultures from wild-type or genetically altered animals are routinely used in our group for mechanistic and drug discovery phase studies, allowing for systematic screening before the implementation of a particular treatment in full animal models. In vitro studies remain limited by the fact that they cannot simulate the systemic impact of the SCI condition on many organs/systems, the complex biological response triggered by a CNS injury with its myriad of cellular players involved, and the long-term pathophysiological changes that occur in the whole body. Our group has thus been investigating innovative ex vivo avenues, such as organotypic brain cultures, and will also be developing combined ex vivo and in vitro methods using patient-derived cells (see below), as these approaches can be used to simulate specific pathological aspects associated with mechanical or hypoxic-ischemic injuries and will be very informative for further developments investigating the use of bioengineered cell constructs. Such in vitro approach could be adapted to spinal cord tissue.

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

Use of human samples.

Why were they not suitable?

Access to human samples is very restricted due to the long chronic phase of the human condition. Post-mortem tissue may become available in some instances but a long time after injury, and the type of analyses that can be carried out on such tissue is rather limited. There is also limited access to acute injured human samples. Furthermore, mechanistic studies in patients remain challenging, due to the variable onset and chronic pathophysiology of the injury, with limited access to randomised controlled clinical trials. However, we will soon be testing an innovative approach consisting of culturing patient-derived neuronal cells in microfluidic chambers, in which neuronal cell bodies and axons are compartmentalized in a 3-D environment for a more naturalistic study of nerve injury and repair. This approach may help us design novel bioengineered cell constructs for SCI repair and allow high- throughput testing of neuroprotective and pro-regenerative compounds. A PhD student has started work on this project.

A retrospective assessment of replacement will be due by 18 November 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 know from previous research in our laboratory, using the models of injury that we plan to use in the current project and taking into account the natural variance, that the level of effect for most treatments for behavioural, biochemical, and anatomical parameters is such

that small experimental group sizes (i.e., 8 to 10 animals per group) are generally sufficient to define a statistically significant effect.

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

Due to the complexity and intrinsic severity of CNS injury, our group is committed to the development and implementation of innovative minimally invasive readouts to maximize the assessment of outcomes while protecting the animal's welfare and reducing the numbers of used animals. The endpoints in our studies have been selected to include histological data (e.g., related to cell death, nerve fibre integrity, activation of neuroinflammatory cells) taken at a single final post-surgery time point, as well as a variety of behavioural measures taken at multiple time points in a single animal. In some cases, longitudinal imaging and tissue sampling (blood, CSF) of the same animal will be carried out, which will maximize readout efficacy while providing a large range of clinically relevant data throughout. We have available a preclinical MRI for imaging of our neurotrauma models. We also have various academic and industry collaboration programs on blood-derived biomarkers of CNS injury and novel imaging biomarkers for neuroinflammation, which will allow us to use such repetitive readouts, with direct impact on the translational value of the data and the number of animals required to reach robust conclusions. Furthermore, anatomical/structural and biochemical data will be correlated with functional behavioural outcome measures taken in the same animal, leading to a reduction in animal use.

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

Whenever possible, tissue from the same animal will be shared within various ongoing projects for assessment of a variety of biochemical, histological, cellular, and molecular parameters, thus optimizing the use of animals.

A retrospective assessment of reduction will be due by 18 November 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.

Rats and mice will be used in the experimental animal models of SCI described in this proposal as their nervous system resembles anatomically the human nervous system, and most of the literature published so far in neurotrauma research has been generated in

these species. Mice, and more recently rats, can also be modified genetically, thus providing information on key molecular determinants of both the injury mechanisms and resulting pathogenesis, and possible drug targets.

SCI in humans is a very heterogeneous condition. It can be caused by a variety of traumatic events, affect various levels of the spinal cord, and may result in contusion, compression, partial or complete section of the spinal cord, in spinal root or nerve injury, and most often occurs in a context of polytrauma where further nerve damage might also be present. The traumatic events cannot be reproduced experimentally but the methods used for producing SCI in animals in this project (spinal cord contusion, compression, or partial section) have been designed to induce SCI symptoms and pathophysiology in rodents that resemble these seen in humans while ensuring, through the use of strictly controlled measures, a minimum level of suffering, distress, and harm to the animals.

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

We have set up various in vitro models (primary neuronal and oligodendrocyte cell cultures, mixed oligodendrocyte and neuronal cultures, organotypic cultures) for high-throughput screening of neuroprotective and pro-regenerative compounds and we will continue to refine these models in line with our needs and emerging technological developments. While these models form the core of our early-stage drug discovery programs, they are inevitably limited and cannot account for the complexity of the injury and recovery process seen in a full organism.

Unlike humans or commonly studied mammalian model systems (rats and mice mainly), some other vertebrates can regenerate crushed or transected spinal cord tissue at the adult stage. It has been long known that regrowth of severed axons, repair of neuronal circuits, and functional recovery to full movement capacity exist in tadpole-stage frogs, adult salamanders, certain reptiles, lampreys, and teleost fish species. Research employing amphibians and reptiles is currently developing, and while SCI in zebrafish is still understudied, several advances have been made over the past decade to understand spinal cord regeneration in this species. These models are very powerful to develop numerous transgenic and genetic tools for dissecting the mechanisms of regeneration that are lacking in mammals. However, testing neuroprotective and pro-regenerative compounds and combining novel pharmacological approaches with innovative strategies for rehabilitation, an approach that is very relevant for fast clinical translation, require the use of animals whose spinal cord pathophysiology in response to injury, and functional abilities (reaching, grasping, coordinated gait), are as close as possible to these seen in humans.

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

Due to the complexity and inevitable severity of animal models of SCI, our experimental program will use an intensive post-operative care protocol, including appropriate peri-operative analgesia, feeding and drinking support, management of bladder function, regular welfare and behavioural assessments, with close monitoring of those animals that will present with long-term impaired locomotion and motor abilities. Our group has extensive experience in running such a comprehensive post-surgery program, which is instrumental in promoting animal well-being and collecting meaningful experimental outcomes. We are particularly interested in pain assessment and management, and testing novel means for assessing neuropathic pain and spasticity will be key to the current project. Thanks to NC3Rs funding, we have recently implemented the use of a novel

automated tracking system to monitor cage activity in animals post-trauma, which represents a new avenue to assess the recovery and well-being of injured animals and refine the management of chronic neuropathic pain when present. Our group will continue to work closely with the HOI, NACWO and the NVS to improve and revise pain assessment and the welfare of injured animals, as deemed appropriate. We are constantly exploring the potential of new behavioural tests to improve the quality of our experimental outcomes, and also to refine animal care and pain management, which is instrumental for the understanding of the welfare of animals with impaired motility. Along with specifically tailored neurological scores for the routine assessment of motor disability in paraplegic animals, we have implemented the use of a gait analyser system which has proved useful for detecting subtle deficits in gait and could also help us identify pain when present. We have also revised the animals' housing conditions, with the implementation of cage enrichment to promote activity and aid spontaneous rehabilitation in the home cage.

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

All researchers involved in the current project will follow the guidelines produced by the NC3Rs. "The Responsibility in the use of animals in bioscience research" general guidance document will be used as a reference document, as it sets out the expectations of the funding bodies for the use of animals in research. The updated ARRIVE guidelines (2.0), designed for transparent reporting of animal research methods and findings, will be consulted for the planning and design of new experiments to ensure reliability and reproducibility of findings. In this respect, the "Guidelines for planning and conducting high-quality research and testing on animals" (2020, ref. 1) and PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) guidelines (2018, ref. 2), published by Smith and colleagues (from Norecopa, Norway's National Consensus Platform for the advancement of "the 3 Rs", 2018) offer comprehensive guidance and a helpful 15-point checklist for planning animal studies. Importantly, the points in the checklist relate not only to the formulation of the study and the quality control of the components in the study but also to the dialogue between scientists and the animal care staff. Finally, we will follow the guidelines of the UK Research Integrity Office (UKRIO) and will aim to register our study protocols in an international online register of protocols for preclinical animal studies. Our group has also been involved in a recent consultation in collaboration with the RSPCA and NC3Rs that has resulted in a reference review paper on the issue of refinement of animals models of SCI (ref. 3).

Smith AJ (2020) Guidelines for planning and conducting high-quality research and testing on animals Lab. Anim. Res. 36:21. doi: 10.1186/s42826-020-00054-0. eCollection 2020.

Smith AJ et al. (2018) PREPARE: guidelines for planning animal research and testing. Lab. Anim. 52(2):135-141. doi: 10.1177/0023677217724823.

Lilley E, Andrews MR, Bradbury EJ et al. (2020). Refining rodent models of spinal cord injury. Exp. Neurol. 328:113273. doi: 10.1016/j.expneurol.2020.113273.

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

I am receiving the NC3Rs' e-newsletter monthly updates. The group is also in constant interaction with the NACWO, NVS, and animal technicians, which has resulted in significant improvement in the wellbeing of injured animals at our facility.

A retrospective assessment of refinement will be due by 18 November 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?

23. Control of equine herpesviruses in the horse.

Project duration

5 years 0 months

Project purpose

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

Key words

Vaccines, Herpesvirus, Equines, Abortion, Neurological Disease

Animal types	Life stages
Ponies	adult

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.

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 research is to test the safety and protection afforded by new vaccines against herpesvirus infections in the horse.

A retrospective assessment of these aims will be due by 07 October 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 it's 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?

Infectious diseases are very common in racehorses in training, breeding and in pleasure horses, the more serious of which can be devastating to the animal and the industry. Some of the well characterised infectious diseases are caused by members of the herpesvirus family and the most problematic of these are EHV-1 and EHV-4. EHV-1 and 4 are abundant viruses that affect horse populations on all continents. While the two viruses share a high degree of genetic similarity, they differ significantly in the disease they can cause. EHV-1 can result in respiratory disease, abortions, death of new-born animals and damage to blood vessels in the brain and spinal cord which can cause neurological disease. Neurological disease outbreaks have occurred with increasing frequency in North America and Western Europe and are a concern for the UK equine population. Outbreaks on stud farms in unvaccinated pregnant mares can lead to extremely high rates of abortion. Commercially available vaccines have been shown reduce the extent of virus shedding of EHV-1 and 4 from infected horses. However, they offer poor protection against abortion and none are licenced to protect against neurological disease. This was highlighted by the abortion outbreak recorded in Hertfordshire in 2016 and the later cases of neurological disease that occurred in Valencia in 2021, both in fully vaccinated animals. EHV-4 can cause severe respiratory illness in horses, these horses are placed in quarantine, cannot travel, and are unable to be ridden or compete causing financial impact sometimes globally.

The Fourth International Havemeyer Workshop on Equid Herpesviruses, held in 2018 in North Carolina, invited the most pre-eminent scientists from around the world working on equine herpesviruses. They highlighted the continued occurrence and impact of equine herpesviruses on equine health, welfare and the equine industry. The workshop concluded the most important direction for research continues to be the development of vaccines that can control the most serious types of disease. Previously published work by other groups has indicated that a vaccine based on a partially disabled EHV-1 virus can offer protection against both EHV-1 and EHV-4 infection, but not the other way round. Therefore, the focus of this project licence is primarily EHV-1.

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

This project will develop and test the safety and effectiveness of new vaccines against EHV induced disease. This will also contribute to an improved understanding of EHV disease processes. Results will be published in peer-reviewed publications and findings will be presented at local, national, and international scientific meetings. In the longer term, there will be intellectual property that may support a planned patent application, support future funding applications/clinical trials or may aid in the licencing of any successful vaccine. The results will directly improve the health and welfare of horses worldwide. Knowledge gained in this project may be directly applicable to the development of vaccines for the prevention of other mammalian herpesviruses.

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

In the short and medium term we will determine whether we can design and make novel vaccine candidates in the laboratory. Data gained from these experiments will contribute to our understanding of EHV-1 infections in horse cells and ultimately in the horse. Long-term we hope to develop a more effective vaccine against EHV-1 which has the potential to benefit all equines, preventing respiratory disease, abortion and neurological disease. These improved vaccines will be of particular interest to the equine breeding industry in

the UK which still suffers significant welfare problems and financial losses due to EHV-1 induced abortions.

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

This project has contributors from a number of international academic and commercial laboratories. Where applicable, results will be patented, published in peer reviewed journals and presented at international conferences. We will attempt to disseminate all findings, including unsuccessful approaches and non-significant data via open access publications or platforms such as F1000Research which offers rapid publication of data without editorial bias.

Species and numbers of animals expected to be used

- Ponies: 15

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.

Unfortunately, the use of ponies for our research is essential. EHV-1 and EHV-4 cause disease that is unique to this animal. We need to measure how the complex pony immune system responds to the vaccines and virus infection in order to maximise the level of protection achieved.

For pharmaceutical companies to licence and market vaccines commercially, they need to be tested according to regulations determined by the competent authority. These regulations state that the vaccines must be tested in the target species, in this case ponies.

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

Animals are kept at grass when uninfected. Blood samples will be taken from the jugular vein for the isolation of blood cells for the cultivation of vaccines and the development of tests. These are routine procedures conducted by experienced staff. Ponies will be maintained on site and may be rehomed at the end of the procedure.

Some animals will be vaccinated with experimental vaccines. Typically, this will involve the injection of the vaccine into the neck muscles in the same way as commercially available vaccines. When undergoing the first vaccination procedure these animals will be held in containment buildings. These animals will then have swabs inserted into the nose to obtain biological material (nasal swabs) and blood samples taken regularly to monitor the immune response induced by the vaccines. At the end of the study animals will be humanely killed. Once a dossier of data has been compiled for each vaccine, animal housing and rehoming will be reassessed to determine whether the animals could be vaccinated at grass and rehomed after the procedure. This procedure typically lasts 6 weeks per vaccination.

A pilot study will be conducted where animals housed within an open barn with bedding will be infected with EHV-1. This procedure typically lasts three weeks. Infected animals will be bled and swabbed regularly to determine viral titres and the immune response to virus infection. At the end of the experiment some ponies will be humanely killed. Others may be kept alive for continued bleeding to assess the on-going immune response to infection.

Some animals will be vaccinated and then infected with EHV-1 and housed within an open barn with bedding. This procedure consisting of a vaccination phase and an infectious phase typically lasts 8 weeks in total. At the end of the experiment all ponies will be humanely killed.

EHV-1 infected animals are typically not rehomed as they are thought to carry the virus for the rest of their lives. This virus may reactivate later in life, spread to other animals and cause disease. Animals that are fully recovered at the end of procedures may be kept alive at the establishment (with agreement of a vet) with a view to their reuse on procedures if appropriate and licenced. Otherwise, animals will be killed humanely using an approved method.

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

When blood samples are taken from equines this will result in temporary discomfort and no lasting harm. Animals that are vaccinated may experience mild local reactions such as swelling or soreness. We expect any reactions observed will be no worse than those seen following the administration of current commercially licenced EHV-1 vaccines that are used in the general horse population.

Animals infected with EHV-1 typically have a self-limiting and mild illness. Unvaccinated ponies typically develop a fever from day 2 after infection which lasts for 1-3 days and they may develop nasal discharge. Naïve ponies can sometimes develop secondary bacterial infections after virus infection and any affected animals will be treated with antibiotics. There is a possibility that ponies infected with EHV- 1 may develop neurological disease as a result of damage to blood vessels in the brain or spinal cord, similar to that seen in some outbreak situations in the field. In most animals this resolves on its own without treatment. Any affected animals will be observed closely for the duration of the clinical signs and appropriate supportive therapy administered. If they fail to respond to treatment or the severity limit is likely to be exceeded they will be humanely killed in order to minimise any suffering. At the end of the project, animals could undergo continuous use if we need to do more work to achieve our objectives.

Animals could also be re-used on another project licence.

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)?

Ponies: Mild 90%

Moderate 10%

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

- Killed
- Rehomed
- Used in other projects
- Kept alive

A retrospective assessment of these predicted harms will be due by 07 October 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?

During the preliminary stages of the vaccine development programme we will use cell lines and horse cells isolated from blood to avoid the need to infect animals. These studies will enable us to select only those vaccines that are most worthy of further investigation in ponies. Post-mortem tissues from animals humanely killed for reasons not connected to this project will also be used in order to avoid the need to infect animals in the preliminary stages. Unfortunately, the use of ponies for our research is essential.

EHV-1 causes disease that is unique to equines. We need to measure how the ponies' immune system responds to the virus and vaccines in order to maximise the level of protection achieved by the vaccines.

For pharmaceutical companies to licence and market vaccines commercially they need to be tested according to regulations determined by the competent authority. These regulations state that the vaccines must be tested in a target species, in this cases ponies.

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

We will use commercially available cell lines for vaccine production and some of the laboratory based vaccine characterisation. Unfortunately, there are no non-animal alternatives that can be used instead of equines for the testing of the vaccines.

Why were they not suitable?

There is no non-animal model that can reproduce the complex interactions between the virus and the horse immune system.

A retrospective assessment of replacement will be due by 07 October 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?

This first part of the vaccine development programme requires horse blood from which we isolate primary cells. We have used 2 animals for this purpose on a number of different project licences over many years. This is because not all animals produce cells that respond in the typical way. We have previously seen up to 10% of cells not behaving as predicted, so we use the minimum biological replicate to reduce the chances having a non-responding blood donor. Blood will be tested at the beginning of the protocol and replaced with alternative donors if necessary. Using two animals also allows us to keep them in a social group at grass.

The second part of this programme is the establishment of the EHV-1 challenge model in Welsh mountain ponies. Historically, we have used 5 animals in these pilot studies for this purpose in order to get an accurate representation of infection in the animals, before we proceed with safety and efficacy studies later in the programme. Previously conducted studies have shown between 75-100% of control animals develop disease so we would expect at least 4 animals to become infected. These data obtained in this pilot study will be used to re-evaluate the number of animals to use in any vaccination study in the future.

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

During the experimental design phase we consulted the PREPARE guidelines (https://norecopa.no/PREPARE) and the guidance on the NC3Rs website which gives advice on how to minimise the number of animals used per experiment. The design of all studies have been checked by a statistician to ensure that the smallest numbers of ponies are used in order to achieve statistically significant results. We have also used additional software such as GPower1 to confirm the experimental design numbers (https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine- psychologie-und-arbeitspsychologie/gpower).
1) Faul, F., Erdfelder, E., Lang, A.-G., & Buchner, A. (2007). G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behavior Research Methods, 39, 175-191

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

We use pilot studies to ensure we have the experimental conditions correct before we use larger numbers of animals later in the project. Pilot studies will allow us to assess the experimental design and identify potential problems, as well as implement improvements early on in the licence.

Animals are randomly assigned to control or treatment groups, while trying to maintain existing social groups within the ponies. The study director and staff involved in animal husbandry and the clinical monitoring of the animals are blinded. An experienced study co-ordinator maintains the study file with the key for the randomisation which can be accessed if required during the protocol.

We will also ensure that we freeze virus and blood samples in a -70C archive freezer in order to reduce the number of animals used in the future. This will also allow us to share samples with other researchers further reducing the number of animals used.

A retrospective assessment of reduction will be due by 07 October 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.

Basic experiments have been developed that help us characterise vaccines in the laboratory before we use them to vaccinate horses. In this way we can reduce the number of animals used for testing. However, we cannot model the complex interaction of the virus with the horse immune system so we must use the natural host to determine whether newly designed vaccines are safe and work. Extensive experience of animal handling and regulated procedures suggests that serious adverse effects of repeated blood sampling, vaccinating and EHV-1 infection are rare. Horses that undergo vaccination only are kept at grass in groups in grass paddocks with freedom to roam with shelter if they require. The research being addressed by the programme will contribute to the welfare of horses in the long term by providing new and improved vaccines. In order for pharmaceutical companies to obtain marketing authority for commercial vaccines they must be tested in the target species, in this case equines.

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

The only way to accurately determine how well an EHV-1 vaccines will work in the horse is to use the natural host. In order for pharmaceutical companies to obtain marketing authority for commercial vaccines they must be tested in the target species, in this case ponies.

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

All ponies will be health-checked by an equine veterinarian prior to purchase. Ponies will be transported by a professional experienced transporter in a livestock lorry with plenty of floor space, the floor will be covered with straw bedding. These procedures ensure that the ponies arrive in good health with the minimum of transport-induced stress.

The ponies will start their acclimatisation process as soon as they have had a couple of days to settle in at the quarantine site. Ponies are easier to handle if they become confident of being around humans. Routines are quickly put into place and the ponies are run as a herd for all handling procedures. Typically any handling procedures are conducted first thing in the morning using the same experienced personnel. Initially the ponies are held in a safe holding pen, then driven through a race system into a crush / veterinary stocks. Once they are comfortable moving freely through this system they are individually held in the crush, with a backboard slid behind them to prevent them from reversing out.

Initially each pony will be offered concentrate cubes from a scoop, but most at this early stage of training will not take any. This process (which can take a couple of weeks) then progresses to ponies being quietly approached in the crush and a halter placed. Following a handling session, the ponies are then released as a group and fed concentrates to provide a source of positive association. Visual assessments of each pony while enclosed in the holding area become part of the daily routine of husbandry management. When the ponies are confident, new procedures will be introduced such as weighing, having rectal temperatures taken, worming and bleeding.

