Home Office

Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted July - December 2021 that require a retrospective assessment

Contents

1. Fish-pathogen interactions

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
- 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

Parasitology, Infectious Disease, Fish-parasite interactions, Fish welfare, Multi-stressors

Animal types	Life stages
Freshwater fish species including invasive non-native species	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 investigate how environmental and biological factors influence fish-pathogen interactions. This will help us prevent and predict disease outbreaks to reduce the impact of these parasites, which includes bacteria, fungal-like organisms, worms and crustaceans, on native and non- native fish populations.

A retrospective assessment of these aims will be due by 19 January 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 present one of the biggest challenges to the sustainable growth of aquaculture, they threaten endangered fish stocks and significantly impact fish welfare.

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

New information and publications covering: new tools for early disease diagnoses; metabolic impact of specific pathogens to their hosts; markers of strain variation; how disease impacts movement of fish around different engineered structures; non-additive effects of multiple stressors on fish welfare; impact of pollutants and bio-alternative products on fish health; assessment of dietary supplements on fish health.

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

Parasites constitute the majority of species on Earth (Windsor 1998). In addition to their huge medical and veterinary impact, parasites are a driving force for host evolution (e.g. Hamilton et al. 1990) and are often transmitted between novel host species leading to the emergence of new infectious diseases.

This can be accelerated if they are accidentally introduced into new ecosystems as Invasive Non- Native Species (INNS) or with INNS hosts. With intensification of farming practices in the aquatic environment, fish parasites are now recognised as the 'weeds of aquaculture'. Even a single parasite species can cost the industry millions £ per annum just to contain (Cable & Harris 2003). The ornamental fish industry also loses billions of £ each year to fish disease. For instance, we urgently need to understand more about the parasites belonging to the genus Saprolegnia because of their enormous global impact on fish stocks. These 'fungal-like' oomycetes are ubiquitous pathogens of freshwater fishes. In aquaculture, Saprolegnia infections are considered to be the single largest cause of economic loss; a staggering 1 in 10 farmed salmon die due to saprolegniasis. Infections

with Saprolegnia have also been associated with serious losses of wild fish stocks, with increasing problems observed in migratory salmonids across England and Wales. All these infections pose a growing threat to fish populations and the socio-economic value of the fisheries they support. By helping to understand the factors that influence the spread and introduction of pathogens, we can inform control programmes, and reduce suffering and host mortality caused by infectious diseases.

Thus, there are direct benefits of this work to:

1. Improving fish welfare by reducing infectious parasite burden and understanding the impact of other stressors on disease symptoms.

2. Food fish aquaculture (addressing basic epidemiological questions can inform farming through direct links with companies and test dietary supplements)

3. Ornamental fish industry (providing direct advice on the care of fish in the hobbyist market).

4. Wild fish stocks (through collaborations with the Centre for the Environment, Fisheries and Aquaculture Science (Cefas), Natural Resources Wales (NRW) and the Environment Agency (EA) the findings of our work can be used to assess the threat pathogens pose to native and Invasive Non- Native Species of fish).

In addition, this research is of fundamental zoological interest, concerned with unravelling the adaptations of parasites and their hosts.

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

Within academia, our work is both multidisciplinary and interdisciplinary, and involves extensive stakeholder collaboration, ranging from governmental organisations (Environment Agency, Natural Resources Wales, Centre for the Environment, Fisheries and Aquaculture Science, Government Advisory groups including Invasive Non-Native Species Advisory Committee) to academic societies (British Society for Parasitology, Parasites and Pathogens Special Interest group of the British Ecological Society), professional societies (European Scientific Counsel Companion Animal Parasites), industry, and other local groups.

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.

Fish are the most diverse group of vertebrates but are vulnerable to anthropogenic changes; in fact available data on species extinction suggests freshwater fish are subject to a higher rate than any other terrestrial vertebrate group (Dudgeon et al. 2006; Adams et al. 2014). With fish playing a key role in food chains and ecosystem stability, the 'silent extinction' of fish may have far reaching ecological implications. For humanity, fish occupy a key role. With the exception of poultry, we consume more fish than all other animals combined, and aquaculture continues to be the fastest growing food sector globally (FAO, 2018). The Five Freedoms of animal welfare (OIE Aquatic Animal Health Code, 2019) is equally applicable to fish as terrestrial animals but is generally less well studied. The third freedom - freedom from pain, injury and disease – is arguably, the greatest welfare concern for fish, particularly as parasites plague the fish industry and wild stocks. This project licence seeks to understand the impact of key parasite species on fish stocks. Particular host-parasite combinations to be studied here will be determined by the most pressing needs as new and emerging infections are recorded, which is why it is essential not to specify particular species. Our fundamental research is based on well-known experimental models for which we already have a wealth of knowledge, namely the tropical guppy (Poecilia reticulata) and the temperate three-spined stickleback (Gasterosteus aculeatus) and their associated gyrodactylid (ectoparasite) species. For all fish, it is essential that we test some of the pathogens on different life stages of the host and sexes as we know the impact of parasites can vary with host age, size and sex.

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

Fish will be experimentally infected with specific pathogens that these hosts would naturally encounter in the wild and or in aquaculture. We will expose the fish to a predetermined infection dose under controlled environmental conditions so that we can assess the impact of parasites on the hosts without any confounding variables. Experimental infections on individual hosts may last for only a few hours to months or even years, but typically we will monitor the infection for less than a month before then treating the fish, and in some cases then assessing if there are any long-term effects of the infection (or treatment) on host behaviour or physiology. Three different protocols are included in this licence reflecting whether fish will be infected with relatively mild endemic infections (Protocols 1 and 2), or a severe infection that has the potential to kill fish rapidly (Protocol 3).

For some experiments, it will be necessary to identify individuals so that we can assess how parasites are spread through populations, and how this impacts individual disease risk. If this is possible using natural colour patterns on the fish then it will not be necessary to conduct any procedure. For fish that look similar, however, we will tag the fish (using either VIE or PIT tags) either for laboratory experiments (where we might be assessing the impact of parasite burden on individual host behaviour over time) or field experiments (where we might be conducting a mark-recapture experiment to look at the change in parasite burden over time under natural conditions). Alternatively, examination of fish from the field might just involve collecting particular wild type parasite strains.

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

Fish with parasitic infections are likely to experience altered swimming behaviour, increased metabolic cost, lethargy and altered feeding behaviour. For many parasites, this will not impact host body weight unless the experiment is continued beyond two months.

Some parasites will be visible on the fish, for instance, resulting in fluffy (mycelial) growths on the body, which can appear within hours of infection. Others will cause indirect clinical signs, such as causing the fin rays to fuse or altering colour of the skin. Individual fish even though exposed to the same pathogen will respond very differently to infection, ranging from asymptomatic condition to death.

There is no evidence that tagging fish (using our methods - VIE and PIT tags) causes any lasting impacts.

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 depend on the pathogen, fish species (but also the stock and even individual) and specific environmental conditions. Approximately 80, 15 and 5% of fish in any population are likely to experience mild, moderate or severe (risk of death) infection outcomes, but this is lower than fish would experience in the wild and much lower than in aquaculture.

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

Killed
Kept alive
Set free
Rehomed

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

Obligate parasites cannot survive for any length of time away from the host, and all parasites, at least to a certain degree, are host specific. Therefore, if there is a need to study a particular pathogen and in vitro maintenance is not possible, then the only option is to work with the specific hosts that are naturally infected with the pathogen. This project studies particular fish pathogens, which requires the use of these vertebrate hosts.

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

For the aims of this project, there are no alternatives because of the inability to culture the parasites in vitro or in vivo on different hosts.

Why were they not suitable?

N/A.

A retrospective assessment of replacement will be due by 19 January 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?

Primarily based on previous experience with similar experiments.

Typically, each treatment (exposing fish to a particular parasite or other stressor) involves 30 fish and for each experiment there is at least one uninfected control group (also 30 fish) so for an experiment with 6 treatments (and this includes the control) = 30 x 6 treatments = 180 fish. Approximately 6 of these experiments would be conducted per year (ca. 1000 x 5=5000). In addition, we would conduct ca. 4 larger scale experiments per year (8 treatments = 4800 fish) and need fish to maintain those parasite cultures, which cannot be kept viable off the host, which based on the previous 20 years experience is ca. 1000 fish per year = 5000). Hence total Project use over 5 years of ca. 14,800 with additional 700 fish added to allow for contingency planning.

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

Through close collaborations with other parasitologists and fish ecologists/behaviouralists, it is possible to combine experiments to reduce animal usage. The methods we currently employ are well established and have been optimised over the last 30 years (at least for the main pathogens we work on) to ensure the minimum number of fish are used for any one experiment while still generating significant and interpretable results in full multifactorial statistical analyses. Where other pathogens for which much less information is available are to be studied, we will always consult with other researchers in the field to check that we have selected optimal methods for reducing the number of animals needed.

For each experiment, experimental design is often the longest part of the process which involves a review of the literature, consultation with additional experts (if necessary) and consideration of sample size and data analyses resulting from previous experiments. The NC3Rs EDA is often not suitable for our use because of the multiple variables we might be testing simultaneously, but when we have used this - it has confirmed our selection of sample size.

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

Computer modelling is an active aspect of our work through active collaborations with mathematicians. Sharing of tissue samples is considered during our experimental design phase and our small biobank of past samples is readily shared with colleagues to prevent further unnecessary use of samples. We also collaborate with many researchers, experts for example in 'omics technologies, so that the scope of our work can be expanded and other research groups at different universities can avoid having to use any animals.

A retrospective assessment of reduction will be due by 19 January 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.

Freshwater fish are chosen based on current knowledge of host specificity, the specific questions we are asking, and the ease with which we can mimic natural conditions for the animals under a laboratory setting. Methods have been developed (many in our lab) to reduce animal suffering; because we are particularly interested in how parasites act as drivers of host evolution in natural conditions it is essential that the animals are kept in optimal conditions and not unduly stressed. A key advantage of using ectoparasites as a model system is that the entire trajectory of infection can be monitored on a single host.

Predator models (which may include visual or and chemical cues) will be used in preference to live predators where possible since they allow the least invasive method of measuring behavioural responses in a repeatable and controlled way. The length of time for which the model is presented will be minimised and the model will be removed after a response is noted. In trials where fish are exposed to a predator stimulus, refugia such as patches of weed, rocks and flowerpots will be provided within which the fish will be able to shelter, ensuring that the environment is enriched, and that the accommodation needs of the animals are met. We now know that this enrichment is also essential to prevent fish stress which impacts host immunity and disease susceptibility.

Surgical implantation of VIE or PIT-tags is the least invasive method of individual tagging available for small and juvenile fish. Each PIT tag weighs 0.1g, which is less than 3% of the total weight of the fish at the time of implantation.

In a comparison of the adverse effects of seine netting, angling and electro-fishing (Bouck and Ball 1966) highest mortality rates were found for angling. There are few sub-lethal effects of electro-fishing, with fish regaining usual behaviour within a few minutes of capture (Beaumont et al. 2000). Steady DC electric fishing will be used as far as possible (when water chemistry conditions allow) since it causes fewer injuries than pulsed DC (e.g. Hudy 1985; Hollender and Carline 1994; Beaumont et al. 2000).

This method will only be deployed by external agencies, such as the Environment Agency Fisheries Team).

Two of the three protocols under this licence are graded moderate to reflect the worst case scenario that occasionally an individual fish may die from an experimentally induced infection. Overall, the work under this licence is graded mild in terms of severity, reflecting that most of the animals we experimentally infect will have lower parasite burdens than they would naturally encounter in the wild, and significantly reduced compared to infection levels in aquaculture. For example, with regard to guppy parasites, although parasite induced mortality in the wild is unknown, the high natural prevalence of gyrodactylids (up to 85%) indicates that these parasites exert an important selective force. Commercially purchased guppies harbour high multi-parasite burdens, which often leads to high rates

(10-90%) of host mortality. In contrast, the majority of infected fish that we will maintain will carry low parasite burdens with <10% host mortality during the course of an experimental infection.

The final protocol has a severe categorisation as Saprolegnia parasitica has the potential to kill fish very quickly, depending on the strain. Conversely, other fish in the same tank may be completely unharmed and may not even acquire an infection. To minimize potential severity, we will check fish hourly post-inoculation (during the period of maximum severity, typically up to 72h post spore exposure). Any fish that are lethargic, show reduced swimming capability or signs of extreme fungal lesions will be euthanised. Therefore, only a small % of fish will die from infection with no intervention (e.g. humane end-point at onset of overt infection). However, we urgently need to understand more about this parasite because of its enormous global impact on fish stocks. The 'fungal-like' oomycetes of the genus Saprolegnia are ubiquitous pathogens of freshwater fishes. In aquaculture, Saprolegnia infections are considered to be the single largest cause of economic loss; a staggering 1 in 10 farmed salmon die due to saprolegniasis. Infections with Saprolegnia have also been associated with serious losses of wild fish stocks, with increasing problems observed in migratory salmonids across England and Wales. These infections pose a growing threat to fish populations and the socio-economic value of the fisheries they support.

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

Fish parasites require live juvenile or adult fish to survive so using these hosts is the only way that we can conduct this research.

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

Over the last few years, we have specifically focussed our research on environmental factors known to impact fish welfare (for example noise, vibration, light, enrichment) and this new information will be used to refine our baseline conditions for all our fish stocks.

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

To a certain extent we are leading the way on this with regard to fish pathogens by providing guidelines on best practices and during the course of this licence we hope to expand this to particular endangered fish species, working in collaboration with the Environment Agency.

All new researchers to the lab are trained in experimental design and asked to summarise this information to the wider research group so everyone's knowledge is regularly updated.