Ponies form associations with other ponies and staff should be aware of these affiliations so that they are allowed to continue during the studies. Accidental splitting up of these attachments can be stressful, therefore animals are managed to avoid this occurring. Difficult or very nervous animals can be separated into pairs or smaller groups to enable a more intensive period of training to get accustomed to the facilities, handlers and procedures.

The procedures of bleeding, vaccination and challenge will be undertaken by experienced staff on site thereby reducing the amount of stress suffered by the animals. By the time the study commences, ponies are used to being handled and entering the handling system and crush. Animals are housed in social groups and acclimatised to their environment. We use environmental enrichment to help ensure the animals are less stressed. To mitigate the effects of containment indoors, environmental enrichment includes the radio, which provides background noise during the day and is thought to calm the animals. Lighting is adjusted to reflect seasonal and daylight-saving changes outside. Root vegetables and fruits are provided such as carrots, swede, white cabbage, turnips and apples with some strung from the ceilings. Apples are provided in buckets of water, along with hay blocks, hay nets, foodballs and licks to encourage play. Scratch mats are also provided for the ponies in easily accessible areas of the containment room or enclosure. These processes are constantly reviewed by the scientists and veterinarians involved to maximise the quality of life and minimise suffering.

Animals will be regularly monitored after EHV-1 infection by experienced handlers who know the animals well in order to minimse any suffering or stress as a result. This will ensure that the humane endpoints are adhered to. Checks will include measuring body temperature, appetite, demeanour, breathing, eye and nasal discharge and assessing the size of the lymph nodes in the neck. Non- abrasive swabs with flexible handles are used. If

animals develop serious respiratory disease they will be treated using antibiotics and/or anti-inflammatory drugs usually given orally as a paste.

We also include a neurological assessment or each animal to determine if the central nervous system has been affected. Any animals that develop neurological disease will be observed by the named veterinary surgeon or other competent person for the duration of the clinical signs and appropriate supportive drugs administered.

When the ponies become eligible for rehoming, they will be well handled but are usually relatively young, so extensive work is undertaken to find knowledgeable, experienced homes for them. Potential new owners are encouraged to visit the ponies prior to departure to ensure they are fully aware and comfortable with each individual and where they are in their training. Comprehensive details are provided for the new owner and advice given in any areas required.

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

We will follow the guidelines published by the NC3Rs which includes the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines. These guidelines make recommendations which help to improve the reporting of research involving animals, thereby maximising the quality and reliability of published research, and enabling others to better scrutinise, evaluate and reproduce it in the future. The Planning Research and Experimental Procedures on Animals: Recommendations for Excellence (PREPARE) guidelines complement the ARRIVE guidelines. They cover the planning of experiments, dialogue between scientists and the animal facility, and quality control of the various components in the study. Advice on use of the guidelines is available on the Norecopa website (https://norecopa.no/PREPARE). The Laboratory Animal Science Association (LASA) also publishes guidelines that make recommendations on good practice (https://www.lasa.co.uk/current_publications/).

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

We will be working closely with the Named Veterinary Surgeon (NVS), the Named Animal Care and Welfare Officer (NACWO) and a number of experienced horse handlers which will help keep us up-to- date about the principles of the 3Rs when working with the equines.
We also have access to on-line resources such as the National Centre for the Replacement, Refinement and Reduction of Animals in Research website (https://www.nc3rs.org.uk/) which often reports on the development of new 3Rs approaches. The website of the Royal Society for the Prevention of Cruelty to Animals (RSPCA) has an Animals in Science section (https://science.rspca.org.uk/sciencegroup/researchanimals) which has advice on implementing the 3Rs. The Norwegian National Consensus Platform for the advancement of the 3 Rs (Replacement, Reduction, Refinement) in connection with animal experiments (https://norecopa.no/about-norecopa) hosts a large amount of guidance for planning animal research and testing. The laboratory animal science association (https://www.lasa.co.uk/) hosts the large animal research network meetings (LARN) which are an important source of 3Rs information and discussion.

A retrospective assessment of refinement will be due by 07 October 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?

24. Provision of control samples to develop and maintain tests for animal diseases

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
    - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
    - Assessment, detection, regulation or modification of physiological conditions in man, animals or plants
    - Improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes

Key words

Positive control material, Notifiable diseases, Endoparasites

Animal types	Life stages
Cattle	juvenile, adult, pregnant, aged, neonate
Sheep	neonate, juvenile, adult, pregnant, aged
Goats	neonate, juvenile, adult, pregnant, aged
Pigs	neonate, juvenile, adult, pregnant, aged
New World camelids	neonate, juvenile, adult, pregnant, aged
Domestic fowl (Gallus gallus domesticus)	neonate, juvenile, adult, pregnant, aged
Horses	neonate, juvenile, adult, pregnant, aged
Ponies	neonate, juvenile, adult, pregnant, aged

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.

- Uses cats, dogs or equidae

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 supply of positive control materials [product] to develop and maintain tests for reference and statutory functions and protection of animal health.

This will be achieved by either:

Sampling of field cases for positive control material

Provision of periodic animal passage of animal endoparasites

Additional protocols may be requested for addition to the project to establish aetiological agent, determine the susceptibility and preliminary pathogenesis of potentially new and emerging infectious agents.

A retrospective assessment of these aims will be due by 15 December 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 is important to meet the organisations responsibility as the competent authority under EU and international law, to protect the health of animals and plants, for the benefit of the environment, the economy and human health.

The strategic aims are:

Aim 1 - Rapid control of pests and disease outbreaks in animals and plants
Aim 2 - Reduced threat from new and emerging animal and plant diseases
Aim 3 - Enhanced food security
Aim 4 - Improved agricultural economy
Aim 5 - Healthier people, plants, animals and environments Aim 6 - Improved policy making in UK and EU

To accomplish these strategic aims, the organisation is a national reference laboratory for 48 specialisms (e.g. Transmissible spongiform encephalopathies, Duck viral hepatitis, Equine infectious anaemia, Glanders, Bovine Viral Diarrhoea) as well as an international reference laboratory across 23 specialisms (e.g. Avian influenza, Bovine tuberculosis, Rabies) for diagnostic organisations both nationally and internationally.

The organisation also undertakes testing for other non-notifiable diseases and has a biological archive to manage the storage and provision of tissues. The archive continually and actively reviews the corporate strategy for the collection and storage of reference materials pertinent to current research, testing and reference laboratory commitments. Prospective tissue collection from either field cases or experimental animals is a key part of keeping this collection current, comprehensive and relevant.

This includes taking advantage of UK field cases for the provision of positive material. Under previous licences there was a requirement for positive control material from cases of Maedi Visna, Leptospira hardjo bovis, Equine Infectious Anaemia (EIA), Chlamydophila abortus, Infectious Bovine Rhinotracheitis (IBR), Caprine Arthritis Encephalitis (CAE), Schmallenberg Disease and Porcine Epidemic Diarrhoea Virus (PEDV).

Key functions of being a reference laboratory include confirmatory testing, diagnosis of new and unusual forms and provision of control material of known status for testing purposes. The objective (and importance) of this licence is to ensure the supply of materials to undertake these functions in the absence of any other source.

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

The supply of materials to develop and maintain tests for reference and statutory functions and protection of animal health. This project will ensure the supply of sample materials to undertake confirmatory testing, diagnosis of new and unusual forms of disease and provision of control material of known disease status for testing purposes. The samples obtained by this project will contribute to research, development, and publications.

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

The demands on this project will be relatively small due to the specialised nature of the work. It is primarily to fill gaps in material availability, replace material that has been used that cannot be sourced by other means. The samples used to replenish archive samples will be made available to encourage innovation in diagnostics and ensure control samples are available for research.

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

The samples collected on this project will replenish archive stocks and meet the competent authority's requirement to accurately identify and diagnose notifiable and endemic disease.

Species and numbers of animals expected to be used

- Cattle: 5
- Sheep: 15
- Goats: 5
- Pigs: 5
- Camelids: No answer provided Horses: 5
- Ponies: 5
- Domestic fowl (Gallus gallus domesticus): 60

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.

The choice of species will depend on the species susceptible to the disease. Field case animals are exposed via natural infection, so species and life stage are determined by natural selection.

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

Animals on the project will be sampled and then treated and released from project (non-notifiable disease) or euthanised and disposed of (notifiable disease) in accordance with government guidance/requirements.

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

Protocol 1:

Pain caused by the insertion of a hypodermic needle according to good veterinary practice, during the sampling. Stress caused by restraining the animals during procedures. Blood sample may cause mild transient discomfort from needle insertion.

Sterile blood sampling procedures and careful handling of the needles plus good restraint will be used to minimise damage to the blood vessels and inflammation caused to the vein.

Heamatomas will be prevented or controlled by pressure on the site immediately on removal of the needle/cannula.

Blood withdrawn from animals, will be kept to the minimum practicable volume, but will not exceed 10% of total blood volume in any 24-hour period or more than 15% of blood volume in any 28-day period. Total blood volume will be estimated as 70 ml/kg body weight.

Protocol 2:

Mild discomfort during single oral dosing.

The acclimatisation to the faecal collection harness and the associated handling by competent staff during collection and change of the harness is well tolerated and subthreshold.

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)?

Subthreshold to mild severity is expected in 100% of animals.

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

- Killed
- Kept alive
- Used in other projects
- Rehomed

A retrospective assessment of these predicted harms will be due by 15 December 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?

Protocol 1: The requirement for animals is based on the requirement for immunological response of naturally exposed animals to non-notifiable and notifiable diseases.

Field case animals were naturally infected and this project allows sampling of those animals that will be euthanised and disposed of according to government guidance.

Protocol 2: Many animal endoparasites have complex life cycles involving alternate hosts, different sexual and asexual stages or fastidious feeding requirements which cannot be undertaken in-vitro. Parasites worked on this licence are also species specific therefore have to be passaged in their natural hosts.

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

Sampling is done on animals naturally infected in their 'home' environment (or holding).

Why were they not suitable?

Protocol 1: There is no suitable substitute for naturally infected animals that are in their 'natural' environment in the UK. Positive control material is required to be taken pre-mortem and there is currently no alternative.

Protocol 2: There is no artificial way to replicate the gut for generation of helminth eggs.

A retrospective assessment of replacement will be due by 15 December 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?

Due to the specialised nature of the project, demands will be relatively small (estimated at 1 animal per year for most species). Due to the diversity of domestic fowl and number of notifiable disease cases seen in 2021 and 2022 within the domestic poultry and wild bird populations, additional numbers are requested. The '60' animals is based on the organisations veterinary field staff model of collecting 20- 20-20 samples across 3 poultry houses when investigating a highly pathogenic avian influenza report case (total of 60).

An additional 10 sheep are requested for the endoparasite portion of the project (total of 15).

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

Animals used will be to fill gaps in material availability and to replace material that has been used that cannot be sourced by other means.

The helminth egg production has to occur every year as the helminth eggs deteriorate once outside the body, so have to be refreshed annually.

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

A minimum number of animals will be used to obtain the required positive control material.

For helminth production two lambs are used to provide company for each other (essential for lambs as flock animals) and minimise the numbers of animals used each year.

A retrospective assessment of reduction will be due by 15 December 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.

Field case animals may have to be transported to the establishment for further tests and/or post- mortem, which we arrange. We have a fleet of suitable and approved vehicles and experienced drivers who are trained under Welfare of Animals in Transport Order (WATO) and are also NACWOs. Animals will be assessed for their fitness to travel prior to transport and only animals deemed fit to travel will be transported. Transport is kept to a minimum length of time and distance.

Male neutered lambs will be used for the collection of faeces using harness and bags. Depending on availability bottle-raised lambs will be selected for this. These lambs are very familiar with people and do not object to being handled or caught. If only a single lambs is required, it will be housed with a companion.

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

The choice of species will depend on the species susceptible to disease. For field cases this will be by natural infection.

Obtaining endoparasites from lambs is required because the helminth required is an obligate parasite of sheep

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

As well as pre-start meetings involving the NVS, NACWO and animal care staff to ensure current knowledge is brought to bear, all projects are followed up by a wash up meeting. All aspects are discussed, was the project a success, what went well and if there was anything that could be done better. If there are any suggestions for refining the procedure they will be considered and if appropriate, incorporated into the protocol.

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

Home Office The Harm–Benefit Analysis Process _Advice note Home Office Guidance to Animal (Scientific Procedures) Act 1986

Home Office Code of Practice for the housing and care of animals bred, supplied or used for scientific purposes

OIE (World Organisation for Animal Health) Manual of Diagnostic Tests and Vaccines for Terrestrial Animals

NC3Rs website

Establishment guidance and current legislation for Welfare of Farm Animals and Transport of Farm Animals

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

By maintaining regular contact with the NVS, NACWO and NIO and regular on-line scientific literature reviews for relevant publications. Use of the organisations Operations Manual to ensure that governmental guidance is followed for the appropriate care and disposable of animals with notifiable diseases.

Engaging with stakeholders such as National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs), Laboratory Animal Science Association (LASA) and RSPCA.

A retrospective assessment of refinement will be due by 15 December 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?

25. Molecular mechanisms in cardiometabolic disease: effects of diabetes on blood vessels

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
    - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
    - Assessment, detection, regulation or modification of physiological conditions in man, animals or plants

Key words

Cardiovascular disease, Diabetes, Obesity, Angiogenesis, Aneurysm

Animal types	Life stages
Mice	juvenile, adult, neonate, embryo, aged, 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?

This project aims to identify the molecular mechanisms which lead to the development of diabetes and cardiovascular disease. It focuses specifically on how diabetes affects the risk of developing diseases of blood vessels and the circulation including atherosclerosis, narrowing of blood vessels after treatment, and aneurysm formation.

A retrospective assessment of these aims will be due by 26 October 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 it's 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?

Cardiovascular disease is the commonest cause of death in people with diabetes. Although much is known about some of the links between diabetes and diseases of the heart and circulation, we still do not fully understand why people with diabetes remain at high risk of cardiovascular disease and do not respond as well to treatment. Increasing our knowledge in this area is vitally important at this time, because changes in human lifestyle have led to large numbers of people with obesity and are predicted to cause a huge increase in the number of people worldwide with diabetes over the next 15 years.

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

We expect to have increased our understanding of the causes of blood vessel disease and how these are affected by diabetes. In particular we will better understand the actions of insulin and related proteins within blood vessels and how these are altered by both diabetes and cardiovascular disease. We hope to have identified new genes or proteins which link diabetes with cardiovascular disease.

Our short time outputs will be scientific papers published in scientific journals and presentations to the scientific community at meetings. We hope that our research findings will allow us generate longer term outputs with new ways to diagnose, prevent and treat cardiovascular disease in people with or at risk of diabetes.

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

In the short term the scientific community will benefit from these outputs, which will increase our understanding of the basis of cardiometabolic disease. In the longer term we hope that our outputs will improve the lives of people living with, or at risk of, diabetes and cardiovascular disease.

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

We will maximise the value of our outputs by dissemination through a variety of means. These include presentations at scientific meetings, publications in open-access scientific journals and release of key findings through our institution's websites and social media streams. We have close links with networks of researchers and clinicians working in this field. Our institution has strong support systems in place to facilitate translation of research findings through to clinical application. We work very closely with colleagues in other disciplines - for example to allow us to develop new drug-like molecules to explore the findings from this research.

Species and numbers of animals expected to be used

- Mice: 10 600

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.

We use mice to study the links between diabetes and cardiovascular disease. This is because it is relatively straightforward to alter the genes of mice and study how that gene influences diabetes or affects blood vessels. Many lines of mice are available to the scientific community in which selected genes have either been deleted or increased. Because genes encode proteins in the body, this allows us to study the effect of specific proteins in diseases. One example is that we study the receptor by which insulin exerts its effects on the cells of the body. By reducing or increasing the numbers of insulin receptors in certain cells within the blood vessel wall, we can see how insulin and its effects on those cells influence the susceptibility to blood vessel disease.

Mice are amenable to studying the effects of most of the diseases that affect humans. Because mice are mammals, findings can be used to mimic what happens in humans. For example, we can study diseases such as atherosclerosis (furring up of arteries), peripheral arterial disease and aneurysms as well as reproducing the effects of treatments such angioplasty. We can examine how the influence of diabetes on blood vessels affects the body's capacity to repair and heal itself, for example in healing of wounds and the growth of new blood vessels. Finally, we can learn how diabetes in pregnancy affects the susceptibility to diabetes and cardiovascular disease in children.

The majority of our research is carried out in adult mice. We use neonatal mice to study the development of blood vessels in the retina. We also study the effects of genes in pregnant mice on the placenta and the developing fetus.

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

Mice used in this project will typically have been bred in another project licence held by the applicants to create genetically altered animals (Molecular mechanisms in cardiometabolic disease: breeding and maintenance of genetically altered animals: PP5104353). The genetic alterations affect the animal's molecular and cellular processes but do not themselves cause direct harm or disease. We use them to examine how specific proteins affect the mouse's susceptibility to develop diabetes and blood vessel diseases. Information obtained from genetically altered mice can be complemented by treating the animal with a drug or infusing it with cells from another animal.

We promote type 2 diabetes in mice by feeding a high calorie and high fat diet. This leads to obesity and diabetes just like in humans. We induce type 1 diabetes by injection of a drug which damages the insulin-producing cells in the pancreas. We assess the diabetes status of mice by taking blood samples after giving insulin or glucose. We can measure energy usage, activity and metabolic rate by housing mice temporarily in a special cage. We gain basic information on blood vessel health by measuring blood pressure with a cuff around the tail and by taking scans of mice using ultrasound, MRI, CT or laser. Blood vessels are studied in the laboratory after the animal has been humanely killed.

We gain more detailed information on blood vessels and their roles in disease in separate groups of mice in which we study particular human diseases. We study atherosclerosis

(furring up of arteries) in genetically altered mice with high cholesterol levels by feeding them a high cholesterol diet. We study the response to injury of blood vessels in mice by inserting a small wire into the artery in the leg under anaesthesia or by performing surgery to place a cuff around the artery in the neck. We investigate peripheral arterial disease by tying off the main artery in the groin, so that blood flow to leg passes through the tiny branches until new blood vessels develop. We reproduce aneurysms either by applying chemicals to the main blood vessel in the abdomen during surgery, or by infusion of a drug. We study wound healing by removing small discs of skin under anaesthetic - a 'punch biopsy' - and letting them heal. New blood vessel development is either studied in the retina of new born mice killed humanely or in small plugs of gel injected under the skin in adult animals. Finally the effects of parental influences on the next generation are investigated by giving injections to pregnant mice and then studying the offspring.

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

Genetic alterations themselves affect only the molecular and cellular processes in the body but do not cause direct harm to the animal. Our research looks at how these genetic alterations affect the mouse's tendency to develop diabetes or blood vessel disease when exposed to the interventions discussed above. As in humans, diabetes can lead to thirst and increased urine production and high fat diets can lead to an oily coat in addition to obesity. Blood sampling and blood pressure measurements lead to temporary discomfort. Ultrasound, MRI, CT and laser scans are performed under anaesthesia from which mice recover very quickly. Metabolic testing requires animals to be temporarily housed in single cases which can sometimes cause distress.

Surgical procedures, for example to injure arteries or tie-off arteries in the groin, are performed under anaesthetic from which mice recover rapidly. Mice sometimes experience temporary weakness of the leg after surgery but are fully mobile within 24 hours. Occasionally poor blood flow to the leg can lead to loss of the tips of the toes. In most cases aneurysms do not cause any symptoms. However, as in humans, aneurysms can sometimes rupture which leads to rapid death of the animal from bleeding into the abdomen. Skin punch biopsies cause pain after they are made which settles as the wounds heal.

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)?

One of the protocols on this licence is mild severity; ten protocols are moderate and one is severe. The following proportions of mice are expected to experience the stated severity ratings:
mild: 400 mice (4%)
moderate: 10 180 mice (96%)

severe: 20 mice (0.2%)

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

- Killed

A retrospective assessment of these predicted harms will be due by 26 October 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 influence of diabetes on the cardiovascular system is complex. Diabetes comprises elevated blood sugar levels, raised insulin levels, resistance to the effects of insulin and activation of the immune system. All of these affect the function of cells in the blood vessel wall. In obesity, many factors are released from fat deposits into the circulation which also affect blood vessels. Development of blood vessel diseases arises from the combined effects of multiple biochemical factors, fats, circulating and locally produced hormones and growth factors on cells with the blood vessel wall. The complex interactions between these processes and the communications between individual cells as vascular diseases develop means that the diseases can only be effectively studied in animals or in humans.

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

We use a wide range of non-animal approaches to address our research aims. We use tissues from humans to identify genes which contribute to diabetes and cardiovascular disease. We perform much of our research in cultured cells from blood vessels to dissect out individual genes, proteins and pathways which influence their function. We mimic the context of diabetes by culturing cells in high glucose or high fat conditions. We generate proteins in cultured cells to assess how these behave and interact with receptors. We use computer-based modelling to design molecules to mimic the effects of these proteins. Finally we conduct clinical studies in humans to investigate the effect of diabetes on clinical outcomes and interrogate genetic databases and tissue banks to identify new targets.

Why were they not suitable?

These approaches complement and inform animal-based studies but unfortunately cannot replace them. As discussed above, the complex interaction between circulating and cellular factors implicated in the development of cardiovascular disease in diabetes means that this can only be studied in an intact animal.