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

We share and receive any new information regarding advances in 3Rs with researchers who are working with similar hosts and or parasites. We are also in regular contact with the NC3Rs committee, our local Animal Welfare and Ethical Review Body, and fish managers and organisations like OATA (Ornamental Aquatic Trade Association) and ESCCAP (European Scientific Counsel Companion Animal Parasites) whose priority is animal welfare.

A retrospective assessment of refinement will be due by 19 January 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. Regenerative neuroimmunology applied to progressive multiple sclerosis research

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

Neuroimmunology, Progressive MS, Brain-Immune Interactions, Regenerative Medicine, Therapy

Animal types	Life stages
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?

The aim of this project is to identify and manipulate important biological processes that control the coordinated actions of the immune system and the brain in the context of progressive multiple sclerosis (MS). The ultimate goal of this project is to increase the brain’s own ability to heal the damage caused by MS, which will prevent the accumulation of disabilities and slow down the progression of the disease.

A retrospective assessment of these aims will be due by 08 January 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?

Multiple sclerosis (MS) is a lifelong disease that affects the brain and spinal cord of people of all ages. MS is an autoimmune disease. As such, MS starts when an unknown cause makes the immune system dysfunctional in a way that it would mistakenly attack the layer that surrounds and protects the nerves. This is called the myelin sheath and in MS this is repeatedly attacked by cells of the immune system, leading to damage and scarring. As a further consequence of these repeated attacks to the myelin sheath, further damage to the nerves the sheath protects occurs. This means that messages that travel along the nerves are slowed or stopped. This can lead to problems with vision, arm and leg movement, sensation, and balance issues.

The main role of the immune system is to protect against infections caused by bacteria, viruses, and parasites. However, we now know that the immune system is involved in the development of the brain and spinal cord. Here, the immune system helps with the maturation of new nerve cells and myelin sheath forming cells. Ultimately, the cells and molecules that make-up the immune system work to prolong the bad inflammation in the brain and spinal cord. This is what contributes to the large amount of tissue damage that occurs in MS.

After many years with MS, a large number of patients go on to develop a progressive form of the disease. This usually happens in people who are 50 and older. The progressive form of the disease is a result of continued bad inflammation that does not allow the healing of the brain. This is an important aspect to consider as the disease changes in patients getting older. In older patients, the symptoms become worse over time. This causes an increase in disability that is permanent. Unfortunately, there are no treatments available for these patients once they have reached this point in the disease.

Therefore, the development of treatments for patients with progressive MS is important.

New information has shown us that immune cells that were once bad can be changed into good immune cells. These good immune cells can then be used to help heal the damaged brain and spinal cord. This will improve the quality of life for patients with progressive MS.

In fact, previous work in the lab has identified new ways to turn bad immune cells into good immune cells. These good immune cells have been shown to help heal the MS brain in laboratory mice.

We have also shown that when you put brain stem cells into mice with an MS-like disease they can turn bad immune cells into good immune cells. This reduces the damage in the brain caused by the bad immune cells. In addition, we have put these brain stem cells into mice with a damaged myelin sheath to help form a new myelin sheath. These experiments have shown that we are able to alter the interaction between the immune system and the brain in a way that it will eventually heal. This could be very important in helping to promote reduction of brain damage and slow down the accumulation of disabilities in the progressive form of MS.

With this license, we wish to further explore new ways to experimentally manipulate the interactions between the brain and the immune system. This will create new opportunities to test new therapies that can stop bad inflammation. This will help to promote the regeneration of the damaged MS brain.

We will focus our efforts on studying the way immune and brain cells produce energy to carry out their complex activities called metabolism. In fact, early evidence exists that interfering with the metabolism of brain or immune cells is a promising new approach to treat MS.

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

The main output of this project will be new information on the function and therapeutic potential of mechanisms that lead to continued brain inflammation. Additional outputs will include the publication of data from experiments in scientific journals. Datasets containing a large amount of information will be made accessible. We will use these datasets to apply for funds for future projects. These datasets will also be useful for other scientists and doctors studying progressive MS. Additional products from this project will be (1) patents to protect the main discoveries, (2) tools to study immune and brain cell function or deliver therapies, and (3) technologies to identify if the therapies are effective.

Ultimately, our approach will lead to the identification of new and important interactions of the brain and immune system. We can then use new experimental methods to target these interactions. These experimental methods could then be tested in patients with progressive MS.

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

In the short term, the main beneficiaries of this project's outputs will be scientists from academic institutions and from pharmaceutical companies. In the medium term, the outputs generated by this project will help the NHS and the patient community. These outputs will advance treatments for progressive MS that are aimed at slowing down disease progression and promoting brain repair. In the long term, these outputs will help

us to better understand how to slow the damage to brain caused by ageing and help the ever-growing senior population maintain healthy brain function.

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

The outputs of this work will be distributed to academic scientists throughout the duration of the license. We will communicate regularly with organized research networks and laboratory groups in the field of progressive MS and beyond. These networks will allow us to share workloads and ideas. This depends on the expertise of each group, avoiding repeat experiments, and accelerating the progress in these fields.

Preliminary data will be shared at national and international conferences and/or workshops in order to gain valuable feedback of the work from peers. This will also provide us the opportunity to build new collaborations locally and internationally. This will improve the quality and rigor of our research for the duration of the licence. We will also present our findings ahead of publication on preprint servers (such as arXiv, bioRxiv, or PeerJ).

When finalized, all our data will be published in peer-reviewed scientific journals. This data will include gene, protein, and metabolic datasets. We will also make these datasets available on appropriate databases for other research groups to access freely.

We are committed to publishing both positive and negative results. This will increase awareness and inform the community of how our findings fit into the wider field of study. It will also help in determining which experimental outputs are worth pursuing further and those we feel would not be worth further exploration.

Finally, we will ensure that the published results are made available to the general public. This will maximize their impact and increase global awareness to both the public and fellow scientists.

Species and numbers of animals expected to be used

- Mice: 2000

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 new-born mice that have been produced on a different licence to collect cells to grow and keep in a dish to do experiments.

We are using mice because currently they are the most commonly used animal in human disease research. It is extremely difficult to accurately and fully model the complexity of MS using cells in a dish. Therefore, using mice we can study many of the changes caused

by MS disease in the patient's cells and tissue. These include the formation of scar tissue, the presence of inflammatory immune cells, and damaged nerve fibres. Additionally, the possibility to use genetically modified mice to study target proteins and pathways involved in the MS disease process is particularly useful.

Since MS is 2 to 3 times more common in women than men, our studies will reflect this. Here, we will use an increased proportion of female mice to male mice in our experimental protocols. We will also use both young and old mice. This will allow us to investigate the biological impact of human adulthood and human ageing more accurately on the progression of brain and spinal cord inflammatory damage. This is highly relevant as progressive MS effects older adults more and is a chronic, life-long disease.

Additionally, we now know that ageing plays a significant role in the ability of the brain to heal itself. This further emphasizes the need to study the disease using both young and aged adult mice.

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

Mice will be subjected to a disease called experimental autoimmune encephalomyelitis (EAE). EAE is widely used to investigate aspects of the human disease multiple sclerosis. In mice, EAE disease results in immune cells normally found outside the brain and spinal cord, collectively called the central nervous system (CNS), invading the CNS. Here, immune cells interact with cells of the CNS to create an environment that is destructive to the tissue. This results in the mice displaying clinical signs of disability similar to those observed in MS patients. These include impairment in walking, paralysis of limbs, and issues with bladder control and output. These mice also display tissue level signs of the human disease such as the accumulation of inflammatory immune cells normally found outside the CNS, damage to nerve fibres, and destruction of the protective wrapping (i.e., myelin sheath) of nerve fibres.

EAE disease induction in mice is as follows. On day 0, mice will be maintained under light anaesthetic and loosely restrained and positioned for injections. A protein of the myelin sheath will be mixed with an oil based solution that has been supplemented with a heat-inactivated version of a disease-causing bacteria. This will create a dense mixture that will be injected under the skin of the mouse. This mixture will produce a local autoimmune response that results in the generation of inflammatory immune cells. These immune cells eventually enter the brain and spinal cord and start a neurological disease like in MS. The mixture will be injected under the skin at three separate locations on the mouse: 1) in front of the base of the tail, 2) in two sites near the left and right shoulder blades. Mice will also receive two consecutive doses into a vein of a solution containing a bacterial toxin. These doses will be given on the day of the mixture injection (day 0) and two days later (day 2). Following each of these injections, mice will be closely observed while they recover from the light anaesthetic until fully awake and responsive. The weight and overall condition of the body of the injected mice will then be monitored daily throughout the length of the study.

Lysophosphatidylcholine (LPC)-induced MS-like lesion model is as follows. Mice will be anaesthetised and remain unconscious throughout the surgery. Mice will be given both pre- and post-surgery pain relief. Anaesthetised mice will then be securely fixed in a device to maintain a stable body position. Then, a small portion of the thin membrane covering the upper and middle part of the spinal cord will be exposed to reveal the underlying tissue. This way we can cause the localized destruction of the myelin sheaths. This is done by slowly injecting a small volume of a chemical that is known to be toxic to myelin sheath forming cells only (i.e., lysophosphatidylcholine-LPC) into the spinal cord tissue through a syringe and ultra-fine needle. Following the injection, the needle will be slowly removed. Mice will receive post-surgery care that includes pain medication, soft bedding, and access to (wet) mashed food.

Depending on the experiments, EAE and LPC-lesioned mice may receive additional injections. These injections can be intravenous (e.g., into a vein), intraperitoneal (e.g., into the abdomen), subcutaneous (e.g., under the skin), intrathecal (e.g., into the fluid filled spaces of the spinal cord), intracerebroventricular (e.g., into the fluid filled spaces of the brain), intraparenchymal (e.g., into tissue), and local into the spinal cord. Injections will include either substances (such as drugs, beneficial small molecules, and agents to induce gene modification), viruses (as a vehicle to artificially carry foreign genetic material into cells of the mice), and cells. These substances are all meant to encourage the interactions between the immune system and the brain in a way that results in regeneration or less damage of the brain and the spinal cord.

Finally, to validate and identify biomarkers of brain and spinal cord damage and their role in the regenerative process, we may also collect bodily fluids from live mice. These include blood and cerebrospinal fluid (CSF). CSF is a clear fluid that surrounds and cushions the brain and spinal cord from injury. These fluid samples are important for verification or discovery research using technologies that enable the rapid testing of large numbers of substances and molecules for activity. Blood is obtained from a surface vein observable to the naked eye using a fine needle. CSF is obtained via a quick, minimally invasive surgical procedure under general anaesthetic so that the mouse is unconscious.

The study lengths for experiments using EAE or the LPC-induced MS-like lesion model may be as short as 7 days or as long as 6 months (maximum limit) to study both early and delayed regenerative responses. The duration of each experiment is determined prior to the use of any mouse study and is variable in length. At the end of the experiment all mice will either be humanely killed or tissues and organs collected under deep, terminal anaesthetic unconsciousness. Tissue and organs collected this way will be stored in a solution that preserves their structure until follow-up analyses.

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

Within the EAE protocol, mice will present with limb weakness that progresses to total paralysis. Total paralysis of a limb or limbs often leads to weakness in other functional

limbs. The effects of EAE begin with weakness of the tail muscles that spreads towards the back legs. This weakness can also spread to the front legs. The severity of the weakness or paralysis can last from 10-35 days after the first sign of disease are observed. The first signs of disease are usually observed at 10-15 days following injection with the disease causing mixture. Within 21 days from the injection of the disease-causing mixture, over 90% of mice show a long-lasting and irreversible disease course. All mice with the disease gradually lose body weight following injection with the disease causing mixture. The amount of weight loss that occurs depends on the severity of the disease in each individual mouse. This weight loss is temporary.

All mice gradually gain back the weight that was lost. This recovery of weight occurs during the 2-3 weeks after the most severe effects occur.

Within the LPC-induced MS-like lesion protocol, mice are expected to display signs of pain after surgery. This can last for up to 10 days after surgery. Steps are taken to minimise the pain by providing pain medications to mice before and after surgery. Mice will also have temporary weight loss after surgery. The weight loss is expected to recover within 48 hours of the end of the surgery.

In mice that receive additional injections of substances, there could be temporary pain and discomfort. This will last for a maximum of 48 hours (depending on the route of injection). Collection of blood through the vein in the tail will only lead to temporary discomfort for the mouse (equivalent of a very fine needle jab). Collection of cerebrospinal fluid may cause changes in (1) food/liquid intake, (2) normal expected weight gain, and/or (3) temporary (max 48 hours) pain at the site of collection.

Within this license, some mice used within the EAE and LPC-induced MS-like lesion protocol will be aged. The majority (>90%) of adult mice remain healthy up to 15 months of age. Mice aged past this point (up to 24 months) may develop health problems due to their old age. This can include: (1) the growth of small, non-cancerous tumours under the skin (10%), (2) hair loss (10%), (3) loss of fur colour (i.e., greying of fur) (10%), and obesity (i.e., non-healthy weight gain) (10%). This should not cause any adverse effects or suffering in the mice.

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: Moderate 25%

Mouse: Severe 75%

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

Killed

A retrospective assessment of these predicted harms will be due by 08 January 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?

Progressive MS in humans leads to permanent and irreversible damage to the brain and spinal cord. This leads to a life-long decrease in the quality of life of patients and their carers. Therefore we need to develop treatments that are able to reduce the impact of non-stop inflammation on the human brain and spinal cord to promote its healing. This is a main unmet need for patients with progressive MS. However, no experimental treatment can be tested in humans without first testing its safety and effectiveness in relevant animal diseases that reproduce aspects of the human disease. Any treatment developed to work on cells grown in a lab dish has to be tested and refined in animals. This way we can see how the treatment works in the complex environment of the adult brain and spinal cord. We can also determine if the treatment causes a recovery of functions.