A retrospective assessment of replacement will be due by 26 October 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 estimated the number of animals needed for each protocol based on our experience of using these approaches in previous projects and plans to continue work in this project along with new lines of investigation. In most cases we have based our assessment on statistical approaches to calculate the minimum number of animals to obtain significant results. However, as new genetic alterations will be studied as informed by ongoing research, we have made assumptions on future requirements based on our best assessment of the science and our previous experience.

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

We utilised available online resources such as the NC3Rs experimental design assistant to plan experiments and perform power calculations to determine sample size. These were based on knowledge of the mean values and variability of the primary outputs for each protocol based on our prior experience and on published data. We designed experiments so that multiple experimental readouts can be derived from a single animal. We use imaging when possible so that disease development can be tracked non-invasively and confirmed by tissue approaches after humane killing.

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

We work collaboratively with other researchers at our institution, so that we can share tissues between projects and avoid duplication of animal use. We optimise breeding of genetically altered animals (performed under the authority of another licence) so that breeding is fully aligned with planned experimental requirements. We use an electronic animal management system so users can track animals remotely and plan experiments to reduce waste. We keep updated with advances in scientific techniques and with ideas for reduction in animal use from the NC3Rs newsletter.

A retrospective assessment of reduction will be due by 26 October 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.

We will use mouse models of diabetes and cardiovascular disease. Typically, genetically altered mice will be used to study individual genes (or combinations of genes) in disease development. This approach will be supplemented by administration of drugs, viruses or cells when required to address scientific questions. We will use a wide range of methods and models to study the full range of human vascular disease. These are described in detail in the 'Project Harms' section of this application. Our general principle is to use the model with the least likelihood of causing suffering to address the scientific question.

We have gained substantial experience of surgical techniques during the course of our previous licence. We have performed >200 surgeries for arterial wire injury, >100 for carotid artery ligation or cuff placement, and >400 for hind limb ischaemia. This has allowed us to develop a number of refinements described in the section below. We have not needed to submit any Condition 18 reports relating to surgical procedures during the term of our previous licence.

We avoid single housing of animals unless essential for scientific reasons or animal welfare. We perform surgical procedures under general anaesthesia with routine use of analgesia. Longer procedures are covered with adequate hydration, warming tables, application of eye lubrication and post-operative warming.

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

It is necessary to use a mammal to study the complex interactions involved in the development of diabetes and cardiovascular disease and to translate the findings to humans. Although certain genetic factors implicated in blood vessel growth can be studied in zebra fish, it is not possible to model type 2 diabetes and more complex vascular pathologies in fish. Because vascular pathologies typically develop over days to weeks, is not possible to study the entire process under terminal anaesthesia in mice. Adults will typically be used as this is the life stage at which the human cardiovascular diseases in which we are interested develop.

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

Surgical procedures will be performed under general anaesthesia. Animals will be recovered in a warmed chamber following longer procedures. Analgesia will be administered routinely to avoid pain developing.

We have taken the opportunity to develop a number of refinements over the last five years during the course of our previous project licence. Induction of diabetes using streptozotocin now employs a low dose regime which in our experience avoids severe hyperglycaemia and ketosis. Induction of aortic aneurysm by angiotensin II infusion employs a modified dosing regime based on our previous studies in which we reduced the dose to minimise the risk of aneurysm rupture. Experience in arterial wire injury surgery has allowed us to reduce operative time to 15-25 minutes; we use minimal contact with the neurovascular bundle and avoid disturbing the associated fat pad; we perform the

arteriotomy in the saphenous branch to avoid ligating the main artery and to reduce complications such as immobility of the leg. For hindlimb ischaemia surgery, we have reduced operative time to 15-22 minutes; we avoid injuring surrounding tissues when ligating the artery; we use 8.0 suture to improve healing of the wound; we massage the leg after surgery to improve perfusion and encourage early mobilisation.

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

We will use the following resources in planning and conducting experiments: ARRIVE Guidelines 2.0. https://arriveguidelines.org/arrive-guidelines PREPARE Guidelines. https://norecopa.no/prepare
NC3Rs Experimental Design Assistant. https://eda.nc3rs.org.uk/

NC3Rs guidance on blood sampling in mice. https://www.nc3rs.org.uk/3rs-resources/blood- sampling/blood-sampling-mouse

NC3Rs guidance on microsampling, including the microsampling decision aid. https://www.nc3rs.org.uk/3rs-resources/microsampling

NC3Rs Mouse Grimace Scale. https://www.nc3rs.org.uk/3rs-resources/grimace-scales/grimace-scale- mouse

NC3Rs guidance on anaesthesia. https://www.nc3rs.org.uk/3rs-resources/anaesthesia
NC3Rs Guidance on analgesia. https://www.nc3rs.org.uk/3rs-resources/analgesia
NC3Rs Guidance on handling and restraint. https://nc3rs.org.uk/3rs-resources/handling-and-restraint

LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery. https://www.lasa.co.uk/wp- content/uploads/2018/05/Aseptic-Surgery.pdf

EFPIA/ECVAM good practice guide to the administration of substances and removal of blood, including routes and volumes. https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/abs/10.1002/jat.727

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

Our group will stay informed through the NC3Rs website. Relevant information, including the NC3Rs newsletter, is circulated within our institution by email to all personal and project licence holders. We will attend local events organised by our Animal Welfare and Ethical Review Committee and information sessions on NC3Rs funding streams organised by our institution's Research & Innovation Service. We will share best practice within our institution and have well developed interdisciplinary networks to facilitate this. We will hold regular local user-group meetings for project licence holders at which the group will receive updates on any changes to best practice or requirements.

A retrospective assessment of refinement will be due by 26 October 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?

26. Characterisation of models of cardiomyopathy

Project duration

5 years 0 months

Project purpose

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

Key words

Gene, Contractility, Adverse remodelling, Stromal and immune cells, Therapy

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

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?

This project aims to i) characterise mouse models of inherited cardiomyopathy with novel gene mutations; ii) develop new drugs and approaches to correct excessive and/or abnormal contractility in mutant cardiomyocytes (heart muscle cells); iii) investigate the role(s) of stressed mutant cardiomyocytes and the non-cardiomyocyte compartment in driving cardiac fibrosis and adverse remodelling in cardiomyopathies.

A retrospective assessment of these aims will be due by 26 October 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 it's 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?

Inherited hypertrophic cardiomyopathy (HCM) is a heart condition characterised by thickening (hypertrophy) of the heart (cardiac) muscle. It is the most common genetic disorder affecting approximately 1 in 500 individuals. In the UK, it substantially contributes to the burden of heart disease (overall NHS costs of heart disease are estimated at £11 billion annually). The outcome of this morphologic alteration may result in fatal disturbances of heart rhythm leading to sudden cardiac death (SCD), particularly in young athletes, symptoms including chest pain, palpitations, exercise intolerance, loss of consciousness, as well as heart failure which may require cardiac transplantation. Inherited HCM largely arises due to 'spelling errors' (known as mutations) in genes encoding components of the cardiac sarcomere, the essential unit of contraction in heart muscle cells. However, a causal mutation is not always identified in people with HCM, which suggests other unknown genes and/or factors that cause this disease exist. Characterisation of rodent models with established and novel gene mutations will enrich our understanding of the consequences of gene mutations to human HCM. Although it is known that HCM mutations typically cause excessive contractility and poor relaxation of heart muscle with increased energy consumption, there are very few specific treatments available with most medications currently in use not having been designed for HCM. These carry a risk of significant side- effects, are frequently limited in their ability to improve patient symptoms and – beyond the use of implantable cardiac defibrillator devices - do not alter the risk of sudden death, with none influencing the risk of developing end-stage heart failure.

Accordingly, HCM is associated with substantial morbidity and risk of premature mortality with socioeconomic consequences to the individual and society. It is our goal to develop novel compounds and therapeutic approaches that preserve or normalise the heart's function and modify the underlying disease process of HCM, with the potential to prevent and/or reverse the disease across the spectrum of mutations. HCM also leads to scarring of the heart, known as cardiac fibrosis leading to risk of SCD and abnormal cardiac function. Cell types in the heart other than cardiomyocytes are believed to make major contributions to cardiac fibrosis and adverse remodelling in response to dysfunctional mutant cardiomyocytes. There are no treatments for cardiac fibrosis in HCM, representing an important unmet medical need. Therefore, it is critically important to understand the role of the non-cardiomyocyte compartment in the pathogenesis of HCM (which includes immune cells, fibroblasts and endothelial cells) and identify novel therapeutic targets to treat disease contributors such as cardiac fibrosis and inflammation.

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

Advance understanding of disease
Research publications
Discover new drugs and treatment approaches
Who or what will benefit from these outputs, and how?

Peer-review publications will enrich the knowledge of the pathogenesis of inherited cardiomyopathies in the scientific community, providing the ability to identify novel targets

for intervention and identifying key disease nodes for proof-of-concept therapeutic evaluation in patients. In fact, we have identified some specific immune cell types that appear beneficial to heart remodelling in HCM and are currently conducting experiments to modulate this. We expect to develop and refine these experiments in the early stage of the project and test novel lines of research in the later stage, both in relation to HCM and other cardiomyopathies. The potential beneficiaries of this work include patients with HCM and their families, physicians and surgeons treating HCM, basic scientists with a special interest in cardiac disease and drug developers working on new therapies for cardiomyopathies.

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

The research group has strong links to internal and external scientific collaborators to support and maximise the outputs of the research. The outcome of this work will be disseminated to the research community with presentations at leading academic conferences and high-profile scientific publications. The development of novel genetic models of cardiomyopathy will be valuable to others in the wider scientific community. By substantiating a key role for the non-cardiomyocyte compartment in the development and perpetuation of cardiomyopathy and heart failure, the experimental approaches and therapeutic methods are likely to have broader relevance in other disorders of the heart. We will look to translate novel project findings into the clinic, including validation using human samples where feasible.

Species and numbers of animals expected to be used

- Mice: 28,400

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.

We use mice as a species because of their ease of genetic manipulation, and cardiovascular, neurohormonal and metabolic system allowing generation of disease models sufficiently close as to be highly relevant to human. The vast majority of our current understanding of the molecular basis of cardiomyopathy and heart failure is based on studies conducted in genetically altered mice. Mouse models of inherited cardiomyopathy are usually genetically altered and may develop characteristic features mirroring that of cardiomyopathy in humans at various stages of their lifetime. To be maximally informative and relevant for treatment of HCM in patients, we aim to understand the full spectrum of disease pathogenesis from mutation through to advanced tissue remodelling and heart failure.

However, every model has its own course of disease progression. Investigation early in the life stage will help us identify targets and approaches to delay onset or prevent emergence of HCM in those who carry disease-causing mutations. On the other hand, studies focused on later life stage with established features of events will facilitate the development of novel therapy or the identification of new targets to tackle or reverse established pathology.

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

A mouse model of inherited cardiomyopathy will typical undergo breeding and longitudinal cardiac phenotyping by using non-invasive approaches [e.g, echocardiography (echo), blood pressure (BP) measurement, electrocardiography (ECG), magnetic resonance imaging (MRI)] and/or a haemodynamic study under terminal anaesthesia within 24 months. Sometimes, a well-established surgical model of pressure-overload induced cardiac hypertrophy (e.g., transverse aortic constriction, TAC) may be used to enable detailed comparison and understanding of the molecular signalling changes characterising acquired cardiac hypertrophy, typically within 16 weeks of surgery.

Mice may also undergo the administration of certain substances to understand the effect of these on morphological and functional changes in the heart and their ability to rescue the disease phenotype. On the other hand, reagents or irradiation may be adopted to temporarily expand or remove or (immuno)modulate specific cell populations (e.g., immune cell types) to reveal their role(s) in the disease. The challenge period will typically last no longer than 20 weeks and the approaches used are well-defined and validated in the field. Where a cell/molecule manipulation is not already available, a genetically altered mouse model with compound mutations to mirror inherited cardiomyopathy and specific deficiencies in cardiomyocytes and/or non-cardiomyocytes (e.g., immune cells, fibroblasts, vascular or other stromal cells) will be generated and bred to study the individual role(s) of these cardiac cells in the pathogenesis of the disease under rigorously defined genetic conditions.

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

Mice with inherited cardiomyopathy can sometimes show weight loss, hunched posture, or enhanced respiratory rate as they age. An increasing rate of sudden cardiac death (SCD) may present at a certain age for some of our genetically altered models. These mice may show very little or no clinical signs before sudden cardiac death. Mice with an immune deficiency may be prone to infections, which are minimised by frequent bedding change and barrier setup. Irradiation can cause temporary weight loss, acute bleeding, acute infection and transient diarrhoea.

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)?

Mice: 45% Subthreshold, 30% Mild, 20% Moderate, 5% Severe

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

- Killed

A retrospective assessment of these predicted harms will be due by 26 October 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 use of animals is essential for our research programme. In studying the pathophysiology of cardiomyopathy, we seek to understand the interaction between cellular/biochemical changes and the work done by the functioning heart. Similarly, studying and modulating heart failure necessitates examining the intact functional heart as it responds to the complex interplay between autonomic nervous/vascular/endocrine and immune/inflammatory systems in the whole body.

The choice of a mouse model of inherited cardiomyopathy is due to its cardiac physiological features that are sufficiently similar to human hearts. It also provides a model for studying the pathogenesis from the onset to the advanced stage of the disease with less confounding effects compared to human samples. In addition, it offers an opportunity to develop new drugs and therapy to treat the disease.
Indeed, a substantial proportion of our current understanding of the molecular basis of cardiomyopathy and heart failure is based on studies conducted in genetically altered mice.

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

We have access to some rare human post-mortem samples as well as cardiac imaging of patients with HCM to investigate functional, morphologic, cellular and molecular changes in patient hearts. We plan to use various multiplex imaging and RNASeq based technologies to understand the complicated cardiac tissue remodelling process. In addition, we are seeking to establish additional international collaborations to acquire cardiac biopsy samples from patients for further investigation.

Why were they not suitable?

Human samples or imaging often provides a snapshot of the disease status. Myocardial biopsies are generally from patients with a specific form of HCM, known as obstructive HCM, do not reflect the full breadth of the human disease and are generally obtained from hearts with very advanced disease. A living mouse model offers an opportunity to study the full course of the disease from onset to advanced stages and most importantly, a tractable platform in which to test hypotheses, identify targets for treatment and evaluate novel treatment approaches as a critical step in clinical translation.
A retrospective assessment of replacement will be due by 26 October 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 estimate the number of animals based on our extensive previous experience with mouse models of cardiomyopathy. Previous cardiac and immune phenotyping experiments generally result in a requirement of at least n=10-15 per group for our least sensitive/most variable modalities. In procedures with attrition (e.g. surgical complications during in vivo haemodynamics), we will increase this to n=20 per group.

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

We routinely employ online tools to help us design the animal experiment such as the NC3R's Experimental Design Assistant. We make every effort to maximise the scientific data acquisition from the smallest number of animals. For example, we have successfully managed to apply serial non- invasive, highly sensitive cardiac phenotyping [e.g., echocardiography, magnetic resonance imaging and a terminal invasive cardiac phenotyping (e.g., haemodynamic study) on the same animal and collect tissues for further ex vivo/in vitro analysis]. We have also developed a multi-parameter flow cytometric approach for detailed cardiac cell phenotyping (e.g. six flow cytometry panels with 17 markers each), which allows us to significantly reduce the number of animals whilst obtaining deep insights into the impact of cardiomyopathy-causing mutations on the immune and stromal contexture of the heart. Pilot studies involving a small number of animals are integral to our experimental design.

Power calculations are routinely performed and consider likely losses incurred during the course of the study and variance in the measurement endpoint.

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

Good colony management. For example, an efficient breeding strategy allows us to avoid or reduce non-experimental genotypes. In addition, we only breed the numbers needed for experiments.

We also adopt a pilot experiment scheme to test unknown substances or novel methods. Only a small number of animals are used to establish a safe and effective dose range.

We share tissues with colleagues, collaborators and other university research groups where possible. We have built up an in-house tissue bank for future experiments and collaboration.

A retrospective assessment of reduction will be due by 26 October 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.

We have only selected rodent models in this project, given their ease of genetic manipulation, and that they are mammals whose cardiovascular system and disease models are sufficiently similar to human beings to provide relevant translational value. This includes mouse models with inherited cardiomyopathy. Mice with genetic mutations typically gradually develop clinical features when they age, which largely recapitulates what happens in patients with HCM. Sudden cardiac death (SCD) phenotype may occur at a certain age group with some genetic modifications and also depending on the strain. This also happens in patients with HCM, especially in young athletes and adolescents and therefore is a key aspect of the phenotype to understand. We will choose the least harmful age with a consistent discernible phenotype to perform experiments in order to reduce the SCD risk. Occasionally, we will use a surgical model in order to: i) investigate whether findings derived from genetic models could have a wider application to other cardiovascular settings; ii) determine whether a subtle or even absent cardiac phenotype in some HCM models could be accentuated by a single surgical procedure compared to those animals without genetic modifications. This is highly relevant clinically, in that it mimics the situation that a higher risk of cardiac hypertrophy and remodelling may present itself when environmental factors interact with inherited genetic defects, e.g. a patient with a sarcomeric HCM mutation who also has hypertension or features of the metabolic syndrome. All surgical procedures are standardised to adhere with aseptic techniques and post-operative care, with analgesia, heat support, access to water-softened chow, routine administration of fluid even in the absence of blood loss, topical eye lubrication all routine practice in our previous and current projects.

Why can’t you use animals that are less sentient?
We have only selected rodent models in this project, as they are mammals whose cardiovascular system is sufficiently similar to human beings and that may provide translational value. Species that are less sentient do not offer these benefits or faithfully recapitulate the consequences of cardiomyopathy mutations observed in humans. Mice with genetic mutations gradually develop clinical features when they age, which largely recapitulates what happens in patients with HCM. Neonatal mice or those at early life stages may not develop detectable pathogenesis. Animals will be anaesthetised for non-invasive cardiac phenotyping. They will be under terminal anaesthetic conditions when invasive cardiac phenotyping is used.

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

We will mainly use non-invasive cardiac phenotyping methods.

Where irradiation is used in a study, infection risk will be minimised by attention to strict hygiene measures and where appropriate the provision of prophylactic antibiotics for the first two weeks of the recovery.

We will choose the least harmful age with a consistent cardiac phenotype of interest (e.g. cardiac fibrosis) to perform experiments in animals in order to reduce sudden cardiac death risk in those transgenic models at risk forthis.

For animals undergoing a surgical procedure, peri-operative analgesia will be administered and continued after surgery for as long as required to prevent and alleviate pain. All surgical procedures are standardised to adhere with aseptic techniques and post-operative care as outlined within LASA guidelines. We also institute a post-operative care scheme to encourage recovery.

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

We will utilise the LASA, ARRIVE, Prepare guidelines and University internal resources in our experimental design.

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

We attend departmental animal welfare meetings where advances in the 3R's are discussed and disseminated. The establishment also has an annual 3R's meeting which we attend.

We have access to a Regional NC3R's manager who arranges seminars to promote best practice and also the Named Information Officer is a source of new advances.

We are also signed up to the regular newsletters from the NC3R's themselves and regularly check the website for information.

A retrospective assessment of refinement will be due by 26 October 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?

27. Developing novel therapies for inherited metabolic diseases

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
    - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
    - Assessment, detection, regulation or modification of physiological conditions in man, animals or plants
- Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

Gene Therapy, RNA Therapy, Exosome Therapy, Liver, Inherited metabolic diseases

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

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 our project is to design and deliver gene therapy in viral or non viral vectors in mouse models, which are mimicking the human presentation of untreatable genetic diseases. This will enable us to study and cure these diseases and translate these findings to the clinic to benefit patients.

A retrospective assessment of these aims will be due by 16 November 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 it's 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?

Inherited metabolic disorders (IMDs) are rare genetic diseases mostly occurring in childhood. There are hundreds of different genetic metabolic disorders, and their symptoms, treatments, and prognosis vary widely. Most people with IMDs have one defective gene that alters the efficacy of a key enzyme in a metabolic pathway. This alteration causes accumulation of toxic compounds upstream the metabolic block or shortage of key metabolites, which in turn will alter the physiology of the cell or the organ. Most IMDs are rare and considered as orphan diseases. They attract limited attention from industry and large pharmaceutical companies due to the limited number of patients and often due to the rapid and severe prognosis. The pathophysiology is poorly understood and treatments are often symptomatic and not curative, hence rarely modifying the course of the disease.

This work is essential for the following reasons:

This work aims to enable the generation of novel disease-modifying therapeutics, which will translate to patients. Our team has long-standing experience of clinical translation of novel drugs or biologics from bench to bedside.

The pathophysiology of these rare disorders can inform the pathophysiology of common diseases, like the involvement of urea cycle in tumorigenesis or the role of lysosome dysfunction in neurodegenerative diseases like Parkinson disease.

Treating these severe disorders with novel innovative technologies can demonstrate clinical efficacy of new biological tools which can then be applied to more common and less severe conditions.

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

The expected benefits of this project are:

Obtain preclinical data of efficacy and safety of new therapeutics for inherited metabolic diseases to allow clinical translation to patients

Refine and optimise therapeutic agents, especially vectors for gene therapies enabling higher efficacy and improved safety. For example in gene therapy, an engineered vector design might allow higher rate of on-target biodistribution, greater efficacy, prolonged gene expression, and/or reduced immune response.