To confirm findings using our cells in a dish, we need to use validated and widely accepted mouse models of diseases that reproduce aspects of MS seen in humans. This allows us to test whether the responses of immune cells in a dish and the effect of treatments are similar to the effects that occur in a mouse with the disease. The response of immune cells in disease is very different in a mouse compared to cells in a plastic dish. In a mouse with MS-like disease, there are many responses by the cells, tissues, and organs of the body which directly impact the ability of the brain and spinal cord to recover. These include inflammation, the formation of scars, and loss of nerve fibres necessary for normal movement of the bodies limbs. It can also affect how well a treatment works. Further to this, injury to the brain and spinal cord and ageing also affects many other cell types that are present. This a further challenge that limits our ability to study cells in a dish.

We are using mice because currently they are the most commonly used animal in human disease research. It is extremely difficult to accurately and fully model the complexity of MS using cells in a dish. Therefore, using mice we can study many of the changes caused by MS disease in the patient’s cells and tissue. These include the formation of scar tissue, the presence of inflammatory immune cells, and damaged nerve fibres. Additionally, the

possibility to use genetically modified mice to study target proteins and pathways involved in the MS disease process is particularly useful.

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

Over the years our team has refined and improved upon our models of cells grown in lab culture dishes. This has allowed us to test whether the treatments will be (1) safe for the cells and (2) how effective the treatments are before testing them in a mouse. Additionally, we have developed a new model of maintaining and expanding human immune cells and human stem cells in plastic dishes that does not involve the use of mice. This new system allows us to (1) capture the response of human cells in a dish and (2) design/perform experiments to test ideas about the response of cells without having to extract these cells from mice beforehand. After the identification of targets, we will then proceed to test our results in our mouse models of disease.

Why were they not suitable?

Immune and stem cells grown in lab culture dishes are useful for studying some aspects of progressive MS and ageing. However, they cannot replicate the complex changes that occur in the cells and tissues that support the function of the brain and spinal cord. These includes changes to the supporting cells of the brain and spinal cord (called glia). This can lead to the formation of scar tissue, the recruitment of immune cells into the injury site, and damage to nerve fibres that is typical of progressive MS. Immune and stem cells grown in culture also behave differently to those found in a living organism. Here, they show loss of cellular heterogeneity (i.e., the unique identity of individual cells) and loss of communication with other cell types.

It is therefore necessary to use animal models. This allows us to assess the complexity of biological and behavioural responses in an animal both following an injury or disease and after treatment. There is also a requirement to demonstrate that a treatment is safe and effective in animal models before progressing to human application.

A retrospective assessment of replacement will be due by 08 January 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?

Mouse numbers were estimated based on a combination of the retrospective review, annual return of procedures, and estimated mouse usage for the duration of the project. With the new mouse colony management system in use, we now have access to our total mouse usage year-over-year.

For the experimental autoimmune encephalomyelitis (EAE) protocol, we are expecting to use 300 mice/year based on our usage over the last 5 years. Each study typically involves 50 mice and lasts approximately two months. Therefore, we can run six studies per year that includes 300 mice. Over the course of this five year licence we will use a maximum total of 1500 mice in this experimental protocol.

For the lysophosphatidylcholine (LPC)-induced MS-like lesion protocol, we are expecting to use 100 mice/year based on our usage over the last 5 years. This will provide us a maximum total of 500 mice used across in this experimental protocol.

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

The National Centre for the Replacement Refinement & Reduction of Animals in Research (NC3Rs) experimental design assistant is a tool which we constantly use to help design and further refine our experiments. We also reference the Planning Research and Experimental Procedures on Animals: Recommendations for Excellence (PREPARE) guidelines.

According to our lab standard operating procedures (SOPs), experiments are constantly assessed at the pilot stage first. This is when a first experiment is conducted with a reduced number of mice to adjust any aspects before running the full experiment. This guarantees that we are using the correct number of mice to achieve reliable statistical results when experiments are ready to be conducted in full. Mice are then placed in the experimental groups randomly, which helps to ensure treatment and non-treatment groups are evenly distributed. Treatments are given ‘blind’. This means that either the person injecting a treatment (or a vehicle, as control) or the surgeon performing the injections have been given no access to the information related to the treatment they are giving. Blinding is also applied to post-mortem tissue and molecular studies. Unblinding is the responsibility of the principal investigator and it is done only after the experiment is concluded and results are analysed. This is to avoid any bias in the generation of the results.

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 always perform pilot studies before undertaking a full experiment to ensure that larger studies are as accurate as possible. These pilot studies allow us to assess the experimental design and identify potential problems, as well as implement improvements

early on in the licence. We are also coordinating 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.

A retrospective assessment of reduction will be due by 08 January 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 will be subjected to a disease called EAE. This is a widely- and consistently used method to investigate aspects of human MS disease. It has undergone continual refinements over decades of research. The induction of EAE disease is the most refined for the purpose to cause the least pain, suffering, and lasting harm to the mice. Despite these refinements, mice that develop physical signs of EAE inevitably suffer and are in pain. We minimise and manage pain by providing mice with pain medications as needed throughout the course of the study. The substances used to induce EAE disease are certified as high-quality from trusted suppliers. The substances do not contain impurities and other potentially toxic components which could impact on the welfare of the mice. To reduce the chance of ulceration (i.e., open wounds forming) from injections, the area is wiped down with a sterile cleaning solution. The substances to induce disease are administered under the skin and into the vein and result in minimal and temporary discomfort.

The lysophosphatidylcholine (LPC)-induced MS-like lesion requires surgery to inject a toxin that damages the protective covering of nerve fibres. This injection is in the bottom part of the spinal cord. This model allows for the study of tissue damage (loss of protective covering of nerve fibres) and regeneration (regrowth of the protective covering of nerve fibres) in the spinal cord. The toxin-induced lesions very closely resembles the lesions observed in MS patients. This method induces moderate pain and suffering with no lasting harm. We minimise and manage pain by providing mice with pre- and post-surgery pain medications. Pain will be managed during the period of study by administering medication

as needed. This MS-like lesion rarely results in mice developing clinical signs or complications from surgeries. This produces extremely limited suffering to the mouse.

Within our mouse models of MS and MS-like lesions we will also be using aged mice (i.e., older than 15 months). This is the closest way to study how increasing age impacts brain recovery. Mice will be naturally aged (i.e., no manipulations to the mouse to force accelerated ageing will be performed). This is the most refined for the purpose to produce the least suffering.

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

We are extremely limited in the use of invertebrates (e.g., worms), fish, or amphibia. These animals are not fully suitable for the development and testing of new treatments for humans with progressive MS. Some preliminary work on regenerative biology can done in non-mammalian species. However, the complexity of the interactions between the immune system and the brain and spinal cord in the context of tissue damage and regeneration can only be studied in mammals. This is because they possess a body structure and immune system with similarities to the human brain, spinal cord, and immune system.

We also cannot rely completely on animals that have been terminally anaesthetised. This is because we need to assess the long term behavioural and pathological outcomes of our manipulations and interventions. We need to know if they promote regeneration of the damaged and diseased brain and spinal cord. Therefore, we need the animals to remain alive for several weeks-to-months after the onset of disease or after making a lesion in the spinal cord using a toxin.

Finally, we need animals with cells that have reached a mature stage of development. This is because we need the cells to be representative of the cellular make-up present in adult human disease.

Therefore, we will use adult and/or aged animals for all pre-clinical animal models in this project. The use of animals during the immature stages of life will be restricted to the collection of cells to be grown in lab dishes where indicated and appropriate.

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

Before starting any study plan, we will discuss all experimental methods with the appropriate staff within the animal unit. This will guarantee that all the necessary equipment is in place to perform procedures under optimal conditions and/or supervision. This is to maintain the best health and welfare of the animals. Prior to running studies, we will determine if the necessary staff and expertise is available to successfully run the whole study. This way we can determine which skills are missing to guarantee the study is successful. As well, which relevant equipment is available to process samples under optimal conditions.

Once the study has started, we will rely on our established step-by-step care packages. This will minimise the harm to the mice and that the welfare of the mice is never compromised.

Over the years we have made significant refinements to our EAE mouse model. These refinements cause the least amount of pain, suffering, and distress to mice. As a result, we have put together a dedicated and comprehensive standard operating procedure (SOP) that provides a detailed step-by- step care package. Refinements to this model are centred around the housing of mice experiencing expected adverse effects and to the daily care and monitoring of the mice.

Refinements made to the housing of mice experiencing expected adverse effects include the following:

- providing bedding that does not inhibit the free movement of these mice, (2) heating pads fixed to the bottom of the cages to maintain stable core body temperature, (3) placing wet mashed food on the cage floor to encourage eating and allow ease of access to disabled mice, and (4) providing cardboard houses to mice with ulcers to distract the mice from continuing to itch and re-open the wound.

- For the daily care and monitoring of the mice we have made a number of improvements. We have increased the number of daily checks to ensure the health and welfare of the mice is maintained. We now use pain medications to ease disease complications, and perform fluid replacement through subcutaneous (i.e., under the skin) injections if dehydration is present. Mice can experience bladder dysfunctions such as increased frequency of urination with decreased urine output. Mice can also have issues with urinating that results in the build-up of urine in the bladder. Here, as part of our daily monitoring, mice will have their abdomen checked by an experienced user to identify signs of an obstructed bladder. If the bladder cannot be emptied by manual stimulation through the skin, a veterinarian will be contacted to provide immediate care.

Having a dedicated SOP ensures that the highest quality of care is provided to mice throughout the course of this severe study. The SOP also significantly minimises the suffering and improves the welfare of mice experiencing expected adverse effects. This SOP has been generated and further refined following recommendations from animal technicians, named animal care and welfare officers, and the named veterinary surgeon. The SOP and protocol for EAE induction are stored on a dedicated external storage device as a living document. The SOP and EAE induction protocol are constantly updated so that any refinement procedure that is found to be beneficial is kept and stored in our system for training (and access) by future users. For example, our new protocol for EAE induction has led to the reduced incidence of ulcer formation. It is now standard in our lab and is used by all new trainees and existing users.

In case of the LPC-induced MS-like lesion model, or any surgery, we have put in place post-operative assessment sheets specific to our model of injury or disease. These assessment sheets help the technicians and users to better monitor the recovery of the mice after surgery. This post-operative assessment sheet is constantly refined during our work, depending on our observations and in collaboration with the animal unit staff. Incidence of pain during the post-surgery recovery period will be controlled by the administration of pain killers, as directed by the named veterinary surgeon. Surgeries will inevitably cause temporary pain that is gone within 48 hours. This temporary pain will be minimised by the use of pain medications. Regular discussion with a named veterinary surgeon will allow us to improve the management of pain if any new and more suitable recommendation appears during the work. In the case mice suffer from complications after surgery, appropriate guidelines are in place for humane endpoints.

Adult mice undergoing surgical procedures will rarely have complications 48 hours post-surgery. However old mice (15 months and older) may have an increased risk of complications. This includes a lack of recovery to normal baseline of activity levels and food intake. Proper precautions are in place to handle old mice after surgery. This includes daily welfare checks to ensure mice are not suffering, in pain, or distressed until fully recovered. If necessary, pain medications will be given either via (1) an injection under the skin by an experienced user or (2) as a voluntary treatment to be eaten by mixing pain medication via flavoured jelly, paste, or milk shake. Appropriate humane endpoints are defined if old mice are unable to recover.

Full training will be provided to new technicians who are unfamiliar with these procedures, as we have filmed previous study procedures to show how we expect our mice to recover. This helps new technicians to learn how to assess our mice correctly. This guarantees that mice recovering in our experiments receive the same high quality and consistent level of monitoring and care they need.

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

We plan our experiments in accordance with the guidance provided in the Planning Research and Experimental Procedures on Animals: Recommendations for Excellence (PREPARE) guidelines. This will guarantee we use the minimal number of animals to answer our objectives and ensure our results are both robust and reproducible. We will follow the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines when preparing our data for publication. In so doing, we will ensure our published findings are complete and clearly presented and easily accessible to other groups. This will lead to a reduction in the unnecessary duplication of animal experiments.

Excellent information is available on our establishment website, which is routinely updated with new 3Rs information. The National Centre for the 3Rs (NC3Rs) website will be regularly consulted to be sure that we are applying the latest recommendations for the refinement of our experiments. The Laboratory Animal Science Association (LASA)

website provides updated information, especially regarding best research practices to perform aseptic (i.e., germ free environment) surgeries. We will also consider any new publications in a peer-reviewed journal relevant to our field offers refinements to our protocols.

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

Our establishment offers continuous training and recommendations via the animal facility and from animal care staff located within. We will keep informed of any changes to animal welfare guidelines by regularly consulting the website they provide. This will ensure that we maintain compliance should any new updates be posted.

The National Centre for the 3Rs (NC3Rs) will be the main reference to assess whether our experiments match the highest standards of 3Rs. We will adapt our protocols if the recommendations evolve throughout the duration of this project. Regular consultations on the latest practical guidance from Laboratory Animal Science Association (LASA), Institute of Animal Technology (IAT), and the Royal Society for the Prevention of Cruelty to Animals (RSPCA) will provide additional sources of new recommendations and advances in animal techniques and clinically applicable models.