Increase our knowledge about the pathophysiology and the function of genes causing rare inherited metabolic diseases resulting in potential new targets fortherapies.

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

Treatments for Inherited Metabolic Disorders are often limited and non-curative. These outputs will directly benefit patients affected by these genetic diseases, their families and their communities, in the UK and worldwide. Preclinical efficacy and safety data generated under this license will enable applications to regulators to initiate early phase clinical trials that might have huge benefits for patients. Although clinical translation is a long process, regulators have generated Breakthrough programmes to facilitate early access to patients, which has been in some recent examples been reduced to less than 2 years from gene therapy design to clinical administration.

Technological improvements will be shared to the community of researchers and can benefit to other research groups for other disorders. Most of vector modifications will be adaptable to other conditions, which will enable optimisation of research protocols for other diseases, enabling refinement of experimental design, or the reduction of animals.

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

Our findings will be published in high impact open access academic journals and presented at national and international conferences.

We are moving towards the use of preprint servers (www.biorxiv.org) to publish our findings even before submitting them to a formal journal with the sometimes protracted peer-review process. This allows rapid access of other researchers to our data. Advertisement of publications and conferences through social media will maximise visibility of these findings.

Collaboration will pharmaceutical companies like Evox Therapeutics and Moderna Therapeutics will enable large diffusion of findings through press releases by these companies.

Our findings will be disseminated to patients' organisations too through conferences as well as appropriate social media outlets. This will target the end-users of this innovative biotechnologies.

Species and numbers of animals expected to be used

- Mice: 7000

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.

We will develop these new therapies in small mammals to enable a rapid and easy clinical translation of therapeutic benefits to patients. We have chosen to use mice and not alternative animal models such as worm, fly, or fish models for this reason. If the

treatments are first developed in the fish model, they will require testing in the mammalian model prior to human patient studies. This is because mice are mammals and thus their basic biology is similar to humans – they contain all of the organ systems of interest such as liver, kidney, immune system, skin, and bone, which lower organisms do not, for example, worms lack an immune system. The biochemical pathways are also similar to humans. There are a few differences such as fur and eye colour – but we can instead identify jaundice and dry skin by looking at non-fur-covered areas.

Inherited metabolic diseases patients can present clinical signs already in utero, at birth or early childhood and therefore it is necessary to use all the stages of development of the mice to determine the best time for treatment intervention.

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

A mouse will typically receive a new therapy administered by a single injection in utero, at birth or later in life and be monitored for clinical signs of the disease mainly with minimally or non-invasive techniques such as blood sampling and live imaging. Finally, it will be euthanized and body fluids and tissues will be collected so we can analyse the efficacy and safety of the candidate therapy applied and effect on disease mechanisms. For some compounds with limited time efficacy, some weekly or bi- weekly reinjections will be studied.

The mice we will study have a disease presentation very similar to humans with similar signs and markers in blood or urine. Therefore the findings will be easy to support translation to humans.

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

The mouse models we will study are associated with jaundice, failure to thrive, sparse fur which starts from birth. Mice will be monitored up to one year of age then will be euthanised. If the suffering exceeds humane endpoints, these animals will be euthanised. In any doubt, advice will be sought from the NVS. The breeding of all strains will be controlled to ensure that over breeding is not practiced.

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 severity of most protocols is mild to moderate. Over 7,000 mice as project's total number, 4,000 mice will likely suffer no or mild severity; 2,950 mice will likely suffer moderate severity; 50 mice will likely suffer a severe severity.

One protocol with severity scored as severe for organic aciduria can cause, when the mouse is fed with a high protein diet, seizures, paralysis, and/or haemorrhages within 48 hours. This will be carefully monitored in liaison with the NACWO/NVS.

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

- Killed
- Used in other projects Rehomed

- Kept alive

A retrospective assessment of these predicted harms will be due by 16 November 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 whole process of designing and testing novel therapeutics, especially vectors for gene therapy is as follows: i) The vector is designed and validated computationally ii) It is tested in vitro, usually in more than one mouse line and, where possible, in cells differentiated from human induced pluripotent stem cells. iii) The vectors are finally tested for efficacy and, where appropriate safety, in the relevant animal model.

The disease we aim to cure multisystem diseases, which cannot be modelled in vitro, neither in single cell cultures nor in mixed culture or organoid systems. To translate these technologies to the clinic, preclinical studies in mouse models are a minimum requirement. Usually, well-performed mouse studies, combined with toxicity studies, outsourced to a CRO, are sufficient and higher animal studies are now no-longer a requirement for progression to the clinic for gene therapy of inherited genetic diseases.

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

We considered other model organisms such as worm, fly or fish models.

Why were they not suitable?

However, these models in non mammalian animals are not accepted to generate a sufficient level of efficacy and safety of preclinical data to allow clinical trial authorisation by regulators.

A retrospective assessment of replacement will be due by 16 November 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?

When performing mouse work, we calculate the minimum number of mice required to be sure that we will get an answer based on pilot data obtained from this protocol, or based on published studies using similar interventions and protocols.

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

By controlling other variables (age and strain of animals, environment), we can minimise the experimental variability and by using the NC3R's Experimental Design Assistant to calculate sample size with 90-95% confidence, we can reduce the number of animals used.

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

We perform pilot experiments for any new technology. Furthermore, we share the tissue collected from the same group of animals with several groups of researchers, where possible, to avoid duplication. From previous experiments, we have learned how best to design our experiments so that they do not need repeating, and that we can reach the standards required by industry and regulatory bodies.

A retrospective assessment of reduction will be due by 16 November 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.

We have chosen to use mouse models of disease which have the least amount of inter-individual variability. This allows us to identify illness more easily as it tends to follow the same pattern. Therefore, we are able to euthanize the animals before they suffer. We also use non-invasive methods such as bioluminescence live imaging to follow the biological processes in freely-moving animals, just by measuring light emission.

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

We aimed to produce preclinical data and for this, a mammalian model is needed, therefore we chose the mouse model. Some inherited metabolic diseases (IMDs) can present at birth and when possible the therapeutic treatment will be given before the onset of the disease (e.g. in utero). However, other IMDs will develop only if the animal is subjected to an altered diet at the more mature stage. Evaluating the efficacy of the new therapies tested is an important experimental outcome therefore utilising only terminally anaesthetised animals would not allow this to be observed.

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

All mice are monitored, by both BSU staff and by our own team members. Mice are provided with environmental enrichment, including tubes and wooden blocks and are habituated to procedures and non-aversive mouse handling methods. For mice where we anticipate disease symptoms, they are monitored daily and weighed as per protocol. Where appropriate, mice may be given dampened food or a specific food diet to alleviate the symptoms of the disease or surgery.

After surgery, animals are closely monitored for any signs of pain or distress. Analgesics are used before and after surgery and inhalation anaesthesia is used as this minimises the duration of anaesthesia and recovery time. The NACWOs and NVS are regularly consulted.

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

We will follow best practice guidance published by the National Centre for Replacement, Refinement, and Reduction.

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

The investigators are subscribed to updates from the National Centre for the Replacement, Reduction and Refinement of Animals in Research and will implement any advances in a timely manner.

A retrospective assessment of refinement will be due by 16 November 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?

28. Investigations into lumpy skin disease virus

Project duration

5 years 0 months

Project purpose

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

Key words

Poxvirus, Lumpy skin disease, Capripoxvirus, Cattle

Animal types	Life stages
Cattle	juvenile, adult, pregnant, aged, neonate

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?

To understand the disease caused by lumpy skin disease virus, and generate knowledge that will enable better control and prevention of lumpy skin disease.

A retrospective assessment of these aims will be due by 14 December 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?
Lumpy skin disease virus (LSDV) is a poxvirus that causes severe systemic disease in cattle and water buffalo. In the past ten years it has spread rapidly from Africa and the Middle East into Europe, Russia and throughout Asia, causing substantial loss to affected farmers and rural communities. LSDV is therefore a rapidly emerging, high impact virus. It is also a neglected virus with scant literature describing studies into the virus and the disease it causes. It is important to undertake the work described in this project licence in order to build our knowledge of the virus and the disease. This knowledge will enable and promote effective, safe and proportionate means of disease control and prevention. These may include better management practices to inhibit vector-borne virus transmission, better diagnostic tests to improve LSD surveillance programmes, and better vaccines to provide cattle and water buffalo with protection against LSDV.

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

The outputs of the project will be new knowledge about the pathogenesis and immunology of LSD, a better understanding of the vector-borne transmission of LSDV, and information about the efficacy of novel LSD vaccines. These outputs will enable improvements to be made to LSD control and prevention. The outputs of the project will be disseminated primarily via scientific publications and conference presentations

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

In the short term, beneficiaries will include other research groups working on LSD. They will be able to make use of more appropriate and well-defined experimental models of LSD.

In the medium term the beneficiaries will include veterinary vaccine manufacturers and companies that produce diagnostic tests for LSD, as they will be able to use the outputs of this project to develop new or improved products. Policy makers will also benefit from the new knowledge which can be used as an evidence-base to underpin decisions.

In the long term the beneficiaries will be farmers and rural communities currently affected by or at risk from LSD.

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

The outputs of this project will be published in open access scientific journals, and presented at national and international conferences. Protocols will be deposited online, and datasets deposited in public repositories. Samples from the studies will be made available to collaborators in the field. Where necessary intellectual property arising from this project will be protected by patenting.

The outputs will be disseminated to industry partners via collaborations, and to policy-makers via direct discussions and participation in expert working groups. Outputs will be communicated to the general public via press releases, articles on science-themed website, and public engagement events.

Species and numbers of animals expected to be used

- Cattle: 696

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.

Lumpy skin disease virus is very species specific and causes disease only in cattle and water buffalo. A less sentient species cannot be used in experimental animal models that aim to mimic natural disease.

Cattle under 8 months of age will be used for ease of handling.

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

Cattle will be inoculated with lumpy skin disease virus (LSDV) and the resultant disease studied.

Expected clinical signs include fever, swollen lymph nodes, skin lesions, and mild lethargy. Samples will be collected during the study including venous blood and skin. Cattle may be vaccinated and challenged. Blood-feeding insects may be allowed to feed on the skin. The typical duration of experiments will be 21-28 days.

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

Cattle may develop lumpy skin disease (LSD). This is characterised by fever, swollen lymph nodes, development of miltuple skin lesions, and mild lethargy. These signs appear between 5 and 14 days post challenge and last for up to 15 days. The animal can develop mild clinical signs, characterised by a small number of lesions and a short fever, or it can develop more extensive signs for example greater than 100 lesions and fever lasting more than 3 days.

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)?

Mild: 80%

Moderate: 18%

Severe: 2%

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

- Killed
- Rehomed

A retrospective assessment of these predicted harms will be due by 14 December 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?

It is necessary to use animals because we are studying the complex interactions between the host and virus. It is not possible to replicate these interactions without using animals.

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

Using non-animal alternatives to cattle has been considered by searching suitable websites (http://www.nc3rs.org.uk/, www.norecopa.no, http://www.frame.org.uk/). The scientific literature directly relating to the work that is being proposed was also searched for replacements for the proposed work (https://www.ncbi.nlm.nih.gov/pubmed/).

A membrane feeding assay was identified as a possible alternative to having insects feed on donor cattle in insect transmission experiments. This has included using thin slices of skin in a membrane feeding system. This area is being developed further. Other potential alternatives such as skin organoids, cutaneous explants and in vitro cell culture systems were also considered, and may be used in some studies.

Why were they not suitable?

The alternatives to animal use that were considered above are not suitable to replacing the work described in this project as they do not replicate the complexity of a systemic infection, as occurs in LSD.

A retrospective assessment of replacement will be due by 14 December 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?

The action plan of this PPL was used to plan the experiments likely to be carried out over the five year span of this licence. I then estimated the group sizes needed for each study following advice from an experienced statistician, incorporating the knowledge gained from previous work by ourselves and others with bovine experimental models of LSD.

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

I used advice from a statistician who is experienced in experimental design. This ensured I was proposing the minimum number of cattle required to obtain reliable and reproducible results. Each individual study will be reviewed by a statistician.

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

We will use the outputs from the research carried out under the previous licence which characterised different models of LSD. This included a large amount of data and archived samples which can inform decisions on the best study design (such as selecting specific time points within the model).

We have close collaborations with other researchers using experimental models of LSD. We will implement the learnings from their studies into our plans, and if possible, combine their studies within our planned experiments, therefore reducing the number of cattle used in LSD research. We also publish our results in a timely fashion in open access journals.

We will maximise the use of tissues from these studies. For example under the previous licence we provided serum samples from LSD inoculated cattle to international agencies. These samples are used for standard setting and as positive controls for diagnostic laboratories around the world. We also donated tissues to a histology slide set which was made available to veterinary schools for use in training vet students and veterinary pathology residents.

A retrospective assessment of reduction will be due by 14 December 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.

We will use a bovine experimental model of LSD that causes disease similar to that described in the field. Humane endpoints will be used to restrict the severity level to moderate in most studies. However some experiments may require the "severe" LSD seen in the field to be replicated experimentally.

Symptomatic treatment as agreed with a veterinary surgeon will be provided to alleviate suffering whenever possible.

Refinement will continue throughout the lifetime of the programme to eliminate or reduce to the minimum any possible pain, suffering, distress or lasting harm to the animals. Assessment of suffering will include both direct and contingent suffering, and take into account the time over which the suffering occurs.

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

LSDV is very species specific and causes disease only in cattle and water buffalo. A less sentient species cannot be used in experimental animal models that aim to mimic natural disease (objectives 1- 4), or in field studies (objective 5).

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

Housing and husbandry: Cattle will be housed in social groups whenever possible. Cannulated calves may be housed singly so that the cannula is not dislodged, however they are in sight and sound of another animal. Cattle naturally spend a large amount of time each day browsing for food and in social interaction. In order to mimic this in high containment conditions, and therefore minimise contingent suffering, we will provide them with ad-lib hay along with a dry lying area (rubber matting) to aid rumination. Enrichment devices will be supplied such as toys and fruit/vegetables as a reward. Salt licks will be available.

Pre-study meetings involving the NVS, NACWO and animal services staff will be held to discuss any advances in animal care. Meticulous records will be kept of behavioural, physiological, immunological and virological measures in order to identify predictive markers and design humane endpoints for future experiments. Pain and distress scoring sheets specifically designed for LSD will be used. Highly trained animal technicians will monitor these animals throughout the day, ensuring they are comfortable and to maximise their welfare status. We have 24/7 CCTV surveillance which can be used to monitor the animals behaviour over time. All experiments will be followed by a wash-up meeting to discuss all aspects of the study and to ensure lessons are learnt.

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

I will follow the PREPARE (Planning Research and Experimental Procedures on Animals:

Recommendations for Excellence) guidelines for planning our experiments (https://www.ncbi.nlm.nih.gov/pubmed/28771074) and ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines for reporting these studies (https://www.nc3rs.org.uk/arrive-guidelines).

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

I will attend appropriate 3Rs conferences, read the relevant scientific literature including the veterinary literature on pain relief, and undertake regular project licence holder training and refresher courses. I will take advantage of news and information provided by the NTCO. I will also use other sources of information such as:

The NC3Rs

(AALAS) American Association for Laboratory Animals Science

(FELASA) Federation of European Laboratory Animal Science Associations (ICLAS) International Council for Laboratory Animal Sciences

A retrospective assessment of refinement will be due by 14 December 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?

29. Pathogenesis and prevention of infections by respiratory pathogens

Project duration

5 years 0 months

Project purpose

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

Key words

pneumonia, Streptococcus pneumoniae, Acinetobacter baumannii, Vaccines, Pathogenesis

Animal types	Life stages
Mice	adult, juvenile, aged

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?

To improve the prevention and treatment of infections of the lung, specifically pneumonia caused by bacteria. As bacteria that cause pneumonia also cause infections at other anatomical sites (for example, the brain to cause meningitis or the blood to cause septicaemia) some of the experimental work will also involve non-lung infection models.

A retrospective assessment of these aims will be due by 05 November 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 it's 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?

Respiratory infections and pneumonia are the commonest cause of death due to infectious disease in the world, estimated to cause several million premature deaths per year (more than malaria and HIV infection combined). A high proportion of these deaths occur in children. In the developed world, pneumonia is the commonest cause of death due to respiratory illness and is the commonest cause of severe infections acquired when in hospital with other illnesses. Common causes of bacterial pneumonia are Streptococcus pneumoniae, Staphylococcus aureus, other streptococci, Pseudomonas aeruginosa, Klebsiella pneumoniae, Acinetobacter baumannii, Chlamydia, and Mycoplasma.

Combined infection with bacteria and viruses is common, and probably increases the severity of pneumonia. The massive clinical importance of pneumonia is compounded by the increasing levels of antibiotic resistance in the common bacterial causes (especially P. aeruginosa, K. pneumoniae and A. baumannii), and the lack of effective vaccines that can prevent pneumonia in adults. Overall there is a strong clinical need for new therapeutic strategies to treat or prevent bacterial pneumonia and other respiratory infections.

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

The mortality and morbidity due to respiratory infections is enormous and any new clinical interventions that can reduce these would have important clinical benefits. This is the ultimate aim of the proposed work under this project licence. Specific outputs will include:

New knowledge on the pathogenesis of bacterial pneumonia, specifically (a) the molecular mechanisms underpinning how pneumonia pathogens are able to cause severe infection and death in the mammalian host, and (b) how the host prevents or combats bacterial pneumonia through innate and adaptive immune responses. The work will mainly concentrate on the commonest cause of pneumonia S. pneumoniae and the increasingly important Gram negative cause of antibiotic resistant pneumonia A. baumannii.

Investigate novel vaccine strategies for preventing bacterial pneumonia. Our work has resulted in early phase clinical trials of two potential novel vaccine approaches for preventing S. pneumoniae infections, and to two patent applications for novel vaccines. Work under this licence will continue to provide the pre-clinical data needed to support these existing vaccine candidates, and will also identify additional potential novel vaccine candidates.

Novel therapeutic approaches for bacterial pneumonia. For example, our work on A. baumannii aims to develop an antibody therapy for acute infection that overcomes the high level of antibiotic resistance in this pathogen. In addition, detailed assessment of the innate and adaptive immune response to respiratory infection is intended to identify components of the immune response that could be manipulated to either prevent respiratory infections or improve the outcome of patients presenting with pneumonia.

The data will provide fresh insights into the molecular pathogenesis and prevention of respiratory infections which will be disseminated within the scientific community by publications in relevant journals, presentations at national and international conferences and invited talks to research organisations. Experimental work performed using the previous project licence for this work supported 3 to 4 research publications per annum.

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

Short term (ongoing over duration of the PPL, occurring within 1 to 2 years post-publication / presentation of data):

Scientific data of interest to academics interested in host-pathogen interactions, clinicians looking after patients with respiratory infections.

Identification of new therapeutic / vaccine candidates

Immunology data that indicates which patient groups are at higher risk of infection with bacterial pneumonia pathogens

Medium term (next 5 years):

Additional pre-clinical data to support vaccine or therapeutic candidates undergoing early phase clinical studies

Development of new early phase clinical products for human studies, with potential for partnerships with biotech or pharma for their further development

New clinical tests to identify patients at high risk of pneumonia Long term
Novel vaccines or therapeutics that improve prevention or treatment of bacterial pneumonia, leading to benefits in patients.

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

Outputs will be maximised by:

Presentation of the data at scientific conferences (mainly international, some national ones)

Publication of research papers describing our findings in scientific journals eg PLoS Pathogines, Nature Communications, mBio, Infection and Immunity, Frontiers Immunology etc.

Publication of reviews and editorials that highlight our work and place the data in context, including their clinical implications

Patents on new vaccine and therapeutic products developed through close partnership with university commercialisation departments (as we have already done for experimental data obtained using the last PPL for two vaccine candidates)

Interactions with biotech and pharma to obtain external support for development of potential vaccine and therapeutic candidates.

Continuing established collaborations in the UK, Europe, USA, Thailand, and Australia and developing when necessary new collaborations

Species and numbers of animals expected to be used

- Mice: 2500

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.

We use adult mice because:

Infection models in mice for bacterial pathogens usually mirror human infection very closely, with considerable overlap betweeen mice and humans in the immunological and histopathological response to infection, and a temporal development of infection that is similar to human disease. The tools for the genetic and non-genetic manipulation of the mouse to characterise the role of different immune components are largely readily available, considerably increasing the power of the data obtained.

Mouse models of infection are already established in my laboratory for multiple S. pneumoniae strains, and for a couple of A. baumannii strains.

Most animal infection work with these pathogens performed by other groups has used mouse models, allowing direct comparison of our results with data obtained by multiple other investigators

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

Typically a mouse will be infected with the bacterial pneumonia pathogen by intranasal (under inhalational general anaesthesia), intraperitoneal, or intravenous inoculation (depending on the infection model). The mouse will then develop infection over 24 to 48 hours, developing physical signs of ill health with reduced mobility, piloerection, reduced response to stimuli, and some loss of weight.
However, most mice will be culled using a schedule 1 procedure at timepoints we have previously characterised are before they have developed significant clinical evidence of progressive symptoms but still have established infection within the lungs or other sites of infection; these time points are typically 24 to 48 hours after inoculation, although longer ones are used for nasal colonisation experiments as colonisation does not lead to active infection. The pre-specified times for mouse culling most procedures will be of mild or moderate rather than severe severity. Target organs. (eg lungs and spleen) and blood will be obtained from culled mice for analysis of bacterial numbers and the immune response to infection using a range of laboratory techniques. In some experiments mice will undergo immune manipulation prior to infection, for example vaccination with novel vaccine candidates by intraperitoneal injection two or three times in the preceding 1 to 2 months, or antibody depletion of an immune effector cell just prior to infection. Occasional experiments will be performed in genetically modified mice to provide more detailed data on host factors affecting the response to infection.

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

Pain at injection sites (for intraperitoneal, intramuscular, intravenous and subcutaneous injections) - temporary, usually self-limiting within a few seconds.

General anaesthesia can temporarily lead to disorientation and poor motor control - duration is less than five minutes, often much shorter.

Physical signs of active infection: reduced mobility, piloerection, reduced response to stimuli, and some loss of weight (<10% body weight), typically lasting for 24 to 96 hours depending on infecting strain and route of infection.

Very occasionally unplanned death may occur due to a procedure (eg inhalational general anaesthesia) or unexpectedly rapid progress of an infection in a particularly susceptible mouse or due to a more virulent infecting organism than planned.

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)?

Severe severity - <5% Moderate severity - 40 to 50%
Mild severity - 40 to 55%

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

- Killed

A retrospective assessment of these predicted harms will be due by 05 November 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 combination of lung anatomy, a highly complex immune response to infection, and changes in the invading bacterial pathogen during infection prevent a comprehensive understanding of lung infections without using animal models of infection. For example, the lungs have a three dimensional structure consisting of an epithelial layer with multiple cell types organised into tubes and air sacs that interact closely with blood vessels that allow different types of cells to be recruited to the site of infection over time. During infection several different types of white cells are recruited to the lungs with their relative proportions varying depending on how long since the infection has started and whether the animal has been infected with that bacteria before or has been vaccinated or not.

Evaluating a new vaccine is only possible using an animal model as a complete immune system is needed for the development of vaccine responses and for assessing how those responses alter subsequent infection. Different types of vaccine will alter the response to the infection in multiple different ways, and this can only be seen in a whole animal. In addition bacterial infection of the lung often spreads to elsewhere within the body, for example to cause septicaemia, and this is important as it makes the infection more severe but can only be identified if an animal model is used.

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

We have considered using the following alternatives to animal models of infection, all of which can be used for modelling some aspects ofinfection: Cell culture - culture of individual types of cells such as epithelial cells or white cells; can be used to make layers of cells that model the respiratory epithelium, and can be used with 2, 3 or even 4 different types of cells to include some immune cell interactions between white cells and / or the epithelium.

Organoids - groups of cells cultured together to form tissue that looks like a lung under the microscope Lung slice models - culture of a section of lung tissue in the laboratory Human infection models - a nasopharyngeal colonisation model is available for S. pneumoniae

Why were they not suitable?

Cell culture - we use these for investigating bacterial interactions with 1 to 3 types of cells to support the results obtained using animal models. They do not have the anatomical structure of the lung and can only include a very limited number of immune cells. Hence they can not be used to model the complex anatomy and immune interactions that occur during pneumonia.

Organoids or lung slice models - both of these can only contain some components of the immune response and these are not replenished / altered due to recruitment from the blood etc. They are therefore are poor imitations of what happens during lung infection, and they absolutely cannot be used for assessing novel vaccines as that requires a fully intact immune system. It is also difficult to assess spread of infection to the blood using these models.

Human infection models - we use these for assessing the effects of colonising the back of the throat with S. pneumoniae but for obvious reasons they cannot be used to assess how severe infection develops or can be prevented. Furthermore novel vaccines or therapeutics have to go through evaluation in an animal model for safety reasons.

All of the above are used to limit the situations when an animal model is necessary eg we can evaluate bacterial interactions with epithelial cells in detail using cell culture or lung slices rather than an animal infection model.

A retrospective assessment of replacement will be due by 05 November 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 yourexperience?

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?

The estimates are based on (a) historical use from my existing project licence (2017 751 mice, 2018 587 mice, 2019 558 mice, 2020 335 mice - these are falling over time and it is likely that the more recent lower numbers will reflect future work: (b) the grant funding and potential future grant funding will maintain my group size at its present level, and it is unlikely that I will expand the number of researchers working using animal models substantially over the next five years.

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

We minimise the number of animals needed during the experimental design by:

By only looking for large biological effects using animal models - these need fewer mice than subtle effects to obtain a statistically significant result.

Using data outputs such as bacterial CFU or cytokine responses that can identify differences between groups using only a small number of mice (eg 5 to 8).

When appropriate using an experimental design that is highly sensitive eg comparing the virulence of bacterial strains using competitive infection experiments that only need 3 to 5 mice.

Our considerable background experience in using mouse models of pneumonia means we have the necessary experience to design experiments that minimise the number of mice required to answer a specific experimental question.

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

We can minimise the number of mice required for our research by:
Using laboratory models of specific bacterial host interactions to fully define how a given bacterial mutant or component maybe interacting with the host before moving to animal models - that way we can be very specific about the information needed from an animal model and thereby reduce the number of mice required.

For some research questions we can replace animal infection models with cell culture and lung slice infection models that can provide the same data eg bacterial interactions with epithelial cells can be investigated using cell culture rather than an animal model.

Whenever possible we can obtain tissue that we may need for laboratory work (eg bronchoalveolar lavage fluid, serum or lung slices) from the same animal to minimise the number of mice needed in total.

A retrospective assessment of reduction will be due by 05 November 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.

Our experiments are performed in mice. Mice are an appropriate species as infection in mice closely mimics human infection and because there are a wide range of methods available for mice that help investigate the immune response to infection. We minimise welfare costs of the infection experiments by: (a) using for the majority of the infection experiments pre-selected timepoints for culling mice – this means that most mice will only develop mild or moderate clinical signs of disease before being culled; and (b) close monitoring of mice over the experimental period to identify any that may develop evidence of unexpected side effects that may need to be culled to minimise suffering. Although experiments may last weeks, for most of that period the mice are perfectly healthy and are between vaccinations. The active infection models are short lived, lasting only a few days at most and occur at the end of any experimental period.

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

The incredibly complexity of lung anatomy, immune responses to infection, and bacterial / host cell interactions prevent these experiments from being done without using animal models of infection. For example, the lungs have a three dimensional structure consisting of a mucosal layer with multiple cell types, and during infection several different types of white cells are recruited to the lungs with their relative proportions varying in a very dynamic way. A vaccine will alter the response to the infection in multiple ways. In addition the bacterial infection may spread from the lungs elsewhere within the body. This highly complex process cannot be fully replicated in laboratory cell culture models, nor by insect or fish infection models and therefore requires a mammalian infection model.

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

The animal models we use have been developed over many years and refined to minimise the welfare costs using the following techniques:

using pre-selected timepoints for culling mice – this means that most mice will only develop mild or moderate clinical signs of disease before being culled

close monitoring of mice over the experimental period to identify any that may develop evidence of unexpected side effects that may need to be culled to minimise suffering

using local or systemic analgesia for procedures that cause pain eg insertion of minipumps using general anaesthesia for more complex procedures such as inoculation with infective organisms by inhalation

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

We will adhere to guidelines issued by LASA and NC3Rs. Furthermore, we will endeavour to report our findings accurately using ARRIVE guidelines.

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

Institutional seminars and workshops will provide continuing professional development in the 3Rs, while the NC3Rs website provides a readily available resource at all times.

A retrospective assessment of refinement will be due by 05 November 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?

30. Investigating brain function and dysfunction.

Project duration

5 years 0 months

Project purpose

- Basic research

Key words

stroke, ageing, vascular disease, therapy

Animal types	Life stages
Rats	adult, aged
Mice	adult, aged

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?

Overall, this project is aimed at understanding how brain function is altered, or becomes dysfunctional, as a consequence of vascular disease and the ageing process.

A retrospective assessment of these aims will be due by 27 December 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?

In terms of vascular disease we are primarily interested in ischemic stroke which occurs as a consequence of a blockage in a blood vessel within the brain. Stroke is a significant cause of mortality and functional disability with limited treatment options available. Ageing is a significant risk factor for stroke and impacts upon the damage produced due to changes within the vascular system during ageing. In the current project, we aim to further understanding of the molecular mechanisms involved in the damage after stroke and aim to identify potential therapeutic targets that may then undergo further clinical investigation.

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

The primary aim of this work is the advancement of scientific knowledge. Secondly we aim indirectly to contribute to the treatment of ill health. Through this advancement of scientific knowledge we aim to contribute to the treatment of ill health by identifying novel treatment strategies that warrant clinical investigation. In addition, we aim to constantly refine the use of experimental stroke models and improve animal welfare.

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

The main beneficiaries of this research are both academic and industry scientists working in CNS function and dysfunction. All major UK institutions have some form of neuroscience department where researchers of multiple research areas are using varied approaches to better understand and protect CNS physiology.

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

Information obtained is disseminated within the scientific field via presentations at conferences and peer-reviewed publications. All relevant researchers may benefit from this research through dissemination and networking. This will be done by presentation at local research events, invites to other institutions and symposia, national (e.g. UK Preclinical Stroke Symposium, held bi-annually) and international (e.g. Society for Neuroscience meeting, held annually) meetings/conferences.
Additionally, dissemination of research findings will occur via publication in high impact and freely available journals. We have previous experience in publishing in open access journals (e.g. F1000), publishing method-based articles (e.g. Journal of Visualised Experiments) and publishing null results. Where appropriate we will openly share data/datasets/tissue.

Species and numbers of animals expected to be used

- Mice: 80
- Rats: 120

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.

Overall, this project is aimed at understanding how brain function is altered, or becomes dysfunctional, as a consequence of vascular disease and the ageing process. Thus, it is necessary to use adult animals and those animals at later stages of the lifespan.

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

Work under this authority forms part of larger multi-disciplinary research programme which utilises a variety of in vitro, in vivo, imaging and epidemiological approaches. Some animals will undergo minimal procedures, such as being used to obtain brain tissue for identification of relevant molecular markers. For animals undergoing experimental stroke it is necessary to create an area of tissue damage which produces pathological and functional deficits. The area of damage is produced by temporarily blocking blood flow within a blood vessel which supply oxygenated and nutrient-rich blood to specific areas of the brain. This period of blood flow blockage/reduction results in cell death and creates an area of damage similar to that seen in human stroke. Following the creation of a stroke lesion animals may then undergo imaging and/or behavioural assessments which may involve some repeated measures. Such procedures are essential in allowing us to track the progression of damage and response to any potential treatments. The stroke lesion may impact on both cortical and striatal areas - thus behavioural assessments allow us to monitor the impact of stroke and/or treatments on cognitive and sensorimotor functions. Imaging studies allows us to visualise and measure changes in brain function as a consequence of stroke, ageing and potential treatments - this can include measures of, for example, lesion area, oedema volume and connectivity.

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

Approximately 70% of animals will only experience mild effects which may include, but not be limited to, injection of a substance (e.g. drug of interest) or injection of anaesthetic prior to undergoing an imaging procedure. We anticipate that ~30% of animals undergoing a stroke will experience adverse effects. This may include the animal experiencing moderate degrees of: paralysis of the contralateral face and limbs, sensory loss in the contralateral face and limbs, hemispatial neglect, hemiparesis and hemiplegia. Affected animals require fluid and food supplementation. All animals post-stroke are carefully and regularly monitored. Post-operative monitoring sheets are completed and kept with the animals ensuring their progress can be identified by any member of technical staff. Weight loss has a significant effect on recovery following experimental stroke and we implement a number of measures to promote feeding and drinking following experimental stroke. We also apply a local anaesthetic/analgesic regime to all animals undergoing experimental stroke.

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)?

We expect ~20% of the animals under this project licence to experience mild severity, ~50% to experience moderate severity and ~30% to experience severe.

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

- Killed

A retrospective assessment of these predicted harms will be due by 27 December 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 overall aim of this work is to investigate how brain function is altered during ageing and vascular disease. Animals are required in order to dynamically study the relationship between vascular supply and neuronal function. In vitro experiments are a complimentary aspect of the overall research programme but cannot replace the use of in vivo animals for studying dynamic changes in blood flow (e.g. via MRI scanning) and the impact of vascular disease i.e. stroke.

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

n/a

Why were they not suitable?

No answer provided

A retrospective assessment of replacement will be due by 27 December 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?

Predictions are based on a 5-year programme of research and take into account research plans/feasibility etc. Animal numbers are calculated per individual studies using power analysis calculations - these draw on, where relevant, data from pervious studies we have conducted or exist in the literature. Good principles of experimental design are followed,

and the NC3Rs experimental design assistant used, to ensure appropriate random allocation, blinding to outcomes and appropriate control groups.

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

The minimum number of animals required to achieve the scientific objectives will be accomplished through good study design largely using the NC3Rs Experimental Design Assistant. Statistical 'inhouse' support is available as well as consulting clinical statisticians through established collaborations with clinical stroke physicians. All Personal Licensees (and dedicated animal care staff) working on this project will be appropriately trained and suitably competent to ensure a high success rate is achieved with the minimum number of animals used. We have previously demonstrated refinements to methodological approaches which reduced variability in outcome measures and a direct reduction in number of animals required per study. The use of MRI imaging to obtain outcome data allows a longitudinal design to be applied and therefore animal numbers reduced. This is particularly relevant for those studies involving aged and stroke animals – as separate groups of animals are not required at each age/time point.

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

For studies investigating potential therapeutic strategies following stroke in vivo experiments will only be conducted once satisfactory outcomes have been obtained using in vitro (e.g. cell cultures) and/or ex vivo (e.g. brain slices) approaches. Depending on the nature of the specific project being undertaken we will look for opportunities for tissue and data sharing.

A retrospective assessment of reduction will be due by 27 December 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 main experimental model being used in this programme of research is an experimental model of cerebral stroke. Stroke models can be divided into two main types: global and focal. Global models (either complete or incomplete) are generally considered less relevant to human stroke as global ischemia is not a feature of human stroke - such models are generally considered to model the cerebral consequences of cardiac arrest

rather than cerebral stroke. Focal models are considered the most clinically relevant models of stroke produces a reliable and reproducible area of infarct whilst avoiding the need for craniotomy, thus ensuring BBB permeability, brain temperature and intracranial pressure are not directly influenced by the surgical technique. Experimental stroke models are demonstrated to have high construct validity but have significant impact on animal wellbeing including weight loss, risk of mortality and behavioural deficits. Of course, following induction of experimental stroke there are expected clinical signs due to this being a clinically relevant model of stroke and the severity of such signs is dependent upon the duration of stroke induced. We limit the length of occlusion to no more than sixty minutes in order to reduce the impact to the animal whilst still producing stroke-damage relevant to our scientific questions.

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

In order to mimic clinical stroke lesions, we require an experimental species high in the evolutionary tree (i.e. mammalian). Rodents are a clear and desirable choice, being mammalian species that are widely-used in scientific research due to their relatively-low neurophysiological sentience. Their cerebral vasculature is relatively well-known and is in many ways similar to the human.

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

An ongoing aim is to constantly refine the use of experimental animal models and reduce the impact on the animal. Evidence suggests that environmental enrichment may improve animal welfare both generally and following injury to the Central Nervous System (CNS) injury, such as experimental stroke but such interventions require validation which we plan to do here. In terms of animal welfare telemetry may be useful to aid in the identification of intervention and/or humane end points. Affected animals require fluid replacement immediately following experimental stroke and access to wet mash subsequently. All middle cerebral artery occlusion (MCAO) animals are carefully and regularly monitored. Post-operative monitoring sheets are completed and kept with the animals ensuring their progress can be identified by any member of technical staff. Animals usually display subdued and limited behaviour and will be housed in a warmed environment for at least the first 24h post-MCAO. MRI imaging is carried out by skilled technicians who have spent a considerable amount of time refining the methods used during the MRI imaging procedure to allow better physiological monitoring of animals.

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

IMPROVE guidelines.

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

Through active participation in our scientific community - we are able to review and read other studies and access information through the NC3Rs portal and via dissemination within the UK preclinical stroke community.
A retrospective assessment of refinement will be due by 27 December 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?

31. Immunity to Parasitic Pathogens of Ruminants

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
  - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - Improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes
- Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

Ruminant, Vaccine, Tropical, Immunology, Parasite

Animal types	Life stages
Cattle	juvenile, adult, neonate, pregnant, aged
Sheep	juvenile, adult, neonate, pregnant, aged
Goats	neonate, juvenile, adult, pregnant, aged
Buffalo	adult, juvenile, neonate, pregnant, aged

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 overall aim of this project is to contribute to the development of improved strategies to control important vector-borne livestock diseases. This includes studies aiming to provide a better understanding of the parasite and host parameters that determine the outcome of Theileria and Trypanosoma infection of ruminants and studies to aid in the development of

modified tick populations that can be used as an alternative means of vector-based disease control.

A retrospective assessment of these aims will be due by 21 December 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?

Theileria, trypanosome and tick parasites are major causes of livestock disease and death in many low-middle income countries, having severe animal health and welfare effects and placing large economic burdens on farmers in the affected countries. Performing scientifically robust studies to i) better understand the interactions between Theileria and trypanosome parasites and the host immune system and ii) aid in the development of novel tick-control strategies are critical to the development of novel methods to help reduce the burden caused by these important diseases.

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

Generation of effective vaccines for many diseases, especially parasitic diseases, remains challenging. The work in this study will provide a better understanding of the immunological mechanisms that determine vaccine success and failure, as well as the strategies used by parasites to evade host immunity that either confound vaccine development or enable vaccine escape. Work conducted under this license will also aim to provide a better understanding of how genetic diversity influences the ability of animals to resist parasitic diseases. The studies will also result in the generation of new reagents/technologies that can be used to refine our understanding of ruminant immune responses.

This information and novel tools will contribute to advances in the development of vaccines to tackle these important veterinary diseases. As a second component of the work conducted under this license we will work with partners on studies to generate modified ticks that can be used as an alternative method to reduce the burdens associated with vector-borne diseases. As with all scientific work, the outputs of the studies conducted under this license will be disseminated to other scientists and relevant stakeholders through a variety of media including publications and conference presentations.

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

Short-term benefits:

Generation of novel data on:

- how Theileria and Trypanosoma spp. interact with host immunity to delay or prevent parasite clearance.

Theileria; we will apply novel techniques (e.g. T cell receptor (TCR) repertoire analysis, transcriptomics) to provide a more comprehensive analysis of the aberrant T-cell responses induced by
T. parva during primary infection of naïve animals.

Trypanosomes: we will expand previous work defining the antigenic variation system in trypanosomes, as well as characterising the immunosuppression induced by trypanosomes - in particular the role of Natural Killer (NK) cells in mediating B-cell (and immune memory) destruction during infections.

For both parasites our group and collaborators (national and international) will be able to exploit the information generated to formulate new hypothesis on how these parasites effect immune-subversion and the potential to exploit this to develop innovative vaccine and therapeutic strategies.

- immune responses induced by different candidate vaccines to evaluate their efficacy, and compare these to protective immunity to identify immunological parameters that confer protection.

In Theileria studies, we will seek to describe in detail the role of cell mediated-immunity (by both CD8+ and CD4+ T-cell lymphocyte populations and non-conventional lymphocyte populations such as NKp46+CD3+ T-cells). For CD8+ T-cells this will include analysis of parameters such as TCR diversity, functionality and transcriptomic profiling of populations induced by different vaccination strategies that differ in their capacities to confer protection. Based on work conducted under previous licences these studies will also be extended to include similar analyses of CD4 and NKp46+CD3+ T-cells. These data will be used to understand which parameters are critical for vaccine success (correlates of protection). This information will be of use to researchers studying vaccine design for Theileria and other pathogens that require T-cells for immunological protection. Consequently, the outputs generated from this licence will be of benefit to a variety of research groups both nationally and internationally and used to inform their own studies and the rational evaluation of vaccine strategies.

In trypanosome studies data on the efficacy of novel vaccination strategies, including the potential use of Trypanosoma theileri and Trypanosoma melophagium as vaccine vectors (in cattle and sheep respectively), the characterisation of newly identified vaccine candidates, and the exploitation of ‘long CDR3’ antibodies as an immunisation strategy against trypanosomes, will be generated. We will also continue to refine tools that facilitate assessment of clinical efficacy, including diagnostic markers of active infection and immune correlates of protection. Much of this work will be conducted as part of international consortia providing highly relevant data in cattle to complement and translate parallel data being generated in murine and human models in a number of local, national and international laboratories.

– the genetic diversity of ruminant loci that influence ruminant immune responses to infection.

In immunogenetic work we will apply Major histocompatibility (MHC) genotyping technologies to provide a comprehensive analysis of MHC variation in British ruminant populations. This will complement work ongoing to study MHC variation in a variety of countries including Brazil, Zambia, Uganda and Kenya, which forms an ancillary body of work to the infection and immunity studies being conducted with Theileria and

trypanosomes under this licence. There are a number of research groups (including from Brazil, US, Kenya and Germany amongst others) who have already requested collaborative projects based on MHC analysis, demonstrating that there will be academic beneficiaries from the data and technologies generated from these studies. In particular many groups are keen to incorporate MHC analysis into genetic association studies with disease resistance/susceptibility. As an example of this we will host researchers from Egypt, Brazil and Germany in late 2022, completing work adding novel functional dimensions to MHC work using in vitro techniques (MHC-peptide elution studies) following on from preliminary immunogenetic studies conducted using these technologies.