Training records for all personal licence holders will be kept up to date using a centralised database. Senior group members will provide extensive training on the relevant regulated surgical or non-surgical procedures to all new lab members who will be working with animals. Further, new lab members will be informed of the mandatory training services available to them. This will guarantee that general practices are firmly adhered to and will ensure the welfare of the animals is consistently upheld.

As a licence holder, it is my own responsibility to stay updated on published best practices. This will be done by consulting information for licence-holders provided by our establishment and by speaking to other project licence holders.

A retrospective assessment of refinement will be due by 08 January 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. The role of the immune system in cardiovascular disease

Project duration

5 years 0 months

Project purpose

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

Key words

cardiovascular disease, immune system, pathophysiology, inflammation, therapy

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

Diseases of the heart and the circulatory system are mostly due to blockage of blood vessels by a process called atherosclerosis (fatty deposits), and are responsible for heart attack, heart failure, and stroke. We want to understand why our immune defence system goes awry and contributes to the development and progression of these diseases, with the aim to find appropriate treatments.

A retrospective assessment of these aims will be due by 06 January 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?

Diseases of the heart and the circulatory system are still one of the major causes of disability and death worldwide. There's increasing evidence that our immune defence system plays an important role in heart and blood vessel diseases. However, our understanding of how this happens is still limited, and this has prevented scientists from finding new effective treatments. We believe that an improved understanding of the role of the immune system in cardiovascular disease may transform the way we treat patients with (or at risk of) the disease.

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

The present research will lead to a better understanding of how the immune system reacts against fatty deposits in our arteries, and how it controls cardiac repair after injury. The results are expected to lead to more effective strategies to combat atherosclerosis (the blockage of arteries due to fatty deposits) and its complications (heart attack, heart failure). We also expect that the work will substantially enhance our understanding of aneurysm (bulging or ballooning of arteries) growth and rupture, and will identify critical targets for treatment. Our results will be published in peer-reviewed scientific journals, and may lead to the filing of patent applications.

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

Atherosclerosis is responsible for the vast majority of cardiovascular diseases (CVD). CVD, including aortic aneurysm, are the largest contributors to disability and death, in Europe and worldwide. There are around 7.6 million people living with heart and circulatory diseases in the UK. CVD cause more than a quarter of all deaths in the UK– an average of 450 deaths each day or one every three minutes. CVD markedly impact on both quality and length of life of patients. CVD carry an important socio- economic burden. CVD’s cost to the UK economy (including premature death, disability and informal costs) is estimated to be £19 billion each year (BHF statistics compendium, https://www.bhf.org.uk/what-we-do/our-research/heart-statistics/heart-statistics-publications/cardiovascular-disease-statistics-2020).

Direct likely immediate benefits include:

1) Development of new and more relevant ways of investigating aspects of CVD in rodents that can be used by other researchers. For example, we are aiming to develop better ways to study cardiac cachexia (weight loss associated with heart failure, which bodes for increased disability and risk of death) to be able to better understand the disease in humans;

2) Better understanding of the pathophysiology of atherosclerosis (blockage of arteries), myocardial infarction (heart attack) and vascular aneurysm (ballooning of the blood vessels);

3) Identification of new targets to better treat patients with CVD. Our results will therefore benefit the scientific community through the provision of important new knowledge about mechanisms of disease.

Indirect long-term benefits include:

1) Better understanding of the role of specific cells of the immune system in CVD;

2) Develop new ways to better diagnose patients with CVD (identify individuals with the disease and quantify the risk of disease progression);

3) Develop better treatments for patients with atherosclerosis, myocardial infarction, heart failure, and aneurysm.

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

We work in collaboration with an extensive network of doctors and scientists. My group is part of several national and international research networks. I am co-Editor, Associate Editor or Consulting Editor of many highly respected international peer-reviewed scientific journals, and I am member of highly respected scientific societies worldwide. Our results, whether successful or not, will be presented and discussed at international scientific meetings, published in peer-reviewed scientific journals, and disseminated to the general public.

Species and numbers of animals expected to be used

- Mice: 44300

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.

For the most part of our work, we are not studying congenital (inherited or present from birth) heart diseases. Therefore, most of our work will be conducted in adult animals.

We have chosen mice because 1) they are widely validated animal models of heart and circulatory diseases, 2) a large number of genetically-altered mice have been developed and are available to the scientific community, which makes possible the study of the role of specific gene defects in the development and progression of heart and circulatory diseases.

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

A typical animal will be put on normal or high fat diet for 6 to 12 weeks, will undergo blood sampling (potentially after fasting up to 16h) less than 3 times during the duration of the experiment, will regularly be monitored clinically, and killed while under terminal anaesthesia (a state of induced unconsciousness) at the end of the experiment. The animal will not recover from the deep unconscious state before being killed.

Another typical example is an animal undergoing a procedure that will cause heart vessel injury, being regularly monitored clinically and through non-harmful imaging to assess disease development and progression, and killed without recovering from terminal anaesthesia at the end of the experiment.

In most cases, the mean number of regulated procedures in a given experiment is less than 5.

A worst case scenario would include the above (from a typical experiment) with 1 to 3 additional procedures, which would include for example, whole body irradiation and bone marrow reconstitution (which is undertaken to better understand the specific contribution of the immune defence system to the disease process), injection (for example in the veins) of gene inducing or disease modifying agents/substances to modulate disease state and the immune system, or single housing for a few days (with or without food restriction) to assess food intake and how the body burns and uses food components.

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

There are adverse effects related to the obligatory procedures in each experiment. These may include pain after a surgical procedure (animal may have hunched posture, may reduce its activity and become immobile), or pain due to heart attack or imminent aneurysm rupture, or shortness of breath or weight loss associated with heart failure.

In most cases, the estimated duration of these effects is less than a few hours, and animals are monitored regularly so we can avoid or treat the adverse effects, e.g., treating pain by use of analgesics (pain killers), or terminating the experiment preventively (humanely killed) before adverse effects approach humane endpoints.

In some experiments, severe levels of suffering may be reached, and they are mostly a consequence of the disease process being studied, e.g., heart failure with severe shortness of breath, or heart failure associated with severe weight loss. In this case, these "adverse" effects may be allowed to last up to 48 hours, because our aim is precisely to understand how and why these effects occur, and be able to find treatments to prevent them, first in animals, and hopefully in patients. In all such cases, animals are very closely monitored (several times a day), so they can be humanely killed before reaching the maximum severity level allowed on the 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)?

Mice: Mild (57.6%),

Moderate (31.8%),

Severe (10.6%).

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

Killed

Kept alive

Used in other projects

A retrospective assessment of these predicted harms will be due by 06 January 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?

Our need to use animals is due to the lack of reliable alternative ways (e.g., experiments done in test- tubes) that would allow a correct understanding of the diseases we are studying in this project. In particular, no test-tube assay will correctly predict the occurrence of heart failure after a heart attack, the occurrence of weight loss after heart failure, or the rupture of a great artery. This is why we need to use living animals to

understand the complexity of the disease process and test the potential effect of new treatments.

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

During the last few years, we have developed the use of human cells that can be manipulated in a dish, to reproduce some aspects of the disease process that occurs in the living animal, with the aim of replacing the use of animals. This has allowed us to replace and avoid the use of animals in certain circumstances. We will continue to test other non-animal strategies with the aim to replace the use of animals.

Why were they not suitable?

Some alternatives have proved to be suitable.

We have also tried to use, in collaboration with other investigators, other animal species that are not protected by law, like immature pre-independent feeding zebrafish. However, we found that these animals were not suited to study the complex disease processes we are interested in (e.g., blockage of arteries).

Other alternatives are just not feasible. In particular, no in-dish assay will correctly predict the occurrence of heart failure after a heart attack, the occurrence of weight loss after heart failure, or the rupture of a great artery.

A retrospective assessment of replacement will be due by 06 January 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 been using mice in our cardiovascular research for 25 years. We therefore have previous data and experience on which to determine the numbers of animals we are likely to require for this programme of research. We will also use statistics and consult expert biostatisticians when necessary.

The total number of mice for this project may seem relatively high. However, my laboratory is relatively big with 15 to 18 researchers (both juniors and seniors) involved in experiments on animals.

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

We follow the PREPARE guidelines (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) and the CAMARADES/NC3Rs systematic review facility recommendations for the design and analysis of our experiments. When designing the experiments, we perform statistical analysis to ensure that we use the minimum number of mice per group that will be informative.

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

Breeding strategies are designed to produce the correct number of animals with the correct genetic make-up (e.g., ensure that the gene or set of genes we have modified are correctly expressed).

When there's little information available, pilot studies are conducted in order to improve our estimation of the numbers of animals required to detect a significant effect.

We always aim to maximise the information that can be recovered from a single animal. For example, the same animal may undergo repeated non-harmful imaging over the course of an experiment (in order to assess the progression of the disease process) rather than killing different animals for each time- point in an experiment. When animals are killed at the end of the experiment, samples are collected from multiple organs to assess the effect of candidate gene mis-expression in multiple tissues.

A retrospective assessment of reduction will be due by 06 January 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 widely validated ways to investigate cardiovascular diseases, in particular experiments in living animals that reproduce the disease process of atherosclerosis (blockage of arteries due to fat deposits), myocardial infarction (heart attack), heart failure, and aneurysm (bulging of arteries) seen in patients.

Animals used here are mice because this species has been shown to be highly suitable to study these disease processes. Our expertise in these animal procedures is internationally recognised. We follow the most updated recommendations for animal experimentation. Animals are housed according to the best recommendations in appropriate and enriched environments. By performing pilot studies and choosing well established experimentation procedures based on extensive previous experience (more than 25 years), we minimise the unknown effects on the mice and subsequently pain, distress and suffering.

Some experimental procedures may be associated with a severe level of suffering. This is inherent to the disease process being studied (e.g., heart failure with severe weight loss, rupture of a blood vessel due to aneurysm), which also causes suffering and distress in patients with these forms of the disease (and is the reason why we are studying the process with the aim to find appropriate treatments). We monitor these animals very closely to pre-empt signs of pain or distress and to be able to terminate the experiment based on appropriate humane endpoints that minimise animal suffering.

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

We have tried, in collaboration with other colleagues, to use zebrafish to study aspects of atherosclerosis (blockage of arteries). Unfortunately, it appears that this type of animal is not suitable because it does not develop the advanced stages of the disease (which are responsible for the suffering and sometimes the death of patients with atherosclerosis).

In a few cases, we will perform the experiment under an anaesthetised state (total unconsciousness of the animal) to address some aspects of the disease process.

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

The number of techniques and surgical procedures used in each experiment is limited in order to reduce the harms caused to each animal while still obtaining scientifically sound information.

We very frequently monitor animal behaviour and well being to detect signs of pain and distress at an early stage.

When necessary, we familiarise animals with changes in their environment. As an example, when there's a need to assess individual food intake and how each animal burns and uses food components, animals must be singly housed in specific cages for up to 10 days. Single housing may be associated with some stress and some animals may show aggressive behaviour after re-housing with littermates. As a refinement control measure, pre-exposure to soiled bedding for several days prior to regrouping may be used in order to reduce aggression. Our refinement measures in this setting agree with the work and recommendations of Jane Hurst (https://www.nc3rs.org.uk/sites/default/files/documents/NC3RsarticleJaneHurst%20making%20sense% 20of%20scents.pdf)

We always aim to maximise the information that can be recovered from a single animal. For example, the same animal may undergo repeated non-harmful imaging over the course of an experiment (in order to assess the progression of the disease process) rather than use different animals for each time-point. When animals are killed at the end of the experiment, samples are collected from multiple organs to assess the effect of candidate gene mis-expression in multiple tissues.

We have developed and refined the procedures we used in mice to enable us to use this type of animal to explore how aspects of aneurysm (i.e. ballooning of a blood vessel) occur. This has been achieved mainly by using a new simplified surgical procedure associated with reduced vessel injury and much shorter duration of surgery. We will pursue such important efforts to improve our use of animals to ensure we minimise as far as is possible animal distress and suffering.

We will use established observation methods for monitoring animals, e.g., scoring sheets and grimace scales (doi:10.1038/nmeth.1455) to reduce suffering and use of pain relieving medication when appropriate.

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

We use widely established experimental procedures, and follow accepted guidelines, including ARRIVE, PREPARE, LASA Aseptic surgery guidance (2017), and NC3Rs recommendations, to ensure optimal planning, reporting and refinement of animal experiments.

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

We are at the forefront of research in cardiovascular medicine. We follow the ARRIVE guidelines (2020, 2nd edition), and other developments and advances in the 3Rs (https://nc3rs.org.uk/3rs-advice-project- licence-applicants-refinement), including Norecopa platform (https://norecopa.no/alternatives/the-three- rs) and the Danish 3R-Centre (https://en.3rcenter.dk/) with the aim to implement them swiftly.

A retrospective assessment of refinement will be due by 06 January 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. Novel treatments for kidney 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

Kidney-Failure, Proteinuria, Diabetes, Treatments, Haemolytic Uraemic Syndrome (HUS)

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

To develop new treatments to prevent kidney disease.

A retrospective assessment of these aims will be due by 07 January 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?

Nearly 700 million people, representing 10% of the global population are affected by kidney disease which can be identified through the detection of protein in the urine (proteinuria) or a reduction of the filtering capability of the kidneys (termed Glomerular Filtration Rate (GFR)). Even small increases in proteinuria or slight decreases in GFR significantly increase the risk of progressing into End Stage Kidney Failure (ESKF) requiring kidney dialysis on machines or a kidney transplant. Furthermore, kidney disease (even slight) significantly increases the risk of developing high blood pressure and suffering a heart attack or stroke[1]. This project involves studying how the kidneys work in detail to identify (and test) new ways of preventing kidney disease and its associated complications.