The broader genetic, transcriptomic and genetic/epigenetic work provides a foundation that will enable other loci that influence immune responses to parasitic infections to be studied - this is important in defining the immune response to what are complex organisms. There is clear evidence from a large number of studies that such factors can be crucial in obtaining a comprehensive understanding of immune responses, and the inclusion of such studies in this license will enable novel details of how epigenetic/genetic variation may fundamentally determine the outcome of ruminant immune responses to parasite infection and/or vaccination to be identified.

- the capacity of novel technologies to improve vaccine production - a collaborative project will provide validation of a novel way of purifying Theileria sporozoites. Such technology could help streamline production of the current Theileria parva vaccine as well as facilitate improved Quality Control and Quality Assurance checks on the final vaccine product. If successful it is anticipated that the technology will rapidly be transferred to vaccine manufacturer's based in Africa. As a consequence this component of the studies has the potential for rapid translation with benefits to industrial vaccine producers, farmers and livestock in the regions where Theileria parva is endemic (eastern, central and southern Africa).

- development of genetically modified ticks with reduced reproductive capacity - working with an industrial partner with expertise in genetic modification of invertebrate vectors we aim to conduct studies contributing to the development of self-limiting strains of Rhipicephalus microplus, a tick that is rapidly spreading in large areas of the tropical and sub-tropical regions of the world.

The results achieved in previous licences provide the foundation for many of the studies proposed in this licence. During the period covered by this license communication to the scientific community will be achieved through direct discourse with collaborators, publication of scientific results in peer- reviewed journals and presentation at national and international conferences. Reagents/technologies generated/validated in the course of our studies will be made available to the scientific community (e.g. through the BBSRC-funded immunological toolbox - https://www.immunologicaltoolbox.co.uk/).

Medium-/long-term benefit:

The data generated on how parasites subvert host immunity and the immunological processes that determine the ability of vaccine-induced immunity to confer protection will contribute to how vaccines (and other intervention strategies) can be designed to combat Theileria and trypanosome infections in ruminants. Previous experience in the broader vaccinology field has shown that progress towards effective vaccines is likely to be an incremental process with information gained at each step informing subsequent work. As such we anticipate that although the work conducted in this project will successfully achieve its anticipated short-term benefits, the aim of generating successful vaccines

(and/or other intervention strategies) may only be achieved over a medium-/long-term time-frame.

Vaccines (and/or therapeutics) that can be used as part of strategies to control tropical parasitic diseases have the potential to have significant impact on animal health and welfare and also on the performance of the agricultural sector in Low-Middle Income Countries (LMICs), where Theileria and trypanosomes have a particularly devastating impact on the rural poor. Effective vaccines would reduce disease severity and consequent production losses and thus substantially alleviate poverty and facilitate sustainable food production in regions where these parasites are endemic.

The successful generation of a reproduction-defective R. microplus would afford opportunities for medium/long term benefits if they can be subsequently deployed as a mechanism to effectively reduce tick, and therefore tick-associated, diseases in areas where the tick is rapidly expanding and causing considerable economic losses due to direct animal injury and/or transmission of disease.

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

The groups operating under this license are involved in a number of national and international collaborations and are active members in the global Theileria, Trypanosoma and invertebrate biology research communities. It is inherent in the research completed under the license that we will actively seek to disseminate our work through both informal (sharing of protocols with collaborators, attendance at workshops) and formal (publications, seminars and conference presentations) routes.

Work that is continuing herein from the previous license has been used by numerous group across the world (e.g. the characterisation of livestock MHC, cattle infection models for trypanosomes) and there are various examples of how we have sought to ensure that the work completed under regulated procedures will have maximal impact and serve to maximise interaction with scientists from other fields of research (e.g. in the transfer of technology from malaria to Theileria to optimise vaccine production). These approaches to maximising the output from work conducted under home office license regulation will be central to all work conducted under this license.

Species and numbers of animals expected to be used

- Cattle: 2800
- Sheep: 1200
- Goats: 500
- Buffalo: 250

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.
The objectives of this project cannot be achieved with the use of experimental animals. The research addresses questions regarding i) diseases of ruminants for which there are no good models available either in small-animal or laboratory based in vitro systems and ii) genetic effects on immune responses, for which it is necessary to derive materials that

allow assessment of the host genetics (i.e. genetic material from the host species). As a consequence of this it is necessary to use the natural host species (cattle, sheep, goats and buffalo) in the studies completed under this license to enable the objectives to be addressed.

Most of the infection and immunity studies will be conducted predominantly using young cattle (generally animals may be recruited post-weaning ~3 months). However, the initial period of these studies involves minimal procedural activity (blood sampling only) for the first 2-3 months as there is a need to archive materials from the animals prior to immunisation (and to generate in vitro cell lines) prior to any immunisation/infections being instigated. Yearling or adult sheep are used for the infection and immunity studies as these are a more appropriate size for the experimental procedures being completed.

For genetic studies, blood may be taken from animals at different life-stages including neonates, juvenile and adult animals. Volumes of blood samples will always be appropriate to the size of the animal being sampled. Embryo transfer work will require the use of appropriately aged recipient females.

For tick colony maintenance (for R. appendiculatus, H. anatolicum and R. microplus) and generation of Theileria sporozoite stabilate young cattle (3-6 months of age) and adult sheep will be used as these life-stages provide the optimal sizes for the housing unit, to meet the other criteria of the study (e.g. sustain the ticks and to transmit the parasite to ticks).

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

The typical experience of the animals in this project will vary depending on the aims of specific experiments. There are a number of different general outlines of studies that will be completed:

Infection and immunity studies: Most of these animals will have multiple blood samples taken (for generating reagents and materials for in vitro studies undertaken as part of the experimental analysis), will be vaccinated (by administration of candidate vaccines either under the skin, into the muscle, into the lymph node or into the vein) and also will be infected with pathogens. The latter of these may lead to symptoms of disease such as lethargy, recumbency and inappetance. The clinical symptoms will be closely monitored and animals will not be permitted to endure more than moderate signs of disease.

For Theileria studies this monitoring includes the taking of lymph node aspirates to measure parasite levels. Scientific endpoints, by which the objectives of the studies are achieved, have been established to be reached prior to the animals developing any more than moderate signs of disease. If recovery from infection is required for scientific objectives to be achieved anti-parasitic and palliative medication will be administered to the animals. All animals used for infection and immunity studies will be euthanased at the end of the study or if humane end points are reached.

Genetic, immunogenetic and epigenetic studies: Most of the animals will have a single blood sample taken to provide access to DNA/RNA used to study the variation of genetic loci that may influence the host's immune responses to infection. Females used for embryo transfer work will undergo a procedures that are standard husbandry practices used on commercial farms. Generally animals involved in these studies will be kept alive after the procedures have finished.

Maintenance of tick colonies: Animals used for maintenance of tick colonies will have cloth bags attached to their ears and ticks applied within the cloth bags. The numbers of ticks used are known not to have direct detrimental effects on the animals. To prevent dis-lodgement of the cloth bags these animals will have to be ‘single-housed’ for a period of up to two weeks (but still in visual contact with other animals of the same species). All animals used for maintenance of tick colonies will be euthanased at the end of the studies

Generation of Theileria sporozoite stabilates: Cattle/sheep-infective Theileria material can only be generated from ticks that have acquired infection from an infected animal. Animals used to generate infective Theileria material will be infected with Theileria parasites leading to the development of clinical symptoms as described above; however due to the need to permit the parasite to mature within the cattle/sheep these clinical symptoms may become more severe and present for up to 3 days.

Symptoms will be mitigated by the administration of NSAIDs and anti-parasitic medications as necessary. To monitor the progress of the infection (key to the timing for the application of the ticks at the correct time for ‘pick-up’ of the parasite as well as to clinically evaluate the animals) multiple blood samples and lymph node needle aspirates will be taken. To prevent dis-lodgement of the cloth bags these animals will have to be ‘single-housed’ for a period of up to two and a half weeks (but still in visual contact with other animals of the same species). All animals used for generation of Theileria sporozoite stabilate will be euthanased at the end of the study or when humane end points are reached.

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

Infection and immunity studies: the main adverse effects will be as a consequence of the development of clinical signs associated with Theileria and Trypanosoma infections. In the 2-3 weeks following Theileria infections animals may become lethargic, and have loss of appetite that may last 12 hours prior to intervention. In Trypanosoma infections clinical signs develop over a longer timeframe (4-6 weeks post-infection) and include anaemia and lethargy. Other adverse effects are associated with blood sampling and lymph nodes aspirates (which are generally well tolerated by the animals) taken to monitor infections, and the administration of vaccines and/or treatments used to either mitigate clinical symptoms or treat disease (which generally cause only mild discomfort).

Genetic, immunogenetic and epigenetic studies: the only adverse effects are associated with the temporary discomfort associated with the taking of a blood sample and, for the embryo transfer work the intra-uterine implantation of embryos and injections required for oestrus cycle synchronisation

Maintenance of tick colonies: adverse effects will be associated with housing in single pens (however animals will always have visual contact with companions close by in the housing unit), the attachment of cloth bags and the feeding of ticks (both of which cause minimal discomfort to the animals)

Generation of Theileria sporozoite stabilates: the main adverse effects will be as a consequence of the development of clinical signs associated with Theileria infections. Animals may become lethargic, have a complete loss of appetite that may last up to 3 days and may become recumbent. Other adverse effects are associated with blood sampling and lymph nodes aspirates (which are generally well tolerated by the animals) taken to monitor the Theileria infection, housing in single pens (however animals will always have visual contact with companions close by in the housing unit) and the

attachment of cloth bags and the feeding of ticks (both of which cause minimal discomfort to the animals).

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)?

Infection and immunity studies: Cattle - 100% moderate, Sheep - 100% moderate

Genetic, immuogenetic and epigenetic studies: Cattle - 100% mild, sheep - 100% mild, goats - 100% mild, buffalo - 100% mild

Maintenance of tick colonies Cattle - 100% mild, sheep - 100% mild

Generation of Theileria sporozoite stabilates - Cattle - 80% severe, 20% moderate, sheep - 80% severe, 20% moderate

For the project overall, if the maximum number of animals were utilized for each protocol the proportion of animals expected to be in each category is:

Cattle: ~88.6% mild, ~10.1% moderate and ~1.3% severe Sheep: ~94.6% mild, ~4.7% moderate and ~0.7% severe Goats and Buffalo: 100% mild

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

- Killed
- Kept alive
- Rehomed

A retrospective assessment of these predicted harms will be due by 21 December 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 objectives of the project cannot be achieved without the use of animals. The research addresses questions regarding diseases of ruminants for which there are no good models available either in small-animal or laboratory-based in vitro systems. By studying the natural hosts of these important diseases we can understand better i) how the parasites manipulate and evade the immune system to cause disease and also ii) define the parameters required for successful immunity that can inform vaccine design, iii)

understand the contribution of genetics to resistance/susceptibility to disease and iv) optimize methods for generation of vaccines; together it is anticipated that achieving these aims will lead to the reduction of disease in farmed ruminants in the longer term.

Infection and immunity studies: In order to gain a comprehensive overview of the complex biological events occurring following vaccination/infection it is necessary to immunize/challenge animals in vivo to define parameters associated with protection or pathology.

Tick colony maintenance and generation of Theileria stabilate: The sporozoite stage of Theileria cannot be generated or maintained in culture; therefore it is necessary to maintain colonies of tick vectors and to periodically passage the parasites through ticks to produce infective sporozoites. Similarly, at present R. microplus ticks need to be fed on animal hosts to be maintained.

Genetic, immunogenetic and epigenetic studies: Analysis of genetic, immunogenetic and epigenetic variation is reliant of obtaining samples from animals.

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

Infection and immunity studies: cell and organoid culture systems

Tick colony maintenance and generation of Theileria stabilate: artificial feeding systems

Genetic, immunogenetic and epigenetic studies: collection of post-mortem samples from abattoirs

Why were they not suitable?

Infection and immunity studies: Recent review of the literature, discussions with colleagues, collaborators and with the NIO have confirmed that currently there are no feasible substitutes that can be used to replace animal experiments to achieve the objectives that look at the host/parasite interactions (for example, for two of the clinically relevant species of trypanosome, there is no in vitro culture system at present for most strains). A consistent feature of studies conducted under previous licences has been attempts to generate better in vitro (e.g. functional analyses of NK and T-cell subset cell-lines cultured in vitro) and in silico (e.g. RNASeq) models to identify which aspects of the immune response appear to correlate with immunity. Such studies have the objective of eventually reducing the amount of in vivo analysis that is required for vaccine validation and will continue to be a large component of ongoing studies. However, in the foreseeable future these techniques will enhance the data derived from in vivo studies rather than replace them.

Tick colony maintenance and generation of Theileria stabilate: Although ticks can be fed using artificial systems, these have only been used on a very limited scale and the success rate is low (collaborators are actively pursuing this area of research and if successful in vitro feeding systems will be adopted). For these reasons and because of the large number of ticks that are required for generating stabilates, feeding of ticks on experimental animals is the only viable method of maintaining colonies of ticks and subsequent generation of Theileria stabilate.

Genetic, immunogenetic and epigenetic studies: As a major factor in variation in immune responses is age, a key factor in the preliminary work conducted to achieve this aim is to

profile cohorts of animals of different ages (neonates, yearlings and adults). Consequently it will not be feasible to obtain samples through post-mortem collection at abattoirs as yearlings and neonates will not be routinely processed for food consumption and so to maintain a standardised approach to sample collection it is required to take samples from animals under license. In addition for some of the analyses undertaken in this component of the work it is necessary to rapidly processing and fractionate cell subsets from the samples which would not be feasible from abattoir-derived samples. For the ability to study Bos indicus animals under appropriate conditions in the UK it is necessary to use embryo transfer to facilitate the availability of these cattle in situ.

A retrospective assessment of replacement will be due by 21 December 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?

The numbers estimated have been based on the planned studies for which we have funding for within the initial period of this license (with the level of studies extrapolated to cover the whole license period at a similar level of activity). These estimates have been based on studies which have undergone review of the experimental design as part of the funding process and our previous experience.

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

Infection and immunity studies: Each experiment will be designed to minimise the number of animals used with advice on the design of experiments taken from Institute statisticians. The most efficient design will be chosen to achieve the objectives of the individual experiments and the number of animals used will allow differences of significance to be detected at significance level of 5% with a power of 80%. Based on previous experience we anticipate that for many of the parameters being examined, and dependent upon the pathogen strain and species used, group sizes of 6-12 would be sufficient to obtain the results required in any particular study. However, for transcriptomic data the group size needed is usually larger as it is known that inter-animal variation is a cause of significant variability in the data output (estimated to be up to 16-24 animals per group using standard RNAseq). To address this we are proposing in this project to use a ‘challenge and re-challenge’ model for some of the Theileria immunisation experiments. In this model animals that receive a non-protective immunisation will be recovered following challenge (using anti-theileria treatment). Such animals will have subsequently generated a protective immunity; re-challenge of these animals will allow analysis of how protective and non-protective immune responses differ within a single individual. Such an approach may

substantially reduce variation, allowing statistically significant experimental data to be generated using fewer animals. We are conscious that in such experiments a balance needs to be achieved between reduction and refinement and would ensure highly focused objectives to minimise the necessary regulated procedures. Due to logistical constraints (the numbers of animals that can be efficiently included in an experiment at any one time is limited to ~4-6) we generally perform experiments using a block design [e.g. 1 naïve control, 2 animals receiving vaccine X (protective) and 2 animals receiving vaccine Y (candidate vaccine)]. This experimental design reduces variability (e.g. animals in each block are age-matched and derived from a common source) and analysis of accumulating data can be used to identify when statistically significance of data for all parameters has been achieved, thereby avoiding unnecessary experiments and reducing animal use.

Controls are essential to verify the efficacy of critical steps in the experimental procedures (e.g. in immunisation trials naive animals will receive the challenge dose of parasite to verify that it was sufficient to cause disease, or the adjuvant alone to ensure any effect observed is due to the vaccination). The minimal number of animals required for satisfactory controls will be used - use of a robust infectious dose (>LD100) ensures that the minimal number of control animals can be used to verify the patency of the infection. Similarly, if pilot studies are required (e.g. to test immunogenicity of novel vaccine candidates) the minimal numbers of animals will be used to obtain the necessary data.

For each study undertaken, a written protocol will be produced providing a detailed statement of the scientific objectives, the data outputs required to fulfil these objectives, the materials/samples required to generate these data, the experimental design and how data will be analysed. In all experiments animals will be randomly allocated to experimental groups and the experiments conducted so that the results can be published according to ARRIVE guidelines.

Tick colony maintenance and Theileria stabilate generation: as ‘non-experimental’ activities the main route to reduce animal use for these purposes is to identify and apply practices that increase the efficiency of tick colony maintenance and Theileria stabilate production. Communication with other groups and collaborators maintaining tick colonies and generating Theileria stabilate will identify best practice procedures (e.g. improved approaches to cryopreservation that increase viability of parasites during freeze-thawing and so increase the number of parasite doses that can be generated from each batch of infected ticks) that will be implemented as appropriate. For example, in this license we have applied for an increased number of ticks to be fed on individual animals, based on our experience and discussion with other groups we are confident that this will not have a detrimental impact on the individual animals but it will allow us to reduce the number of animals required (i.e. allows us to achieve a reduction of an animal use without any compromise on refinement)

Genetic and epigenetic studies: Extrapolating from human studies the minimum number of animals required to achieve the objectives of each study will be determined. For example, to obtain a robust baseline dataset that will enable the generation of a suitable model of the links between the genome and transcriptome in immune cells we estimate that samples from 60 animals will be required in the preliminary analysis. Any increment in the numbers of animals included in studies will be estimated based on analysis of the preliminary data generated and justified to ensure the minimal numbers of animals are subjected to regulated procedures. For establishing a cohort of Bos indicus animal’s transportation from the US was considered as an alternative to embryo transfer but was disregarded as the loss of refinement associated with transportation of sentient animals outweighed the benefits of the reduction in procedures.

Immunogenetic studies: as a ‘survey’ protocol looking at sequence diversity at a population level the main routes to reduction of animal use will be to i) identify opportunities to derive the required materials from other available resources to reduce unnecessary additional procedures. To achieve this we will seek opportunities in which to obtain samples as residues from veterinary procedures (i.e. excess blood from herd health surveys or other medical reasons). We are also currently attempting to adapt the molecular approaches used to generate the sequence to use DNA (rather than RNA which is the current starting material) which will enable us to exploit archived material; ii) to generate models to optimise the sampling framework. Currently the breadth of MHC diversity in British cattle and sheep breeds is unknown and the data to establish a rational sampling set is not available. However, it may be anticipated that the diversity will be substantial. In consultation with a statistician we have decided to adopt a reiterative statistical analysis framework to allow an appropriate model to estimate required sample sizes to be generated as data is accumulated. In this framework small/moderate cohorts of animals will be sampled (e.g. three independent cohorts of 50 animals from a breed) and based on the results and appropriate meta-data (e.g. structure of population sample, size of breed population relative to sample population, distribution of individual MHC haplotypes between separate cohorts and other factors) a model will be established to estimate optimal sample sizes. Until this model is established we have based our predicted samples size (2000) on the preliminary data we have on bovine MHC diversity and the complexity of the British herd structure. As the work to be conducted in sheep, goats and other ruminant species is more preliminary we have requested fewer animals (1000, 500, 250 animals respectively). Obviously, if the other measures used to reduce and refine animal use for this work are achieved the number of animals experience a regulated procedure will be lower.

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

Infection and immunity studies: a principal application of the MHC typing platform developed is to be able to develop efficient breeding of MHC-defined animals for the infection and immunity studies. Application of this platform to samples taken as excess from veterinary procedures conducted on the herd from which we predominantly source animals for use infection and immunity studies, has allowed a substantial reduction in the number of animals required to be sampled to identify those carrying the requisite MHC genotypes.

A retrospective assessment of reduction will be due by 21 December 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.

Infection and immunity studies: the models used for these studies will be infection of natural hosts (sheep and cattle) with Trypanosoma and Theileria parasites. As the natural hosts for the Trypanosoma and Theileria pathogens being studied, cattle and sheep are the only animals in which the specific host-pathogen interactions that are pivotal to the scientific objectives of this project can be studied. For trypanosomes, while T. brucei can be grown in culture, most strains of T. congolense and no strains of T. vivax can be grown in vitro. There are no small animal models of Theileria infection, and although studies in mice provide some relevant information for trypanosome infection, many aspects of immune responses are not translatable across species – a key example being the potential role of antibodies with 'long CDR3' which are present in cattle but not mice. Use of cattle and sheep in infection and immunity studies enables direct evaluation of the parameters associated with induction of immunity or progression of the disease to be defined, which is not possible using other models/methods; consequently they offer the most refined (and in many respects only) model available to address the scientific objectives of the project.

Tick colony maintenance and Theileria stabilate generation: generation of Theileria stabilates (Protocol 5) requires the parasite to establish a high level of piroplasm parasitaemia in infected cattle/sheep; this requires the clinical course of infection to be sustained longer than that for infection and immunity studies. Consequently the adverse effects suffered by these animals may be greater (e.g. longer period of inappetance, dyspnoea and elevated temperature) and in a substantial proportion (up to 80%) of animals will be severe. As it is the parasitaemia rather than the clinical symptoms that are critical to achieving the objective of this protocol all efforts to ameliorate clinical symptoms and refine the animals experience will be made (administration of palliative treatment [NSAIDs and diuretics], supplementary feeding, provision of fresh bedding, regular re-positioning, oral fluid therapy and physical support if recumbent etc.). Once animals have developed marked clinical signs the observation of these animals will be continuous.

Genetics, immunogenetics and epigenetic studies: currently the protocol for obtaining materials for this objective requires blood sampling (mild severity procedure) of each animal. In this project we will explore the potential to derive materials from non-regulated samples (e.g. hair pluck or mucosal swab). If these materials can be successfully used the experience of these animals will be refined to a degree that they are no longer experiencing any regulated procedure. However, for some studies it is unlikely to be feasible to use alternative sources of material. At present embryo transfer represents the most refined method of establishing a Bos indicus cohort of animals in the UK.

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

Infection and immunity studies: As stated above the scientific objectives of understanding how host/pathogen interactions determine biological outcome can only be achieved by studying these interactions in the relevant host species (cattle and sheep). As the interactions occur over a period of weeks - months, it is not possible to conduct these studies on animals at a more immature life-stage or on animals that have been terminally anaesthetised

Tick colony maintenance and Theileria stabilate generation: the tick species used in this license ticks show host specificity and so there is a need to use the relevant host species

(i.e. cattle and sheep). As the tick feeding can require a period of days to weeks, it is not possible to conduct these studies on animals at a more immature life-stage or on animals that have been terminally anaesthetised.

Genetic, immunogenetic and epigenetic studies: as the aims of these studies are to study the genetics of the relevant host species it is not possible to substitute with other species. The adverse events experienced by animals for these studies are mild so terminal anaesthesia is not a suitable approach to refinement.

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

Infection and Immunity studies: The experience accumulated by the principal investigators on this licence will ensure the most refined experiments possible will be conducted in vivo and there is a commitment to continue to explore options to refine studies further. For example, under the previous licence we verified that the standard index for scoring severity of Theileria infection (known as the Rowlands index) that was routinely used in previous immunisation-challenge experiments could be substantially rationalised. Use of the Rowlands index required measurements to be taken for up to 21 days post-challenge to achieve a scientific endpoint (unless a humane endpoint was reached before this). The scientific endpoint we define in this proposal is generally reached by day 13-15 post-challenge, markedly decreasing the time during which animals are experiencing adverse effects and the amount of regulated procedures required for clinical/parasitological monitoring. It therefore allows the earliest practicable initiation of theilericidal treatment/euthanasia so that the experience of the animals is as refined as possible. In both Theileria and trypanosome models consistent and intensive monitoring of the clinical symptoms will be used to ensure that the scientific objectives of experiments are achieved with the minimum of animal suffering possible.

Tick colony maintenance and Theileria stabilate generation: As it is the parasitaemia rather than the clinical symptoms that are critical to achieving the objective of this protocol all efforts to ameliorate clinical symptoms and refine the animals experience will be made - administration of palliative treatment (NSAIDs, corticosteroids and diuretics), supplementary feeding, provision of fresh bedding, regular re-positioning and physical support if recumbent etc.. Once animals have developed marked clinical signs the observation of these animals will be continuous to ensure they receive the best care possible.

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

The NC3R guidance (https://nc3rs.org.uk/the-3rs) will be followed to ensure optimised refinement protocols are adopted.

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

Guidance on the 3R's will be sought from the relevant literature (the University sends regular newsletters with continuing education and other opportunities to remain up to date with 3R's information). Routine interaction with the AWERB and NVS is inherent in the University's systems regulating animal use and ensure that license holders remain informed of the most up to date information and how to implement any changes that enhance the application of the 3R's to their studies.

A retrospective assessment of refinement will be due by 21 December 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?

32. Regulatory Aquatic Ecotoxicology

Project duration

5 years 0 months

Project purpose

- Translational or applied research with one of the following aims:
  - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
- Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)
- Protection of the natural environment in the interests of the health or welfare of man or animals

Key words

Ecotoxicology, Regulatory, Fish, Aquatic

Animal types	Life stages
Zebra fish (Danio rerio)	neonate, juvenile, adult, embryo
Medaka (Oryzias latipes)	embryo, neonate, juvenile, adult
Rainbow Trout (Oncorhynchus mykiss)	juvenile, neonate, embryo, adult
Sheepshead minnow	embryo, neonate, juvenile, adult
Salmon (Salmo salar)	embryo, neonate, juvenile, adult

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 purpose of this project is to obtain acute and chronic effect endpoints of test substances supplied by sponsors for product development and submission to regulatory authorities. The vast amount of the work conducted under this project will be conducted in accordance to Good Laboratory Practice (GLP) with the exception of range finding tests

and those tests where the regulator does not required GLP compliance.

A retrospective assessment of these aims will be due by 12 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 it's 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?

To ensure the environment is protected from any adverse effects of the test substances we have gathered data from. The regulatory authorities will use the data generated to ensure the test substances are safe for use and apply restrictions to manufacture, use, transport and disposal to mitigate risk. The data are used to ensure the test substance is appropriately labelled with risk phrases and packaging.

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

The outputs from this project will enhance the data packages of test materials through a client's regulatory submissions. The data will be used as part of regulatory submissions for test materials.

The protocols in this project licence will result in the following outputs;

- The studies conducted as part of this project licence will form two parts, firstly range finding/ preliminary studies which will provide important information used to design the main Definitive study. A summary of the data generated during these range finding/ preliminary studies is generally incorporated into the final report. The Definitive studies are reported in full and supplied to the client for regulatory submission. On occasion the study outputs may be published in peer reviewed journals.

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

This work will produce quality assured data, in most cases compliant with GLP for statutory assessments of a test material in support of environmental risk assessment requirements required for the manufacture, supply and licensing of materials. As a CRO (contract research organisation) this is predominantly a regulatory driven model to support our clients submissions.

The relevant regulatory authorities will benefit from the outputs produced from this project to assist them in fully assessing a test substance submission. The clients and/or regulatory authorities generally publish the data produced on public databases for access to all. The driver to complete the studies listed within this licence are to support the development of products and to ensure the environmental profile of chemicals is known to protect the environment.

The use of animal models is legally required in some circumstances to provide a holistic assessment of the risk posed to natural populations and the information derived from regulatory tests is used to protect the environment and human/consumer health. This project licence will also include participation in ring tests, these are studies conducted at multiple laboratories using the same test methods and reference substance to assist in the validation of a new test guidance document. By participating in ring tests and similar applied research we will be benefiting the progression and development of regulatory test guidelines for future use.

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

All work conducted under this project licence will produce a report or support a study that will produce a report. The reports produced under this project licence will be in accordance with the test guidance documents followed to ensure they are acceptable for regulatory submissions globally under the OECD Mutual Acceptance of Data (MAD).

Species and numbers of animals expected to be used

- Zebra fish (Danio rerio): 77050
- Medaka (Oryzias latipes): 22650
- Rainbow Trout (Oncorhynchus mykiss): 10000
- Salmon (Salmo salar): 3150
- Other fish: No answer provided

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.

We are using fish for the work conducted under this project licence at our laboratory to satisfy global regulatory requirements for environmental testing and risk assessment. The choice of life stages is chosen based on published test guideline criteria, these life stages are suitable for obtaining the appropriate information for the regulators to make informed decisions.

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

Typically the animals used in this project will be exposed via an aqueous media to a test substance, in one protocol the animals will be exposed to the test substance via feed. Aqueous media refers to the water the animals live in (i.e. Seawater or freshwater).

The effects of the test substance which include lethal and sublethal endpoints are observed over the study duration and the animals are humanly killed using schedule 1 methods after use. Some of the protocols in this project do not expect visual adverse effects of toxicity but assess bioaccumulation and endocrine disrupting properties of the test substance. These animals will be be used once for a protocol and will not be re-used. The protocols range in duration from 96 hours for an acute (lethality) study to many months for a multigeneration study, however typically chronic studies are approximately 28 days in duration. The animals used in this project licence will be expected to experience adverse effects for the duration of the exposure period, however the adverse effects will

differ in severity depending on the study type. Humane endpoints may be triggered to end the suffering where required.

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

The fish used in this project are tested for acute and chronic effects which are likely/expected to cause mortality or sublethal adverse effects. These sublethal adverse effects may include but are not limited to excess mucus production, loss of equilibrium, abnormal respiration or feeding.

The fish will be exposed to single concentrations as well as a range of concentrations therefore the maximum severity of the protocols will not be met for all animals tested.

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 table below shows the animal type, severity classification and estimated numbers for the whole project.

Animal type	Severity classification	Estimated numbers per severity classification	Estimated total by animal type	Estimated total number of animals for the project
Zebrafish (*Danio rerio*)	Mild	36465	80050	217550
	Moderate	36635		
	Severe	6950		
Fish	Mild	60700	137500	
	Moderate	62200		
	Severe	14600		

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

- Killed

A retrospective assessment of these predicted harms will be due by 12 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?

Living organisms accumulate and respond to materials and mixtures of materials in a more complex manner than can currently be demonstrated by cellular assays or in silico techniques. Consequently, ecotoxicological assessment using animal models represents the final stages in evaluation of a material and is enshrined in national and European legislation for the protection of the environment and human health. Regulatory authorities require fish testing to understand the effects the test substance may have on the environment. The recognised regulatory guidelines require fish and amphibian testing as a data requirement for the submission of approvals of test substances.

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

There are a few OECD test guidance documents for alternative *in vitro* models (e.g. OECD 249, 250). The OCED 249 Fish Cell Line Acute Toxicity, used to predict fish acute toxicity in product testing and range-finding and pre-screening before conducting a full fish acute or other fish-based toxicity test. The OECD 250 (EASZY) utilise transgenic embryos and are technically *in vivo* exposures however the assay is conducted prior to free feeding which classifies them as a non-protected animal. These guidelines may be used in addition to the *In vivo* studies or used to provide additional information for study design and conduct.

Other lines of evidences (e.g., Quantitative Structure Activity Relationships (QSAR), weight of evidence (WoE)) within Integrated Testing Strategy (ITS)/Integrated Approach to Testing and Assessment (IATA) will also be considered prior to conducting studies with animals.

Why were they not suitable?

We will always consider the use of alternative methods and approaches in determining whether an *in vivo* test is required, or where it may reduce or refine the animal work. These alternative methods are new test guidance documents and as such are not currently fully accepted or requested by the global regulatory bodies.

A retrospective assessment of replacement will be due by 12 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?

The numbers of animals used in the project are based on business predictions for the next 5 years. There is an overestimation added into this project licence as some test guidance documents give a number of test species for use and global regulators prefer certain species. As such all are included but it is unlikely all will be used to the degree specified.

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

The experimental design of the studies we will conduct under this project licence will be depicted by the test guidelines we follow.

Where we are conducting screens and range finders we will, where possible, share controls, reduce replication and number of animals required per replicate to give us a guide of toxicity.

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

Preliminary Range finding studies will be used in the majority of the studies conducted, this will ensure we employ a suitable dosing regime to meet the requirement of the test guidance document followed. This may include a full range or a slimmed down limit study. A limit study is a study with a single test concentration rather than a range, it is used to show no effect at that concentration and is based on other data available (e,g, range finding or threshold approach utilising algae and *Daphnia* data).

We will use invertebrate and other vertebrate data to assist in experimental design. The OECD threshold approach will be used where appropriate, which will utilise the invertebrate data generated.

A retrospective assessment of reduction will be due by 12 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.

Regulatory authorities require fish testing to understand the effects the test substance may have on the environment. The regulatory authorities have programmes of testing to meet their data requirements. At our laboratory we provide these studies for clients to submit to regulatory authorities and as such need to adhere to their data requirements.

Test Type	Test guidance document
Fish Acute	OECD 203 OCSPP 850.1075
Fish early life stage	OECD 210 OCSPP 850.1400 USEPA Method 1000.00
Fish Sexual development test	OECD 234
Short term reproduction test	OECD 229
21 -Day fish Assay	OECD 230
Juvenile growth test	OECD 215
Fish Bioaccumulation	OECD 305 OCSPP 850.1730 ASTM E1022-94
Multigeneration/fish full life cycle	Combination of OECD 210, 234 test guidelines ZEOGRT OECD Draft MEOGRT OECD 240 OPPTS 850.1500 (draft)

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Multigeneration/fish full life cycle	Combination of OECD 210, 234 test guidelines ZEOGRT OECD Draft MEOGRT OECD 240 OPPTS 850.1500 (draft)

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

Regulatory authorities require fish testing to understand the effects the test substance may have on the environment. At our laboratory we provide these studies for clients to submit to regulatory authorities and as such need to adhere to their data requirements.

We will always consider the use of alternative methods and approaches in determining whether an *in vivo* test is required, or where it may reduce or refine the animal work. These alternative methods are new test guidance documents and as such are not currently fully accepted or requested by the global regulatory bodies.

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

The use of range finding tests and data generated from other testing (invertebrate) will be utilised to ensure the range or limit chosen will fulfil the regulatory requirements and produce a valid study. Where possible, in acute studies we will employ the OECD threshold approach or a limit study to reduce the number of fish used in testing. For the more severe tests we preform at the laboratory, the animals will be monitored frequently by study staff, named persons and licence holders and humane endpoints will be applied to minimise the number of fish dying and the time they suffer adverse effects.

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

This project will either follow or base the study design on the methodologies published in recognised international test guidelines (e.g. OECD, OCSPP, ISO).

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

We will maintain a continual awareness of 3Rs advances in the area throughout the life of the project, and will review possible alternatives wherever animal studies are being considered. In addition advice is available via regular AWERB meetings and NVS visits.

A retrospective assessment of refinement will be due by 12 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?

33. Cellular and molecular mechanisms of organ fibrosis and regeneration

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
    - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
    - Assessment, detection, regulation or modification of physiological conditions in man,animals or plants

Key words

Fibrosis, Scarring, Tissue repair, Tissue regeneration, Cancer

Animal types	Life stages
Mice	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?

We aim to identify the key cellular subpopulations driving organ fibrosis (scarring), liver regeneration and liver cancer as this will allow us to identify unique attributes and markers for these cellular subpopulations. We will use a range of experimental models that have specific human parallels and have highly complementary disease mechanisms in order to achieve this.

A retrospective assessment of these aims will be due by 05 November 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?

Our research will not only further understanding of the process of organ fibrosis, disease and tissue repair, but will also directly facilitate the rational design of highly targeted, cell-specific anti-fibrotic (scarring), pro-regenerative and anti-cancer therapies.

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

We aim for the following outputs by the end of this project:

A greater understanding of the cell biology of organ fibrosis and repair, especially with regard to the identification of the major pro-fibrogenic cell subpopulations driving fibrogenesis and tissue regeneration, defining key interactions with other cells such as immune cells. This new information will benefit scientists by allowing sharper focus on the major pro-fibrotic subpopulations, accelerating the discovery of key anti-fibrotic targets to pursue within this subpopulation with the aim of developing therapeutics.

The development of effective inhibitors that limit organ injury, accelerate tissue regeneration and limit fibrosis in animals. Taking the therapeutic targets identified in 1 above, we will then assess small molecule inhibitors/antibody-based therapeutic approaches to inhibit fibrosis production in pre-clinical models in vivo.

Increase our experience and further refine the use of cutting-edge imaging techniques (e.g. MRI, ultrasound, optical imaging, fluorescent imaging) in the diagnosis and monitoring of organ injury and fibrosis and haemodynamic changes in preclinical models of liver fibrosis. This will also reduce the numbers of animals used for biomedical research, which if adopted more widely in research institutes around the world will have a major impact on reducing the numbers of animals used worldwide.

A greater understanding of the cell biology and mechanisms driving liver cancer in relation to fibrotic liver injury. This will benefit scientists by providing focus on cellular interactions between subpopulations of cells within the fibrotic and cancerous niche, allowing in depth characterisation of key cellular sub-populations and accelerating the discovery of the crucial therapeutic targets.

We will disseminate our findings widely via publications / lectures / conferences.
Longer term benefits we aim to reach that may project past the end of this project are as follows:

The use of small molecule inhibitors or antibody-based therapies to modulate organ injury and regeneration in patients with organ injury and fibrosis, including the development of both anti-fibrotic and pro-regenerative medicines. We will take forward candidate small molecule inhibitors/antibodies identified in pre-clinical studies in this PPL, and aim to

commence phase I clinical trials with these novel therapeutic agents. There is a huge unmet clinical need in organ fibrosis, so if we can identify potent new anti-fibrotic therapies this could have a massive impact on human health.

The effects on organ transplantation are potentially far reaching. Effective therapies for acute liver injury, as seen in paracetamol poisoning, might avoid the need for liver transplantation altogether. This would expand the potential pool of donated livers for patients with chronic liver disease and cancer. Additionally, developing treatments to delay the progression of liver and lung fibrosis once injury has taken place could reduce the need for transplantation and therefore reduce the burden of morbidity and mortality associated with these therapies. New treatments which can reduce or completely negate the need for transplantation could have a very beneficial impact on the massive number of patients with end-stage organ fibrosis.

Identification of other therapeutic targets that may modulate liver injury or scarring or accelerate hepatocyte regeneration, this may be clinically relevant in extending the resection of liver tumours or expanding the applicability of living related liver donation.

Identification of non-invasive biomarkers of fibrotic disease and response to therapy in fibrosis of the liver or lungs. Successful identification of non-invasive biomarkers for fibrotic disease would negate the need for invasive tests such as liver biopsy, which is currently the gold standard for diagnosis of human liver fibrosis, however liver biopsy can result in significant morbidity and even mortality.

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

The overall field of fibrosis and tissue repair research will benefit from the outputs of this project (1-5 years). Furthermore, if successful in achieving the outputs previously detailed, the medical field will also benefit from the outputs of this project (5-10 years) in the form of advances in therapeutics and non-invasive biomarkers of fibrosis.

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

We will aim to publish all of our studies regardless of outcomes. We also intend to maximise our research outcomes through collaborations as well as presentations in both local, national and international conferences and meetings.

Species and numbers of animals expected to be used

- Mice: 30,000

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.

We will use mice for this research, as injury models are available that closely replicate what is observed in the human disease equivalent. We aim to use these models to study the complex cellular interactions involved in organ injury and repair in the context of

different types of disease. We will use predominately adult mice as these will most closely replicate the injury and disease aetiologies that we are interested in researching.

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

Animals used in this project will likely undergo induction of organ fibrosis, substance administration by injection or oral gavage, surgery and live imaging. Experimental durations will range from 24 hours to several weeks depending on the protocol. Within an experiment, up to four procedures can be carried out depending on the procedure. All procedures are protocol dependant.

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

Potential adverse effects for animals are procedure dependent but may include: possible haemorrhage during or post surgery, rapid weight loss, rapid weight gain and clinical signs of distress.

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)?

Severities are considered to be moderate and mild for the majority of procedures, with the exception being protocol 8 that has a severity rating of severe. We estimate that approximately 33% of all animals under this project licence will experience mild severity, around 62% will experience a moderate severity rating, and that no more than 5% of animals will experience a severity rating of severe.

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

- Killed

A retrospective assessment of these predicted harms will be due by 05 November 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?

While we strive to avoid the use of animals whenever possible in our research, they are still crucial to furthering our understanding of organ injury and repair, and to ultimately develop therapeutics for different types of illness and disease. Until further scientific

advancements are made towards alternatives that completely mimic the complex interactions that occur in vivo there is no choice but to continue to use animals where no other option in available.

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

Culture of scar-forming cells on tissue culture plastic allows the identification of important mediators produced during this important pathological process. We have used such in vitro modelling in our previous publications. Where possible our studies will be informed by in vitro human studies which are also conducted in our laboratories as we have set up a workflow to obtain fresh human fibrotic explant liver tissue, which means we can study multiple human liver cell lineages in vitro, including mesenchymal cells, from human fibrotic livers allowing us to further replace and refine our in vivo animal studies. We are currently in the process of setting up 3D organoid culture workflows for both murine and human primary cell lines in order to further reduce the need to use animal models in our research as the use of organoid models mimic in vivo conditions to a far greater degree than standard cell and tissue culture systems. We also have an active collaboration involving the use of human precision cut liver slices which closely emulate in vivo conditions and retain the structure and composition of the liver they originate from. Furthermore, we will actively seek to continue optimising and fully utilise alternative non-invasive imaging techniques (e.g. MRI, high resolution ultrasound) to further reduce the requirement for animals to undergo the more invasive imaging procedures detailed in this project licence.

Why were they not suitable?

These alternatives greatly reduce the need to use animal models in our research and we actively use these alternatives whenever possible, although unfortunately it is currently impossible to fully recreate in vitro the complex cellular interactions driving organ injury and fibrosis. Currently rodent injury models are the closest representation we have to the diseases that we are researching.

A retrospective assessment of replacement will be due by 05 November 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?