1. Matsushita et al.; Chronic Kidney Disease Prognosis Consortium, Association of estimated glomerular filtration rate and albuminuria with all-cause and cardiovascular mortality in general population cohorts: A collaborative meta-analysis. Lancet 375, 2073–2081 (2010).

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

The data generated by this work will reveal the important cellular signalling pathways in the kidney and how they can be manipulated in a beneficial manner to prevent or slow the progression of kidney disease. The study will generate new information on how the kidney works, identify new drug targets, and assess the effectiveness of novel treatments to prevent kidney disease. It will result in peer reviewed scientific publications to share with the world and potentially identify new products that will be helpful for patients with kidney disease throughout the world.

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

The ultimate long-term beneficiaries of this work will be patients with kidney disease. This will be due to the identification of new treatments, either through repurposing of drugs that have been used for other non-kidney indications previously, or the identification and development of brand-new treatments. In the short term the beneficiaries will be the scientists involved in the study in terms of their career development. In the medium term the work will benefit other biomedical scientists through the publication of our findings in peer-reviewed scientific publications and presentations at scientific conferences. Finally, society will benefit economically as our work will hopefully enable people with kidney disease to remain in good health and to continue to live a fruitful and productive life.

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

We will maximise our outputs by continuing our extensive national and international scientific and academic-industrial collaborations. We will endeavour to make our work open access to the world at the earliest time possible. We will also publish findings in peer reviewed scientific journals. We intend to be part of the new national animal research study directives (including the UKRI National Mouse Genetics Network to be established in 2022) to improve animal research through the formation of new world-class collaborations

and the establishment of the best “disease” models to be used by the wider scientific community focused on the reduction, refinement, and replacement (3R) of animal models.
Species and numbers of animals expected to be used

- Pigs: 40
- 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.

We are using predominantly mice as these are the species with the lowest neurophysiological sensitivity for studying the mammalian kidney. They have functional nephrons like humans and a perfused kidney allowing the dynamic study of cellular signalling pathways. We will study mice throughout their lives as kidney disease afflicts both the young and the old.

We will also undertake a small number of gene therapy studies using pigs, as a pre-requisite for the translation of this treatment into human clinical trials. Pigs have been chosen for these studies as they are of sufficient size to model the delivery of the treatment to the kidney in a manner that replicates that which would be used in the medical setting (i.e., through both peripheral blood vessels and blood vessels leading to the kidney). Studies may be performed in both young and old pigs as ultimately the therapy will be used in both children and adults.

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

Most of the mice used will be genetically engineered to switch "on" or "off" genes in the cells of the kidney to understand their importance in the filtration, or cleaning of blood, and the production of urine. For most mice this will be painless and the animal's kidney function will be studied by episodically testing their urine (for protein) and blood sampling. In some mice the genes will be switched "on" or "off" by giving the mice antibiotics in their feeds or injecting them with substances such as Tamoxifen.
Injections are typically given once daily for 5 days. In a subset of mice blood pressure will be measured using telemetry. This will require the insertion of a cannula into a blood vessel and the placement of a small measuring unit on the mouse. The unit is normally placed either under the skin (subcutaneously) or in the abdomen (intraperitoneal). This will be done under recovery anaesthetic and pain relief will be administered during and after the procedure. A similar approach may also be used to deliver medicines to the mouse via an implanted slow-release device. These procedures normally take approximately 1 hour to complete. Thereafter the blood pressure monitoring equipment and/or drug delivery system can remain be in place for weeks to months. The placement of a drug delivery or monitoring device will only be undertaken once in any mouse. In some mice a single kidney may be removed under general anaesthetic to cause partial loss of kidney function as occurs generally in prolonged kidney diseases. This procedure takes less than 1 hour to complete and is generally well tolerated by the mice.

Some mice will be used in models that mimic kidney disease found in humans. Disease will either be induced through genetic manipulation or by giving the mice substances that

initiate kidney disease. In most cases the disease models do not result in the mice suffering ill health. However, the models used to replicate diabetic kidney disease (the leading cause of kidney failure in the world) and haemolytic uraemic syndrome (the leading cause of rapid kidney failure in children) do cause the mice to show signs of ill health (like our patients). To minimise suffering, the mice used for these studies will be closely monitor and promptly killed if their suffering reaches a set humane endpoint.

For the gene therapy studies conducted using pigs, the animals will be anaesthetised, and the therapy delivered by a catheter inserted into the vascular system and given peripherally or guided into the renal artery with the aid of imaging. This will happen once in a procedure lasting around one hour. Following the procedure, the pigs will be given post-operative pain relief until they are pain free. Thereafter, periodic blood and urine samples will be collected to assess the effects and duration of the treatment. The sampling procedure will cause no more than mild transient pain.
What are the expected impacts and/or adverse effects for the animals during your project?
Mouse studies

Genetic manipulation studies

Most studies will be painless and not adversely affect the behaviour of the mice. The small number of mice that develop kidney failure may lose or gain weight over a few weeks. Normally they do not suffer adverse symptoms due to these weight changes.

Diabetes

When inducing diabetes commonly the mouse will become polyuric (produce a lot of urine) and polydipsic (thirsty) (approximately 50%). At this time, they will require more fluid intake. They will also be more prone to getting infections which may make them unwell (approximately 10%). Generally, they do not develop painful conditions except as a result of an infection.

Haemolytic Uraemic Syndrome (HUS)

This is a life-threatening condition for children that causes them to become very sick. Over the course of our previous studies, we have found ways of minimising the adverse effects of inducing HUS in mice, such that only approximately 10% of the animals used for these studies are expected to develop visible signs of ill health.

Surgical procedures in mice (implantation of telemetry or drug delivery devices or the removal of a kidney)

All surgical procedures will be performed aseptically under general anaesthesia and all mice will be given drugs to relive post-operative pain. Normally mice resume normal behaviour within 24-hours and thereafter do not show signs of pain. Although unlikely (due to good aseptic technique) there is a low risk of infections associated with these procedures. These would normally become apparent within 72- hours and if detected would be treated with antibiotics.

Pig studies

The gene therapy will be delivered aseptically in a procedure performed under general anaesthetic. Analgesia will be given peri-operatively and maintained until the animals are

showing no further signs of pain. The risk of infection will be minimised using a good aseptic technique and prophylactic antibiotics where appropriate.

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 -85% mild 10% moderate 5% severe Pigs - moderate.
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 07 January 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 outlined studies require a functional kidney in its normal physiological form, i.e., perfused by blood and producing urine. These criteria can only be met in a living animal. Most studies will be performed using mice as they are the least sentient mammalian species with a renal system that resembles that of humans and are amenable to genetic manipulation. Pigs have been selected for the translational studies as they can be obtained at a suitable size to enable the procedure to be undertaken in a manner that closely replicates that which would be used in a clinical setting.

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

We commonly develop our research ideas by studying kidney cell lines (human and mouse) in dishes. We have also developed kidney organoids to study kidney function in which different kidney cells are cultured closely together so they can signal or "talk" to each other.

Why were they not suitable?

Non-animal alternatives are not suitable for the outlined studies because to understand the physiology and biology of the kidney requires it to be perfused with blood and producing urine since these massively affects the signalling pathways involved in regulating kidney

cell function. In addition, to translate new treatments into clinical trials, it is essential to demonstrate both efficacy and safety in a representative animal model.

A retrospective assessment of replacement will be due by 07 January 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?

Pigs: This is a proof-of-concept study and the estimated number of pigs required is based on a review of the literature, discussions with researchers undertaking similar studies and the need to include the full range of controls required to evaluate the novel treatment being assessed.

Mice: The number of mice needed for the outlined studies is based on our previous experience working in this field and is estimated on the assumption of studying 2 molecular signalling pathways per year for the duration of the licence. The studies will assess the role of specific signalling pathways during renal disease in male and female mice in both diabetic and non-diabetic situations.

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

All our studies are designed in alignment with the ARRIVE guidance and using the NC3R's Experimental Design Assistant tool.

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

Smart breeding techniques will be used to increase the probability of deriving mice containing the required genotypes and to minimise the number bred that are unsuitable for our studies.

A retrospective assessment of reduction will be due by 07 January 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.

Most studies will be undertaken using genetically altered mice. Most of the genetic alterations will have no impact on the wellbeing of the mice. In some instances, genetic alterations may lead to early renal failure however, this will be detected at an early stage by our monitoring system and the animal humanely killed before their welfare becomes compromised. A few kidney disease models will be induced using drug treatment. These models have been extensively refined, during the course of previous work, to have the minimum impact on the wellbeing of the mice, this combined with our monitoring system for detecting renal disease ensures that studies are concluded, and the animalshumanely killed, before their welfare becomes compromised. A small number of mice may be implanted surgically, under general anaesthesia, with devices to either monitor blood pressure or deliver drugs over a prolonged time-period. Following surgery, the animals will be given drugs to control post- operative pain and are expected to make a full and uneventful recovery and resume normal behaviour within 24 hours.

All pigs used in the outlined studies will undergo a minor surgical procedure, that replicates that which will be used clinically on human patients, during which the treatment will be delivered to the kidney using a per-cutaneous catheter inserted into a blood vessel and guided into the renal artery by non- invasive imaging. All pigs will be given drugs to control post-operative pain and are expected to make a full and uneventful recovery from surgery and resume normal behaviour within 24-hours.

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

Mice are the least sentient mammalian species that meet the three criteria needed for the outlined studies, namely: 1) being amenable to genetic modification. 2) having a kidney system that resembles that of humans. 3) pre-established models of renal disease.

Pigs are the species of choice for the translational studies as they have a renal system that resembles that of humans and are of sufficient size to enable the treatment to be delivered with the percutaneous catheter systems that will be used clinically for human patients. Pigs are also more tolerant of surgical procedures conducted under general anaesthesia than similar sized farmed species, such as the sheep.

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

The procedures used in these studies have been extensively refined under my previous PPL to have the minimal effect on the wellbeing of the animals. Surgical procedures will be performed under general anaesthesia using full aseptic precautions. Following surgery, all animals will be given drugs to control pain. Mice used in studies modelling kidney disease

will be closely monitored, using a scoring system developed under my previous PPL to ensures that disease is detected at an early stage. Clear end point are set for all studies that ensure the animals are humanely killed before overt suffering occurs.

The study will use well established mouse models to investigate treatments for three clinically important human kidney diseases (diabetic kidney disease, Focal Segmental glomerulosclerosis [FSGS] and Haemolytic Uraemic Syndrome). The models used have been further refined by my group, through the use of mice with nephropathic susceptible genetic backgrounds, to optimise their induction. Wherever possible, refined methods of urine collection, such as the use of hydrophobic sand, will be used obtain urine samples in preference to the use of metabolic cages. Where it is necessary to use metabolic cages the maximum time that animals spend in these will be limited to 6 hours.

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

All procedures will be conducted in accordance with LASA, and NC3Rs Guidelines on best practice for the administration of substance and performing aseptic surgery. For planning experiments the ARRIVE guidelines will be employed.

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

I am part of the Great Western 4 (GW4) alliance of universities that champion 3R approaches to animal research. I receive their newsletters and myself and/or my team regularly attend and present at their annual symposium. I am also currently involved in the new UKRI directive in animal research in which 3Rs are a major driving force for the establishment of national and international mouse model consortia.

A retrospective assessment of refinement will be due by 07 January 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. Targeting antioxidant and proteostatic pathways to afford cardiometabolic protection across the life-course

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

vascular, diabetes, stroke, obesity, pregnancy

Animal types	Life stages
Mice	adult, neonate, juvenile, pregnant, 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 investigate the relative contribution and effect of pharmacologically targeting antioxidant and proteostatic pathways to improve cardiometabolic health across the life-course. Our work focuses on 2 key areas:
Understanding how these pathways contribute to maternal and offspring health following healthy and adverse (e.g. obese/diabetic) pregnancy

Understanding how these pathways can afford protection against ischaemic stroke, particularly in the presence of comorbidities such as diabetes

A retrospective assessment of these aims will be due by 09 January 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 1 in 4 expectant mothers are obese and 1 in 10 will develop gestational diabetes (GDM), with the number of mothers affected by these conditions expected to increase dramatically in the next few decades. Both obesity and GDM increase the risk of pregnancy complications (e.g. pre-term birth, miscarriage) and additionally both mothers and their children are at greater risk of developing cardiovascular (high blood pressure, heart attack and ischaemic stroke) as well as metabolic (obesity, diabetes) complications in later-life. Whilst excessive weight gain and glucose control in pregnancy can to some extent be managed, the risk of poor child health including later-life cardiovascular and metabolic disease is not reduced and therefore new treatment options are needed.

Oxidative stress caused by the production of harmful 'free radicals' is known to be strongly associated with these adverse pregnancy conditions and exacerbates cardiovascular and metabolic dysregulation, but the underlying mechanisms are still under investigation. In human samples we and others have previously identified problems with the activation of the bodies protective antioxidant defense genes. .
Using our human cell model we have identified ways to restore these protective antioxidant defenses ti protecting them from damage from oxidative stress and thereby keeping them healthy. We now need to test whether these intervention(s) can protect against maternal and fetal cardiovascular and metabolic disease using an intact organism and organ systems. Whilst many of the interventions we are trialing are natural compounds unlikely to cause harm, expectant mothers are a high-risk clinical population. Therefore it is important we obtain this data using an appropriate animal model of adverse pregnancy to provide evidence of safety as well as determine how effective our intervention is in preventing vascular and metabolic dysfunction in animal mums and their offspring.