Our group and others within our institute have many years of experience in the design of animal and human studies of organ injury and repair. Experimental design software (such as Experimental Design Online and G Power) will be used to determine the required

number of animals to achieve statistical significance and to help reduce the number of animals used for experimental protocols overall. We will also utilise data available from previous peer reviewed publications to determine the number of animals required for our experimental research. Biomedical statisticians are also consulted in order to keep our estimates accurate and updated. Furthermore, animal numbers required for breeding and maintenance and out-breeding of lines are derived from estimates provided by the animal facility.

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

In order to improve the reporting of research using animals, maximising information published and minimising unnecessary studies we will use the ARRIVE and UKCCR guidelines. We also plan to perform pilot studies and dose finding procedure studies where possible before carrying out experiments with large cohorts. Furthermore, group sizes for experiments will be calculated following consultation with a statistician and with the use of freely available experimental design software.

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

We will implement efficient breeding procedures and will perform well-designed pilot studies whenever possible to assess the potential for publishable results. We will additionally perform computational modelling and analysis of datasets to maximize the information extracted from our datasets. While our initial studies will be carried out in male mice due to higher incidence of fibrosis, animal numbers will be optimised by use of females rather than solely males to carry out additional experiments (e.g., sex comparison studies for potential therapeutic targets). All relevant tissue will be harvested and stored from each experiment carried out in order to reduce the replication of experiments unnecessarily. Stored tissue will also be made available to lab members and collaborators in order to further reduce the number of animals required. Furthermore, where possible tissue that is not relevant to our research but to others within the institute will be offered to them in order to further reduce the number of mice used within our institute overall. In addition, some of the named procedures within our project licence reduce the need for the use of multiple animals or for multiple experiments to be carried out, subsequently reducing animal use overall.

A retrospective assessment of reduction will be due by 05 November 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 mouse models to be used have been extensively evaluated in the literature, we have experience of these clinically relevant models and parallel studies in humans will ensure refinement to best model the human conditions under study. Where possible we will administer substances during surgery, and intra and post-operative analgesia will be administered to all animals. In addition, over the past few years we have further refined some of our injury models to reduce their severity. For example, we now routinely house mice at 28°C following acute paracetamol-induced liver injury, which has reduced mortality rates from 30% to less than 5%. We will apply presently available clinical scoring systems and modify and refine these relative to our specific injury models as the experiments progress if additional unexpected adverse effects are seen.

A number of potential models of liver cancer development are reported. We have decided to use the DEN model, which we have extensive prior experience with. This model is a simple, reproducible model which involves a single injection to young male mice and results in liver cancer development within a reasonable time frame, in contrast with other methods (eg CCL4, aflatoxin, peroxisome proliferators). This model also provides a good representation of human liver tumours and is widely reported in the literature. The DEN model is also more widely available than the liver cancer models using genetically modified mice, and would avoid us having to derive double genetically modified mice to allow liver cancer development.

With regard to our research involving models of fatty liver injury, we will always opt for a diet which has mild adverse effects and any diet combinations highlighted in pilot studies that cause unexpected adverse effects will be halted immediately.
For repeated or prolonged administration of substances we will use subcutaneous osmotic minipumps to avoid the adverse effects associated with repeated injections.
We will also make use of transgenic rodents that are phenotypically normal in order to reduce the risks of adverse effects.

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

In order to study the complexity of organ fibrosis and repair as accurately as possible, rodent models are the closest equivalent to what we observe in human disease and injury. The use of a species that is less sentient is unfortunately not possible as they simply do not provide an accurate model of human organ fibrosis. Furthermore, adult mice are required for these injury models in order to provide the most accurate representation of adult human fibrotic disease.

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

All of our studies involving surgical or invasive procedures will adopt appropriate intra and postoperative/procedural pain management and post-operative care.

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

We will stay up to date on best practice guidelines from the NC3Rs website (Guidance on the Operations of ASPA -https://www.nc3rs.org.uk/).

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

We will stay informed about advances in the 3Rs by attending events locally and provided by the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs).

A retrospective assessment of refinement will be due by 05 November 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?

34. Neural Circuits and Immunity in Psychosis

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
  - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
- Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

Schizophrenia, Psychosis, Perception, Neural circuits, Immune system

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

This project is aimed at elucidating the biological mechanisms that give rise to psychotic symptoms. The goal is to develop new biological treatments for brain disorders such as schizophrenia.

A retrospective assessment of these aims will be due by 04 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?

Psychosis is characterised by disturbances of perception and thought such as hallucinations and delusions. Psychotic symptoms are a typical manifestation of severe mental disorders including schizophrenia, bipolar disorder, depression and dementia. The prognosis of these disorders has not improved over the past decades, and the development of new treatments has been slow. One main reason for this stagnation is that we still do not understand the biological roots of psychosis.

We will study how brain and immune dysfunctions lead to psychosis-like behaviour. In particular, we will investigate nerve cells that are inside or connected with the striatum. The striatum is a brain region that is believed to be involved in psychosis. We will observe how different types of nerve cells signal during psychosis-like behaviour, and how changing signalling in these nerve cells reduces psychosislike behaviour. This will allow us to identify new antipsychotic treatment strategies that target specific nerve cells. Moreover, we will study how immune signals lead to psychosis. While the exact causes for psychosis are unclear, a variety of genetic and environmental factors point to an involvement of the immune system. We will identify immune signals that are altered in patients with psychosis and induce these immune signals in mice. We will then observe how these immune signals induce psychosis-like behaviour, and test different strategies to block these immune signals to reverse psychosis-like behaviour. This will allow us to identify new treatment strategies that modulate the immune system.

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

This project will generate new scientific knowledge about the biological roots of psychosis. We will publish our results through publications in high-quality peer-reviewed scientific journals.

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

This project will advance our understanding of the biological roots of psychosis. We expect to generate fundamental insights into the mechanisms underlying behaviours relevant to psychosis. This will benefit future research on psychosis in the short-term. Moreover, by identifying new strategies to reverse psychosis-like behaviour, we hope to provide the basis for developing new treatments for psychotic symptoms. This will create opportunities for pharmaceutical companies to develop more targeted therapeutic compounds with less unwanted side effects. This will benefit people affected by psychotic disorders in the medium to long-term.

These benefits are substantial given the high individual, societal and economic costs of psychosis. Psychosis is a syndrome of various major mental disorders including schizophrenia, bipolar disorders, depression and dementia. Mental ill health is the single largest cause of disability in the UK, contributing up to 22.8% of the total burden, compared to 15.9% for cancer and 16.2% for cardiovascular disease (Department of Health and Social Affairs, 2011). The wider economic costs of mental illness in England

have been estimated at £105.2 billion each year (ibid.). This includes direct costs of services, lost productivity at work and reduced quality of life. Moreover, because of the devastating interpersonal repercussions of psychotic symptoms, psychotic disorders pose a high emotional burden on families.

In addition, this project will advance our understanding of immunological conditions of the brain more broadly. By studying how disordered immune processes give rise to impaired brain function, we expect to generate fundamental insights that will benefit research on immunological conditions of the brain beyond psychosis. By identifying ways to rescue dysregulated immune processes affecting the brain, we hope to contribute to the development of new treatments for neurological and psychiatric autoimmune conditions such as autoimmune psychosis, autoimmune encephalitis, multiple sclerosis, or neuropsychiatric lupus. This will benefit people affected by autoimmune brain conditions in the medium to long-term.

These additional benefits are significant given the burden associated with autoimmune brain disorders. Autoimmune diseases affect approximately 4.5% of the population (Hayter et al. 2012), and 30 out of 80 known autoimmune diseases can affect the brain (Theofilopoulos et al. 2020). Multiple sclerosis, for example, is a common autoimmune disease of the brain that affects more than 100,000 individuals in England with almost 5,000 new cases diagnosed each year (Public Health England, 2020). The condition is associated with disability, reduced quality of life and significant costs for the healthcare system with annual per-person costs of up to 57,500€ (Kobelt et al. 2017).

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

During this project, we will actively collaborate with colleagues from academic psychiatry who are working on related questions in humans. We will further engage ourselves in studies in psychotic patients. We will use our own and our collaborators' results to shape our project on an ongoing basis. We plan to disseminate the new knowledge through publications in scientific journals and through presentation at national and international conferences. We will further actively work on making the data generated in this project open-source so it can be used by other scientists in their future research.

Species and numbers of animals expected to be used

- Mice: 5400

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 the species with the lowest sentience that is still suited for studying the brain and immune system. Therefore, mice have been extensively used in neurophysiological and immunological research. As a result, all the necessary assays and reagents for this project are available for mice. As psychosis typically manifests in early adulthood, we will focus on adult mice. However, as some of the biological risk factors affect the developing organism, we will study mice at earlier stages of life as well.

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

The majority of animals will undergo behavioural experiments. During these experiments, animals will be presented with stimuli such as lights and new sounds. All the stimuli are below the pain threshold, and animals will be habituated to them over the course of days. In response to these stimuli, animals will poke their snouts into openings and receive water as a reward. In some behavioural experiments, animals will be unable to move their heads as they will be mechanically fixed. This is necessary to allow for measurements of brain function that require a still head. To make sure that animals are motivated to perform the experiments, water may be restricted outside the experiments. However, we will make sure that animals maintain good health and weight by supplementing them with additional amounts of water outside the experiment or by providing them with free access to citric acid water.

Such citric acid water is not as palatable but equally hydrating as normal water.

Some animals will receive injections of a neuroactive or immune-modulating substance. Animals will typically be injected with a small volume of a substance under the skin of the neck, in the belly, through the nose, or in the muscle. In some cases, injections will be given directly into the space along the spine that contains the fluid that bathes the spinal cord and the brain. For this procedure, a small cut of the skin in the neck may be performed under general short-term anaesthesia. These injections into the space along the spine are necessary to test the effects of substances that cannot otherwise reach the brain because of the barriers between the brain and the rest of the body. This procedure is similar to intrathecal injections used in humans to treat cancer and autoimmune diseases of the nerve system. Animals will experience the effects of the substances. In most animals, these effects will be mild and transient, such as altered perception, increased activity and general sickness behaviour. A portion of the animals (<10%) will be treated with substances that induce an immune reaction against the brain. These animals might experience more prolonged and severe effects such as paralysis and urinary retention. We will very carefully monitor and control for these effects. Animals will typically receive one injection per day and a total of two to twenty-four injections over the course of the experiment. In some cases, more than one injection needs to be given on one occasion. In these cases, injections will be performed under general short-term anaesthesia.

Some animals will receive immune cells from another animal. For this procedure, animals will first undergo radiation or chemical treatment to ablate their own immune system. This procedure leads to a transient suppression of the immune system, which is usually well tolerated. Some animals might experience infections or gut inflammation, which we will monitor for and treat. The procedure is similar to a stem cell transplant that is used to treat human blood cancer.

Some animals will undergo surgical procedures. A portion of the experiments will involve the implantation of a device such as a glass fibre or cannula into the brain. Some experiments will also involve the implantation of a small pump under the skin of the back. This small pump is used to deliver drugs continuously or on-demand without the need for an injection. This is similar to insulin pumps used in the treatment of diabetes mellitus in humans. To alleviate pain and prevent infections, the procedures will be conducted under anaesthesia and aseptic conditions. Postoperative pain and inflammation will be closely monitored, and animals will receive preventive pain killers during the surgery and when they show signs of distress after the surgery. Animals will be left one week to recover before undergoing any other experiments.
Experiments typically run for 3-12 months.

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

During the behavioural experiment, the animals will experience stress which we plan to minimise through gradually habituating animals to the new environments. Animals typically show signs of distress in the form of increased activity for around five to fifteen minutes after entering the experimental environment for the first two to three times until they get used to this situation. Water restriction outside the experiments might lead to thirst and weight loss, which we will minimize by ensuring a minimum water intake or giving free access to citric acid water. Animals may experience pain during injection of substances which typically lasts for minutes. Animals may experience effects of the neuroactive drugs which might induce agitation or sedation which typically last for hours. Animals may experience the effects of immuno-active substances which may include sickness behaviour, less movement and less feeding and are typically mild and last for days. A portion of the animals (<10%) receiving immune-active substances will experience an immune reaction against the brain that can lead to acute or chronic paralysis starting at the tip of the tail and then progressing to the hind limbs, and in some animals, to the forelimbs. This can impact on the animals' ability to feed and groom. Some of these animals might experience urinary retention. We will carefully monitor and control for these effects with daily observations, regular weighing, facilitated access to food and adapted cage environments. A portion (20%) of the animals undergoing immune cell transfer may experience infections or gut inflammation which typically spontaneously resolves after days. Animals will experience pain and distress after surgery, which we aim to minimise with pain killers and careful monitoring, and which typically resolves after a few days.

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)?

Mild - 41%
Moderate - 41%
Severe - 8%

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

- Killed
- Used in other projects
- Kept alive

A retrospective assessment of these predicted harms will be due by 04 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?

Psychosis exclusively manifests itself through behavioural alterations, therefore a living and behaving organism is necessary to study psychosis. Because humans cannot directly be studied with invasive methods that enable to study the biological mechanisms in sufficient detail, studies in animals are necessary.

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

Following the PREPARE guidelines and RSPCA suggestions, we considered cell cultures, simpler nonvertebrate organisms, mathematical and computer simulations, and studies in human volunteers.

Why were they not suitable?

We will supplement our research with the identified non-animal alternatives to refine our hypotheses whenever possible, but our objectives cannot be achieved by non-animal alternatives alone. Isolated cell cultures are not able to describe behavioural alterations of a living organism affected by psychosis. Similarly, simpler non-vertebrate organisms are not suited to reproduce the complex behavioural alterations related to psychosis.

Mathematical and computer simulations are unable to recapitulate the unknown biological links between immunity, brain and behaviour related to psychosis. In human volunteers we cannot study the biological mechanisms underlying psychosis in sufficient detail because we cannot directly access the brain for experimental measurements and manipulations. The available methods for studying brain function non-invasively (functional imaging, electroencephalography, transcranial magnetic stimulation) lack the temporal, spatial and biological resolution required to achieve our objectives.

A retrospective assessment of replacement will be due by 04 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 used our previous research to estimate the number of animals. When interventions are required, we expect that 5-10 animals per treatment group will usually be sufficient to obtain robust results.

Experimental design is based on the PREPARE guidelines. When effect sizes are known, we will use power calculations to determine the number of animals needed. When effect sizes are not known, we will use the minimum number of animals to provide an adequate description or perform power calculations after the first experimental pilot animals, when expected animal numbers are comparably large (>10 animals).

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

To complement my statistical expertise gained during 15 years of research, I will consult with the biostatisticians at my institution whenever necessary for advice on sample size based on power analyses and pilot studies. I will use online tools such as the NC3R Experimental Design Assistant to adequately design the experiments with the minimum number of animals needed, whenever applicable. We will mainly use repeated-measures experimental designs, which will reduce the number of animals needed as compared to standard grouped designs.

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

To achieve our objectives, we will need transgenic mouse lines. Efficient breeding will minimize the number of animals during breeding. This will include breeding from homozygous breeders to ensure that all offspring have a suitable genotype as well as cryopreservation of embryos to enable breeding only when animals are needed. To further minimize the number of animals, we plan to minimize individual variability by using in-bred strains with genetically homogenous backgrounds. To further reduce animal numbers, animals will be efficiently used whenever possible without adverse effects on animal welfare. For instance, when animals have successfully undergone non-invasive behavioural training in a task, they may be transferred to another protocol to test the effects of different experimental interventions on behaviour. This will reduce the number of animals required as compared to the alternative of establishing a behavioural task by training one cohort of animals and testing the effects of experimental interventions by training and testing a new cohort of animals. Moreover, we will minimize the number of animals by maximizing the amount of data gained from one animal whenever possible. For instance, we plan to use imaging or electrical recording methods that allow studying multiple nerve cells at a time, as well as molecular analyses that yield information about single nerve or immune cells. All experiments will be conducted in animals of both sexes, unless a scientific reason suggests the use of one sex. For instance, when a certain autoantibody is exclusively present in female psychosis patients, we will focus on female mice to investigate the biological role of this autoantibody in psychosis.

A retrospective assessment of reduction will be due by 04 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.

Experiments will be performed in wild-type animals (around 60%) and genetically altered animals (40%). Genetic altered animals will carry mutations that help us to measure and manipulate specific kinds of nerve and immune cells. These animals are not expected to show any clinical signs or harmful phenotypes. In some cases, we will use genetic alterations will model genetic and immune alterations associated with psychotic disorders. These animals typically show mild behavioural changes as assessed by specialised tests but no signs of adverse effects that impact materially on their general well-being.

All animals included here will undergo behavioural experiments that capture psychosis-like behaviour. During some of these experiments, animals are trained to respond to sensory stimuli such as lights or sounds in order to get a water reward. In most cases, animals will freely move around during these experiments, which is associated with less distress as compared to head restraint. Head restraint will only be used when valid neural recordings cannot be obtained without it. To make sure that animals are motivated to perform the experiments, water will be mildly restricted outside the experiments. Mild water restriction is a method to motivate animal behaviour that is associated with less pain and suffering as compared to aversive motivations using painful punishments.

Some animals will receive repeated injections or be drawn blood through a needle. Although the harm of these procedures is transient, repetition can contribute to cumulative suffering. However, this repetition is necessary for the efficient experimental repeated-measures design which will reduce animal numbers (reduction). Some animals will be drawn very small volumes of cerebrospinal fluid through an inserted cannula under short-term general anaesthesia. This procedure is similar to a lumbar puncture that is routinely performed without anaesthesia in human patients in neurology and oncology. This procedure allows assessing the immune compartment associated with the brain. Withdrawal of cerebrospinal fluid does not result in lasting harm and is associated with less harm than alternative methods involving the removal of brain membranes or tissue. Some animals will experience paralysis and weight loss. We will follow established best practices for refinement, and closely monitor the animals on a daily basis using established scoring systems. We will use adapted cage environments that can be easily navigated and provide specific high-calory food in easily accessible locations to ensure that the animals can continue to feed. Animals that become too unwell and unable to feed or groom will not be kept.

Some animals will undergo surgical procedures. Surgery is necessary to allow access to the brain. We will use the least invasive surgery method suited to answer our scientific question. For instance, to test the role of one brain region in psychosis-relevant behaviours, we will use drugs to transiently block signalling in that region instead of surgically removing that brain region. Good surgery techniques and pain killers after the surgery reduce the pain and suffering of the animals.

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

Psychosis affects patients of all ages with a peak in early adulthood. The investigation of the biological mechanisms underlying psychosis therefore requires the study of animals of all ages and cannot be exclusively performed at most immature life stages. Because psychosis affects patients as a whole behaving individual, psychosis-like behaviours cannot be studied under terminal anaesthesia.

Moreover, for the work to be translatable to human patients, mammalian species are needed. Mice are well established in neuroscience and immunology research.

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

To reduce the stress associated with behavioural experiments, all the delivered stimuli are below the pain threshold, and animals will be frequently handled and habituated to the experimental setup over the course of days. To minimize thirst caused by the water restriction, we will either ensure that animals receive a minimum of 40ml/kg water (roughly equivalent to 1/3 of their body weight) or we will provide animals unrestricted access to citric acid water (less palatable but equally hydrating as regular water). Whenever possible, neural recordings will be carried out in freely moving animals using lightweight implants that are easily supported by the animal, but, in some cases, head restraint may be needed to enable valid results. In this case, animals will be habituated to the recording setup in incremental steps starting with short durations on the order of several minutes.
To reduce the stress associated with the injection or blood draws, animals will be habituated to being held in the hand of an experimenter. Moreover, injection volumes will be small, and single use of needles ensures a sharp and clean needle per animal. Suitably small needle sizes will be chosen, in accordance with current best practice. To reduce the stress associated with cerebrospinal fluid draws, animals will undergo this procedure under short-term anaesthesia.

To reduce the risk of infection during immune cell transfer, animals will be housed in clean facilities and closely monitored. If signs of infection are detected, a veterinarian will be consulted about the possibility of prescribing antibiotics.
Surgical procedures will be conducted under anaesthesia and aseptic conditions to alleviate pain and reduce the risk of postoperative infection. Postoperative pain and inflammation will be closely monitored, typically twice a day. Animals will receive preventive pain killers during the surgery and when they show signs of distress after the surgery. Animals will be left one week to recover before undergoing behavioural experiments.

Animals will be observed every day by a person experienced in animal husbandry to identify potential adverse events and ensure that humane endpoints are adhered. We will typically group-house animals and provide enrichment including nesting material to increase animal welfare.

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

Whenever applicable, we will follow the best practice guidelines provided by the NC3Rs (e.g. for blood draws or for husbandry). For surgical and non-surgical procedures, we will follow the recommendations of the Laboratory Animal Science Association (https://www.lasa.co.uk/wpcontent/uploads/2018/05/Aseptic-Surgery.pdf) and of the Procedures with Care website (https://researchanimaltraining.com/article-categories/procedures-with-care/).

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

I constantly monitor best practices and new information available in the international literature and on the NC3RS and RSPCA websites (www.nc3rs.org.uk/our-resources www.rspca.org.uk/adviceandwelfare/laboratory). I am also subscribed to the newsletter of the NC3RS (www.nc3rs.org.uk), and follow the RSPCA twitter account dedicated at laboratory animal welfare (@RSPCA_LabAnimal).

A retrospective assessment of refinement will be due by 04 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?