We are also interested in whether these same compounds and pathways can be targeted to treat vascular diseases such as ischaemic stroke, making our findings more applicable to a wider clinical population beyond that of expectant mothers and their children. Ischaemic stroke is a leading cause of vascular related death in the UK, yet only a single drug therapy is available and <4% patients are eligible to receive this treatment. Whilst historically preclinical stroke research has largely failed to translate to viable alternative medicines, recently published scientific study guidelines (RIGOR and STAIR guidelines) have been developed to bridge this gap. A considerable focus is now being put on providing proof-of-principle evidence for long-term functional recovery, as well as inclusion of relevant and under-represented co-morbidities such as diabetes which make ischaemic

stroke harder to treat. As shown from our work plan, we will use a well-established ischaemic stroke model following induction of diabetes as a clinically relevant comorbidity. Similar to expectant mothers, this information is necessary before clinical trials can be conducted in humans (even when using natural dietary compounds).

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

This study will generate new insights into the causes and consequences of vascular and metabolic transgenerational disease transmission as well as allow us to test new treatment strategies. We will publish this information in scientific journals as well as present our findings at patient accessible talks and scientific conferences. We also aim to publish methodological information and or improvements/refinements which could be relevant to other researchers in the field.

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

Findings from this Project will allow us to test whether potential treatment strategies are successful in reducing the incidence or severity of cardiovascular (high blood pressure, heart attack, stroke) and metabolic (e.g. diabetes) disorders associated with adverse pregnancy (e.g. gestational diabetic /obese pregnancy) or later-life vascular diseases such as ischaemic stroke which are exacerbated by comorbidities such as diabetes. Information gained as part of this Project will give us important information as to the causes and consequences of these diseases, and whether by activating protective pathways within the bodies cells (e.g. antioxidant pathways) could be an effective treatment strategy. By the end of this project we will have addressed the objectives set out within our application, which ultimately will help enable us to make an informed decision as to whether our potential therapeutic intervention(s) may be suitable for human clinical trials.

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

Our findings will be presented at patient accessible seminars and to other researchers through scientific publications as well as scientific seminars/conferences/working groups. Disseminated information includes positive and negative findings as well as methodological approaches or refinements which may be useful to other researchers.

Species and numbers of animals expected to be used

- Mice: 4900

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 experimental plan uses the least sentient species (i.e. mice) which we can use to model these diseases and test our proposed treatment strategies. Our experimental plan uses new technological advances such as telemetry to improve animal welfare and minimise suffering as well as good study design practices to ultimately reduce the number of animals we need to study.

To assess the impact of adverse pregnancy (e.g. obese/diabetic pregnancy) and potential treatments to improve maternal and offspring health, we may need to assess both parents and their offspring at different stages across their life-course. This may include stages in pregnancy or in embryos. It is very important such information is obtained as expectant mothers represent a high-risk clinical group and are therefore typically exempt from clinical trials even of dietary supplements. We need to ensure these treatment options are safe and effective before we consider human trials. To model ischaemic stroke disease, we will use adult mice which may be given special diets to induce obesity and diabetes as understudied risk factors for stroke. This will allow us to more robustly test our treatment strategies in the presence of real world variables.

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

Typically, an animal in this Project License will:

will undergo or be exposed to an adverse pregnancy or ischaemic stroke by using special diets and/or surgical induction

Undergo cardiovascular and metabolic phenotyping assessments (e.g. measuring blood pressure, obesity, diabetes)

Treated with therapeutic agents to potentially improve cardiometabolic outcomes

Humanely killed.

The duration of the experiment will depend on the Aim of the study and the individual Protocol used. However, all protocols have been refined to that the minimum experimental duration to achieve the scientific objectives.

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

For the majority of animals used, no adverse effects are expected either in wild-type or genetically altered animals. To ensure that animals do not suffer unnecessarily because of repeated testing (e.g. assessment of blood pressure and glucose control), we will only conduct the minimum number of procedures needed to answer our scientific research questions and will ensure that animals have a suitable recovery period in-between these procedures. Although not anticipated, animals will be monitored regularly for signs of distress or suffering such as weight loss, abnormal behaviours such as lack of grooming, hunching or reduced mobility.

A relatively small proportion of animals used (~10%) will undergo surgical induction of stroke, which is associated with a severe decline in neurological, behavioural and functional performance mimicking the human disease. Where possible, our scientific research questions will be answered without the need to awaken animals following this surgery, to minimise suffering and distress. In cases where these animals are recovered, animals will receive special care including pain relief following surgery and will be studied for a maximum of 1 month to allow us to assess potential protection against long-term brain damage as well as recovery of mobility and sensation. We will only keep animals for the minimum period needed to answer our scientific research questions. Similarly, we will conduct the minimum number of tests needed to answer our scientific research questions and will ensure that animals have a suitable recovery period in-between these procedures.

Animals will be carefully monitored for signs of distress to ensure no unintended adverse effects occur.

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)?

This project is expected to use 5000 animals.

~52% animals will experience a mild severity banding (P1 2000+ P3 320+ P4 300/5000)

~36% animals will experience a moderate severity banding (P3 480+ P4 1200+ P5 50+ P6 50/5000)

~3% animals will experience a non-recovery severity banding (P2 100/5000 + P5 50/5000)

~9% animals will experience a severe severity banding (P6 450/5000)

Protocols (P) broken down by total animal numbers () and % maximum anticipated severity banding: P1 (2000): 100% mild banding

P2 (100): 100% non-recovery banding

P3 (800): 60% moderate, 40% mild banding

P4 (1500): 80% moderate, 20% mild banding

P5 (100): 50% moderate, 50% non-recovery banding

P6 (500): 90% severe, 10% moderate banding

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 09 January 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 require the use of animals because:

Data generated from this body of work may be used to inform whether to go forward to human clinical trials. Regulatory agencies require animal data to demonstrate safety and efficacy before clinical trials, especially when considering high-risk individuals such as expectant mothers

To validate the mechanisms and efficacy of specific pharmacological agents, these studies cannot be conducted in humans for ethical and scientific reasons (e.g. unable to isolate and examine various body tissues, cannot examine direct contribution of specific pathways by gene silencing).

Most aspects of these cardiometabolic pathologies can only be studied in live animals (e.g. development of diabetes, priming of offspring, ischaemic stroke) because there are complex interactions between different body systems, which cannot be replicated in anything other than an intact animal.

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

Where possible we have already used non-animal alternatives to address our research questions (e.g. human and animal cell culture models) regarding redox-sensitive pathologies associated with adverse pregnancy and ischaemia- reperfusion injury.

We will continue to use these in vitro methods to first validate the efficacy of novel potential intervention compounds or strategies to regulate proteostatic pathways before considering animal studies

Why were they not suitable?

The complex interplay between different organs and cell types seen in adverse pregnancy and ischaemic stroke are not currently possible to model sufficiently using either cells or computer systems.

A retrospective assessment of replacement will be due by 09 January 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 produced comprehensive preliminary data, including pilot protein, qPCR, telemetry, histological and behavioural data and cell culture measurements. Animal estimates are based on this information which was used to produce peer and statistician-reviewed experimental designs to allow interrogation of underlying pathogenic mechanisms alongside testing potential therapeutic intervention strategies.

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

Where possible we have already used non-animal alternatives to address our research questions (e.g. cell culture models) regarding redox-sensitive pathologies associated with adverse pregnancy and ischaemia- reperfusion injury.

We will continue to use these in vitro methods to first validate the efficacy of novel potential intervention compounds or strategies to regulate proteostatic pathways before considering animal studies

To address specific new research questions, small scale (n=3) pilot studies will be performed prior to conducting larger, powered studies. These pilot studies will primarily be used to assess the feasibility of new therapeutic compounds, regimens or timing of experimental endpoints. Pilot experiments will provide statistical data allowing animal number estimations for definitive larger experiments

Where possible, our experimental designs have avoided duplication of existing phenotype data and unnecessary inclusion of additional sham or control (normal pregnancy) groups to test therapeutic interventions. We have and will continue to keep scientific records allowing the validity of our findings to be independently assessed and avoid duplication.

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

Breeding of genetically altered strains will be carefully managed to ensure sufficient but not excessive numbers of animals are bred for use on subsequent protocols, whilst ensuring no phenotypic drift within the colonies.

Where possible, we will use the same animal to assess multiple scientific endpoints where it is compatible with good animal welfare in the context of the overall animal experience (e.g. use same animal for longitudinal assessment of blood glucose homeostasis).

Surgical induction of ischaemic stroke will be primarily achieved using the Longa method. This approach is described in the literature as resulting in approximately half the adverse mortality of the previous Koizumi method, which involved permanent ligation of the common carotid artery. In addition, the Longa method results in a more reproducible infarct volume thus representing a reduction in animal usage for similarly powered studies.

Where possible our investigations are blinded. Where operator blinding is not feasible, to avoid need for data duplication, we will use automated analyses where possible and if applicable appropriate positive/negative controls. Laboratory records and/or raw data must be conducted and/or kept in a manner to enable re-analysis of qualitative or quantitative findings where possible by an independent third party.

A retrospective assessment of reduction will be due by 09 January 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: Mice are the lowest relevant phylogenetic species to serve as an adequate model for a clinically translatable study. Assessment of foetal priming in adverse pregnancy must, by necessity, be performed in mammals, while lissencephalic rodents are regarded as the baseline species for pre- clinical translational stroke research.

Animal models: Environmental modulation during development will be conducted through dietary feeding (e.g. feeding a 'western' style high fat high sugar diet). This model represents a close approximation of the clinical presentation of obese GDM pregnancy and allows clinically relevant cardiovascular and metabolic outcomes to be assessed in both breeders (dams and studs) and subsequent offspring. The same or similar diets will also be used to induce diabetes in some adult rodents who subsequently undergo surgical induction of stroke. To interrogate the role of specific antioxidant or proteostatic pathways, our models may incorporate both wild-type and transgenic (global or cell-specific) animals where these pathways are modulated and may use known pharmacological or dietary activators or inhibitors of these pathways.

Methods: The use of these models of adverse pregnancy and ischaemic stroke are necessary to pre- clinically validate new or improved treatment strategies to prevent cardiovascular and/or metabolic dysregulation resulting from these specific disease states. These models will also be used to interrogate the underlying pathophysiology of these disease processes, which may help to identify new treatment strategies or disease signatures/biomarkers.

In both breeders (typically dams, sometimes studs) and offspring alterations in vascular and metabolic outcomes will be phenotypically and functionally assessed:

To increase chance of viable litter production from breeders, particularly in transgenic animals more sensitive to dietary modulation, breeders may be subject to altered husbandry practices/environmental enrichment (e.g. cages fitted with noise cancelling lids, altered cleaning schedule to avoid disturbing nests close to term). Where compatible with the scientific endpoints of the study, we will also where possible pair house breeders until late gestation or in rare cases (e.g. absence of valid copulation plug) the early post-weaning period. Breeders will be monitored for signs of fighting, especially following temporary separation for procedures requiring single housing (e.g. metabolic cage).

Where possible we will use clinically apposite indices and methods considered gold standard in terms of scientific data integrity and animal welfare. For example, changes in blood pressure will be assessed using radiotelemetry and glucose tolerance will be assessed by oral/IP glucose tolerance test (GTT) or continuous glucose monitoring (CGM) by radiotelemetry. These represent a substantial REFINEMENT in animal welfare and data validity over alternative methods such as plethysmography used to measure blood pressure as well as keeping abreast of recent advances in glucose monitoring using CGM in patient populations.

Where compatible with the scientific endpoints of the study we will use alternative strategies allowing indices of cardiovascular or metabolic function to be assessed by non-invasive means. E.g. using isolated tissues/cells collected at the point of termination to assess outcomes such as adipose mass/bodyweight, markers of tissue function by histology/gene expression profiling and/or vascular reactivity by myography.

Where possible, administration of potential therapeutic agents will occur via voluntary ingestion in dietary substances (e.g. nutella). This method has been used successfully in our previous work (under PPL 70/8934) and represents a substantial REFINEMENT compared to previously published studies using like-for-like compounds (e.g. all published studies with Nrf2 activators have given either repeated
s.c injections - typically daily over 3 months, or have used dietary feeding of the precursor in the chow pellet preventing adequate assessment of dosing and suboptimal bioavailability).

In adult rodent's alterations in cerebrovascular and neurological function will be assessed using both recovery and non-recovery preparations:

As previously developed in PPL 70/8934, acute changes in cerebrovascular haemodynamics and neuroinflammation during ischaemia and reperfusion will be performed where possible in a non- recovery surgical preparation. This REFINEMENT avoids the distress associated with recovery following cerebral ischemia-reperfusion.

The Longa method will be preferentially used for surgical induction of ischaemia over the Koizumi method (in which the patency of the common carotid is not maintained). Whilst the Koizumi method is preferential to induce infarcts with the absence of cortical involvement, the Longa method is associated with greater infarct reproducibility and enhanced survival with reduced incidence of haemorrhagic transformation.

As previously developed in PPL 70/8934, we will be implementing a modified anaesthesia protocol during surgical induction of cerebral ischaemia. This REFINEMENT minimises incidence of post- surgical respiratory depression from anaesthesia and reduces the incidence of adverse effects within the immediate post-recovery period associated with the development of maximal infarction (day 0-3 post stroke).

For data related to functional behavioural rescue and its relationship with neuroinflammation and post-inflammatory angiogenesis, we will rely on a refined surgical approach, careful observation and the clearly defined humane end-point's described within the Protocol to limit the distress associated with this severe protocol category. Long-term behavioural tests have been validated for their suitability and where possible our analyses of post-stroke behaviour will be blinded and automated to remove experimenter bias.

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

Less sentient animals cannot be used as they do not have a fully developed cardiovascular and nervous systems to replicate the complexity of the diseases of interest.

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

Will we undertake the following refinements to procedures to minimise suffering:
Pregnant dams subject to dietary modulation will be regularly monitored and where necessary adapted husbandry practices will be implemented to avoid potential distress and fetal loss (e.g. continued pairing, avoiding cage changes close to birth, use of noise cancelling lids on cages)

Animals will be given appropriate time in between procedures to recover and will be monitored regularly (e.g changes to bodyweight, presence of pilo-erection, wounds, poor grooming, hunched posture).

For animals undergoing procedures using non-standard housing conditions (e.g. metabolic cages), animals will be where possible be housed with environmental enrichment and where not possible, environmental enrichment will be gradually removed to minimise distress.

For animals undergoing surgical procedures involving implantation of telemetry probes or biosensors, a programme of enhanced care and monitoring will be implemented in the immediate post- operative 3 day period. Animals will be given additional enrichment (e.g. wet mash) and pre and post- operative pain management and monitoring.

For ischaemic stroke recovery protocols and data related to functional behavioural rescue or the measurement of neuroinflammation and post-inflammatory angiogenesis, we will rely on a refined surgical approach. Animals will be carefully monitored, given pre- and post-operative analgesia and enhanced care for a minimum 3 day period. Experiments will be conducted using clearly defined humane endpoints described within the Protocol to limit the distress associated with this severe protocol category. We will continue to liase with BSU staff to ensure expected adverse and unexpected adverse effects can be readily identified and appropriate action taken (e.g. adverse effects and monitoring information posters left in holding rooms).

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

All animal work will be conducted with the aim of being compliant with the ARRIVE guidelines for publication of animal research. This ensures minimum standards are met with regards to experimental design, powering, randomisation, inclusion/ exclusion criteria, blinding, outcome measure reporting, statistical methods and results reporting. Critically these guidelines also set out the minimum standards for reporting experimental animal use and experimental procedures, including the rational for proposed procedures to ensure experiments are performed with sufficient scientific rigor without compromised animal welfare.

For stroke protocols, animals may be subjected to modified diets (induction of co-morbidities such as diabetes and obesity), as strongly recommended by the STAIR and RIGOR panels for translational stroke research.

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

As general practice we stay informed about methodological advances in the field, through information disseminated in published literature as well as at scientific conferences. We have formed working groups (e.g. 'glucose telemetry' and 'fetal programming' groups) with researchers using similar methods or scientific objectives to enable effective discussion of experimental design, protocol refinements and data validity. As general practice, we also keep regular contact with our local NTCO and have where necessary consulted our designated NVS to discuss potential changes to anaesthetic and as well as pre and post-operative analgesic regimens where appropriate to successfully balance scientific benefit with animal welfare.

A retrospective assessment of refinement will be due by 09 January 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. Understanding and correcting the pathology of alkaptonuria

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

metabolic disease, therapy, genetics, drug, tyrosinaemia

Animal Types	Life Stages
Mice	adult, aged, juvenile, neonate, embryo, 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?

Alkaptonuria and other metabolic diseases are complex and cannot be investigate in cell culture. Our group has developed an animal model for AKU and a therapy through the use

of a drug called: nitisinone. However, this therapy reduces the culprit molecule, HGA, but results in increased tyrosine in the serum. Therefore, in this project we want to:

1, Test biochemical and biomaterials to reduce tyrosine increase in serum following nitisinone therapy 2, Understand how HGA leads to osteoarthritis in AKU and 3, Correct the disease using gene or cell therapy.

A retrospective assessment of these aims will be due by 23 January 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?

It is vital that we build on our expertise and experience gained over many years of treating patients to develop a complete cure for AKU so that those affected will only need one treatment and be able to eat a normal diet. The drug nitisinone was trialled by our group and this year has been adopted as a treatment for AKU by the European agency. However, children can not be placed on this drug because it increases Tyrosine in blood, and this has consequences for brain development. Our plan is to understand how HGA produces ochronosis in AKU, and how elevated level of tyrosine in blood leads to additional pathologies such as cataract. We will establish interventions to block the uptake of tyrosine in the gut and /or repair the damaged gene, and so enable a cure. Two approaches will be used to repair the faulty AKU gene and then ensure that the technique is safe and has no side effects. The first method uses a virus to deliver a healthy copy of the defective gene, while the second repairs a defined single mutation.

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

AKU is an iconic disease; it was the first human disorder that was recognised to conform to Mendelian autosomal recessive inheritance by Archibald Garrod over a century ago and represents the prototype “inborn error of metabolism”. AKU is therefore an outstanding exemplar disease to develop and evaluate innovative approaches including gene therapy for the treatment of monogenic diseases of metabolism and beyond.

In this project we are hoping to develop a sustained therapy for AKU patients in which we block or eradicate the accumulation of high tyrosine levels. If we succeed, we can provide a complete therapy for life, but this is not a cure. Our parallel approach is to develop gene

therapy that cures the genetic defect by introducing the missing or mutated gene that causes the disease.

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

Our main focus is our patients. However, all 1325 patients around the world that have been diagnosed currently with AKU may benefit from a therapy. The global incidence is approximately 1 in 250,000, although in the Dominican Republic and parts of Slovakia the prevalence is 1 in 19,000.

The benefits will also be advantageous to patients affected by mutation in the phenyl alanine pathway as a whole. A gene therapy protocol may also be beneficial to most liver diseases as our targeting strategy may help improve this process.

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

We have national, European and International meeting dedicated to this cause and all our results are published in peer review papers and in the press releases that accompany these scientific papers. We are collaborating with major companies that develop mice model of the disease and close collaboration of other UK universities who help with the viral and gene therapy. There will be no doubt at our ability to disseminate the new knowledge to global audience.

Species and numbers of animals expected to be used

- Mice: 1000 AKU or HGD conditional mice, 100 HT1 mice and 150, humanised FRG mice

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 animals used represent models of human diseases such as alkaptonuria and hereditary tyrosinaemia type I, both of which are in the phenylalanine pathway. We have generated a conditional model for AKU which will allow us to understand the role of the kidney in the disease having extensively used this model to study and document the critical role of Liver in this disease. Our group has also used this model to show that the drug nitisinone can ameliorate both of these animal models but unfortunately increases the level of tyrosine in the blood which is detrimental in early brain development amongst other manifestations. In this project we want to explore new strategies to remove excess tyrosine from the diet or reduce its intake in the blood.

Our alternative plan is to repair the damaged gene, and so enable a cure. In this research we will develop two methods to repair the faulty AKU gene and then ensure that the

technique is safe and has no side effects. The first method uses a virus to deliver a healthy copy of the defective gene, while the second repairs a defined single mutation in the cells that are implanted back in a humanised liver of an animal.

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

In some experiments, we will selectively delete the HGD gene in the kidney and measure the level of metabolites that are taken up in the blood and identify the transporter in order to manipulate its function. Other mice will be given nitisinone and we plan to either break down the elevated tyrosine using enzymes that we add in the gut or use molecular chaperon that can bind to tyrosine and excretes it rather than being taken up into the blood. Mice that are given NTBC may develop cataract in older mice, and we plan to understand the mechanism of this by imaging, biochemical analysis that can lead to topical application to ameliorate the disease.

In alternative approach we plan to repair the defective HGD gene by either delivering it to the liver via a virus or correct the liver cells and implant them back into a humanised mouse model.

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

In the majority of case, we will be ameliorating the final outcome in these mice as we treat the initial defect. However, while manipulating these experiments, the mice will have adverse effect from substances that we are administering or recovery from anaesthesia following surgery, these adverse effects will hopefully be transient, and the animals will be monitored during this time.

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 have suggested a moderate severity since the initial animal represent disease models with known adverse effects. the protocols used in this study will ameliorate the final outcome. However, the procedures undertaken to deliver the therapy or investigations may be more than mild.

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 23 January 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 enzyme deficient in AKU patients is present in at least two tissues and its impact appear to affect connective tissue which do not produce this enzyme. Therefore we cannot replicate these conditions in test tube because we do not know yet how and why these changes occur.

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

Enzymes against tyrosine have been tested on liver and kidney cells as well as fibroblasts and established Km and Vmax in culture. Gene therapy vectors were tried in cell to ensure that they express the missing enzyme. We identified the transporters for tyrosine in kidney cells in vitro. However, these experiments do not replicate what happens in organs that have multiple cells such as liver and kidney.

Why were they not suitable?

Individual cells and their matrix in vitro can not answer how all these cells interact together. Therefore there is no alternative to a mouse model especially when we are proposing to cure the disease. Mice are the smallest mammalian system that we can use where genes can be genetically altered to improve our understanding of the pathology.

A retrospective assessment of replacement will be due by 23 January 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 carried out this type of experiments over many years and in our experience, we calculated the number of mice we expected to use in each experiment, and added all the experiments over 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?

We have used the AKU mice for over 10 years and we have used the past results in power calculations in order to obtain the minimal number of mice that allow us to show differences between test and control. Any new experiments using these or other mice will be put through the NC3R experimental Design Assistant to help us establish new regime

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

We have used the AKU mice for over 10 years and we have used the past results in power calculations in order to obtain the minimal number of mice that allow us to show differences between test and control.

A retrospective assessment of reduction will be due by 23 January 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 animals used represent models of human diseases such as alkaptonuria and hereditary tyrosinaemia type I, both of which are in the phenylalanine pathway. We have generated a conditional model for AKU which will allow us to understand the role of the kidney in the disease having extensively used this model to study and document the

critical role of Liver in this disease. We will also use HT1 mice on immunocompromised background in order to allow transplantation of cells to correct the disease.

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

In the past, we have used these mice to test a drug nitisinone, NTBC, that has been approved for human patients as it prevents the accumulation of toxic molecules that afflicts the disease. However, while this presented a therapy, it is not perfect and leads to other problems such as elevated tyrosine which leads to cataract in eye and central nervous complication. In this project we want to tackle these problems and therefore we need to use the mice on NTBC to ameliorate the side effect of this therapy.

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

We will always try to provide alternatives in route of administration of substances: for example through food or drinking water rather than injection. Provide analgesic following surgery and increased monitoring and weight measurement to ensure animals are recovering. Terminate experiments when new unexpected phenotype occurs or consult local vet in case of scientific importance.

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

In the scientific community that work on animals , we all follow as the Animals (Scientific Procedures) Act 19862 (ASPA) and guidance. New development that focus on " lessons learnt" disseminated by the NC3R website, the UK office of research integrity and other organisations.

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

As an animal user, I currently receive updates from the NC3R through emails and the website and through the meetings held locally by the NC3R coordinator in our region.

A retrospective assessment of refinement will be due by 23 January 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. Mechanisms underpinning the gut-microbiome-liver axis during 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

Liver disease, Microbiome, Intestinal barrier function, Bacteriotherapy, Immunometabolism

Animal types	Life stages
Mice	juvenile, adult, embryo, 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 understand the role of the microbiome-gut axis in the progression of liver disease. We aim to define new strategies to treat liver disease.

A retrospective assessment of these aims will be due by 19 January 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?

Chronic liver disease is the fifth cause of death in the UK. Increasing evidence point to the implication of the microbiome in mediating the progression of liver disease that associates with disturbances in intestinal function.

To improve our mechanistic understanding on how the microbiome interacts with the gut-liver axis and influences its function will enable us to propose therapeutic strategies to preserve health and treat disease.

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

We expect to improve our understanding on how the microbiome influences the gut-liver axis communication with a focus on the impact of host-microbe interactions in regulating liver metabolism and immunity. We anticipate this knowledge will enable us to propose new therapeutic strategies to treat liver disease progression based on the modulation of the microbiome and its communication with the host.

We anticipate we will continue generating high-impact publications in international peer-reviewed journals. We also anticipate we will communicate our findings to colleagues in academia via invited talks to conferences and other academic institutions.

We anticipate our studies using bacteriotherapy to improve gut-liver health has the potential to generate intellectual property (IP) and attract the attention of industrial partners interested in commercialising specific bacteria-based products to preserve health and treat disease.

The results obtained in this project will be included in grant applications to different UK and EU funding bodies.

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

During the life-time of this project, our results will benefit basic researchers investigating the mechanisms mediating the host-microbe interactions including basic hepatologists and gastroenterologists. Our results will also be of interest for microbiologists and immunologists.

We will also engage with the public in several activities that will allow us to communicate our findings.

In the longer-term, clinicians (gastroenterologists) may also benefit from our results defining the therapeutic effects of the modulation of the microbiome-host interactions in

treating liver disease. Our results will pave the way for the development of therapeutics that may benefit the public in the future.

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

We will continue collaborating with researchers locally, nationally and internationally.

As a measure of success of these collaborations, we anticipate we will publish peer-reviewed articles in international journals as we have done during our previous PPL where we have published several collaborative papers in high impact journals. We will present our work in conferences and invited talks to national and international institutions. Also, we will use the results obtained from our work in grant proposals.

Species and numbers of animals expected to be used

- Mice: 13500

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 interactions amongst the gut-microbiome-liver axis that include the regulation of cell metabolism and the immune system are multifactorial and very complex. Thus, these cannot be precisely reproduced in vitro. For this reason our research requires the use of mice.

The mouse models that will be used in this project mimic different aspects of human disease and are thus indispensable to dissect the complex interactions occurring in the gut-microbiome-liver axis during disease progression.

Most of the work proposed in this project will be done in adult mice (8-12 weeks). Nonetheless, age has a great impact on the microbiome, which becomes less diverse during old age. These changes impact on host function and associate with an increased intestinal permeability contributing to systemic low- grade inflammation leading to ill-health. In this project, we will investigate new ways of improving intestinal barrier function also during old age and thus will use a limited number of aged animals (>20mo) to investigate this.

Likewise, following studies demonstrating that the manipulation of the microbiome (using antibiotics) has a great impact at early age, we will also investigate how changes in the microbiome early in life can impact on the immune response as well as on the gut-liver axis later in life. Thus, occasionally, we will use young animals (pre-weaned, early post-weaned).

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

Inflammation will be induced by the administration of substances. Protocols involving the induction of inflammation will generally have a short duration ranging from a few hours up to a few days. The routes of administration will depend on the agent inducing

inflammation, generally by injection or oral gavage. These procedures will generally induce only a mild discomfort to the mice.

Some protocols will involve surgical procedures like partial hepatectomy, the removal of two liver lobes. Mice generally will be maintained up to 72 and rarely up to 10 days, when the liver is fully regenerated in mass and function. Only in specific experiments, mice may be maintained for up to 12 months to determine the impact of specific challenges in tumorigenesis.

Mice having surgically induced cholestasis will be generally maintained up to 14 days and rarely up to 21 days. All mice having surgical procedures will receive analgesia pre- and post-surgery and will be closely monitored daily for the duration of the experiment. Mice will be provided with supportive measures (e.g. soaked food, warm environment, and increased monitoring) if needed.

Drug-induced cholestasis is induced by administration of xenobiotics/substances orally and in food pellets and experiments generally last up to 1 week and occasionally up to 2 weeks. We will also induce liver fibrosis chemically using well-established compounds (e.g. CCL4) that will be administered (i.p.) generally up to 8 weeks and occasionally up to 16 weeks.

The modulation of the microbiome will be achieved by the administration of substances (e.g. antibiotics), live bacteria/bacterial products (e.g. probiotics) or food bioactives (e.g. fibers, proteins) that may be administered combined with the different protocols proposed in this project. These substances will generally be administered orally by gavage on one or several occasions or integrated in food pellets.

Upon completion of experiments, mice will generally be humanely killed after blood extraction under terminal anaesthesia.

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

Based on our experience, we anticipate that the induction of inflammation with substances, bacteria or bacterial products will have a maximum moderate severity. When administering pathogenic bacteria, mice may present weight loss for a limited time (e.g. 1-2 days).

Mice after partial hepatectomy show mild/moderate severity due to the surgery procedure (laparatomy) and usually show only a mild phenotype from 34h/48h after surgery as the liver is mostly regenerated by that time. Only rarely and when mice with specific genetic alterations are maintained alive for extended periods after partial hepatectomy, tumours may develop.

Mice after surgically induced cholestasis generally show a moderate phenotype. However, a small percentage of mice may show a severe phenotype (including ascites), in which case mice will be immediately killed using an appropriate Schedule 1 method. Mice will be daily monitored and their clinical signs precisely scored to avoid severity to be more than moderate up to the duration of the exepriment (generally 7 days, occasionaly 14 days and rarely up to 21 days).

Generally, recovery from all the surgical procedures used in this project is quick. Supportive care measures will be administered pre- and after surgery, including pain relief substances, provision of warm environment and soak food.

Drug-induced cholestasis may induce transient weight loss (3-5 days) that is recovered afterwards and up to the duration of the experiment (1-2 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)?

We anticipate that most of the mice will have a subthreshold (breeding) (75% mice) or mild severity (10%; administration of substances, dietary challenges, microbiome modulation). 15% of mice may show a moderate phenotype and less than 1% of total mice may show a severe phenotype.

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

- Used in other projects
- Killed
- Kept alive

A retrospective assessment of these predicted harms will be due by 19 January 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 complex interactions among the microbiome, the intestine, the liver and the influence of the immune system and metabolism in regulating these cannot be accurately modelled in vitro.

Likewise, the multiple cell-cell and organ-organ interactions (gradually/sequentially) we aim to investigate cannot be fully modelled in vitro/ex vivo/in silico and thus we require the use of mice to investigate the contribution of these multifaceted interactions to the progression of chronic liver disease, the regulation of inflammation and liver regeneration.

In addition, a large proportion of the components of the microbiota cannot be cultured, supporting the need to perform our experiments in mice in vivo where we will analyse the interactions between (specific components of) the microbiome and the liver-gut axis and their impact on host metabolism and immunity.

The translational nature of our research requires testing microbe-based therapeutic strategies in mice prior to the translation into the clinical setting.

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

When possible, we will perform analyses in human samples obtained from patients/volunteers in collaboration with the NNUH and other hospitals in the UK. These analyses in human tissue will complement our mechanistic studies using mice.

When possible, we will perform in vitro studies using human cells. Nonetheless, human immortalised cell lines are generally (transformed immortalised) tumoural cells that present marked alterations in proliferation, metabolism and immunoregulatory functions and are therefore not optimal for investigating cell metabolic pathways/responses.

We are also exploring the possibility to use novel microfluidics-based organ-on-a-chip technology during the lifetime of this project although this methodology is still not developed in our lab. Once we identify specific bacteria/products that may influence liver metabolic function we could apply those in this ex-vivo system.

Likewise, we will explore the use of precision cut liver slices, an ex-vivo model using slices of human tissue in collaboration with colleagues in the UK that have developed this technology.

Why were they not suitable?

These in vitro and ex-vivo techniques are useful to test specific aspects of our research and will we use them accordingly.

However, none can fully replicate the complex interactions among the microbiome, the intestine, the liver and the immune system that occurs in vivo in mice, which are still needed for our research.

A retrospective assessment of replacement will be due by 19 January 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?

Most of the procedures and protocols included in this project have been already used in the previous PPL. These have generated meaningful and robust results that have been published in high impact factor peer-reviewed journals. Thus, we have estimated the number of mice we will use in this project based on our usage in the previous PPL and taking into account the new studies we propose to carry out.

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

• We have liaised with a biostatistician to determine the minimum number of animals we'll need to use to obtain meaningful and statistically robust results and have performed power calculations using results generated in our laboratory during the previous PPL. For new protocols, we have used results generated in previous studies (please see specific protocols).

• We will carefully monitor and control our breeding colonies to avoid over-production of animals.

• We have broad experience in the use of the animal models proposed in this project and thus we can precisely design our experiments and choose the timepoints, dosages and administration routes, in a way that we will obtain informative results to address our different research questions.

• When possible and when we anticipate we will obtain a meaningful result we will perform in vivo imaging of mice. This will allow us to visualise the activation of specific factors and signalling pathways as well as the activation/translocation of immune cells and bacterial products in the intestine, liver and systemically. We will also use in vivo imaging to monitor the whole-body and liver fat content that will allow us to calculate the fat vs lean mass in mice after dietary challenges. These determinations will allow us to perform longitudinal measurements from the same animal throughout different timepoints, reducing the number of mice used.

• When possible, we will perform longitudinal sampling for the analysis of the microbiome (composition/metabolites) to determine the impact of the different disease models/ageing/interventions on the microbiome composition, function and metabolic properties. This will reduce the number of animals used.

• When possible we will perform in vitro studies, with primary cells isolated from mice, which will reduce the number of animals used. Our experience in the precise isolation (hepatocytes) and differentiation (bone marrow derived myeloid cells) of liver and immune primary cells allow us to obtain large numbers of cells per mouse, reducing the use 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?

- We will carefully monitor and control our breeding colonies to avoid over-production of animals but provide us with sufficient number of animals to perform our studies.

- We will use homozygous mice in our breedings unless we are generating a new strain. In our GA strains generated with the Cre-loxP system, we will use both Cre+ and Cre- littermates for our experiments.

- For most of the procedures proposed we will not need preliminary experiments as we have previously characterised and published most of the models proposed including induction of liver regeneration, feeding with modified diets, induction of cholestasis, induction of fibrosis and sterile and non-sterile inflammation. During the lifetime of our previous PPL, we have generated results from pilot studies where we have modulated the microbiome that give us an indication of how many mice we will be needing in this new project.

- Our research involves the study of the microbiome, the intestine, the liver and the immune system. Generally, we will obtain and analyse these different tissue/cell samples from the same mouse in addition to other samples (e.g. blood, spleen and bone marrow) when appropriate. This way we will maximise the number of samples we obtain from each single mouse, reducing the overall number of mice needed and avoiding the repetition of experiments.

- As we have done in our previous PPL, we will continue collaborating with research groups locally, nationally and internationally with whom we will continue sharing samples from our mice. This allows us to maximise the number of analyses we can perform per single mouse.

A retrospective assessment of reduction will be due by 19 January 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.

I have proven experience in most of the animal models and methods proposed in this project that are optimised to cause the least pain and distress to mice. Also, our deep knowledge on the different molecular events undergoing in response to the procedures we propose in this project allows us to reduce the duration of the experiments to the minimum to generate meaningful results. All models have been chosen on the bases that they mimic specific aspects of the different phases of human liver disease.

Breeding of genetically altered mice: Mice are the standard specie of choice to transgenesis. We expect that the majority of GA mice bred under the authority of this project will have no clinically deleterious phenotype. Nonetheless, mice will be monitored and in case any welfare problem is identified due to the genetic modification, the animals will be humanely killed as soon as possible.

Liver regeneration:

This can be induced i) surgically by partial hepatectomy and ii) by administration of drugs/xenobiotics.

i) Partial hepatectomy. This method was established by Higgins and Anderson in 1931 and nowadays it is still the main model used by scientist aiming to investigate liver regeneration (there are more than 9000 publications using this method), supporting its appropriateness. Moreover, we have wide experience using this method (7 research publications since 2003) that has allowed us to refine this method to minimise both the number of animals used and the suffering that the animals. We will perform these surgeries with the highest surgical standards (following the LASA Guiding principle for Preparing for and Undertaking Aseptic Surgery (2010)), using aseptic techniques and high quality pre-/post- operative care, including the administration of analgesics and provision of soak diet and a warm environment when appropriate.

ii) We will use drug-induced model of liver regeneration after acute injury using e.g. acetaminophen (paracetamol). This drug is the main cause of A&E admissions world-wide and thus relevant to investigate the mechanisms underpinning liver detoxification and regeneration mechanisms. Short-term carbon tetrachloride (CCL4) treatment is also a well-established method to induce liver regeneration after acute injury that allows to study the early (regenerative). I have experience in using these methods where mice generally show mild discomfort due to the intraperitoneal administration of the substances.

Induction of cholestasis and fibrosis:

We will use a i) surgical model of Bile Duct Ligation (BDL) or chemically induced with ii) xenobiotics.

i) BDL model of intrahepatic cholestasis is a one of the most widely used preclinical models of human Primary Biliary Cholangitis (PBC) and Primary Sclerosing Cholangitis (PSC) as it closely mimics the ductular reaction undergoing during human cholestasis. Our experience in performing this model (8 research papers published and two in preparation; 4 of which under the previous PPL) ensures that we will meet the highest surgical standards and post-operative care and therefore minimise the suffering of mice (following the LASA Guiding principle for Preparing for and Undertaking Aseptic Surgery (2010)). Our experience and knowledge of the molecular events undergoing during the progression of cholestasis will enable us to refine the use of animals and precisely target the signalling pathways/cell processes we know occur at specific timepoints. This will significantly reduce the number of mice used.

In detail, following on our previous work under the PPL (70/8929) we will investigate the mechanistic implication of the microbiome in contributing to hepatocyte cell death by performing short-term experiments up to 3 days after BDL. Next, to study the mechanisms underlying the inflammatory response we will obtain samples from mice at 3-14 days after BDL. Rarely, when interested in analysing cholestasis-mediated chronic fibrosis, we will prolong the experiment up to 21 days after BDL. BDL is a generally a moderate model, which has associated with adverse effects such as initial weight loss that stabilises after a few days and ascites in a small number of animals. In our experience, the actual severity of this procedure is moderate in most of the cases and we have only found a severe phenotype in <1% of the animals after BDL.

ii) As a model of intrahepatic cholestasis, we will administrate different xenobiotics that are accepted as preclinical models mimicking human Primary Biliary Cirrhosis (PBC) and drugs/infections-induced cholestasis in patients. In our experience (2 research papers published in the last 2 years) these models have a moderate severity and will be chosen when possible/appropriate to answer our research questions.

iii) We will induce liver fibrosis by administration of a xenobiotic (e.g. CCL4) by intraperitoneal injection that we anticipate will cause a transient discomfort. Rarely, CCL4 may cause pyrexia and weakness with local pain (<1%). Animals may experience transient drawsiness briefly after administration (<30min). Administration of CCL4 as a model to study liver fibrosis and test anti-fibrotic drugs has been refined over decades in multiple laboratories and the extensive available experience of this model (including ours) ensures this is a predictable and reproducible model.

Dietary challenges:

Based on our previous experience (7 published articles) and in the numerous literature, generally the dietary challenges we will use in this protocol do not compromise the welfare of the animals. Our experience also enables us to precisely design our experiments depending on the research question we aim to answer as we have previously characterised the impact of most of the dietary modifications that will be used in this work; including high fat and/or sugar content, varying protein, aminoacids and/or fibre content and customised diets including bioactives.

Inflammation:

Our broad experience in the use of models that induce sterile and non-sterile inflammation through the administration of substances (8 research papers) will enable us to accurately design our experiments and use the optimal dosage, route of administration and most informative time points in order to obtain significant biological responses and relevant data by using the minimum number of mice. We also have experience on the impact that specific treatments may have on the welfare of animals and when we expect these to happen, allowing us to set up the appropriate measures (provide analgesia, soak food, warm environment) to minimise the suffering of the animals.

Our current collaboration with researchers with expertise and know-how in microbiology and the use of mouse models involving the administration of bacteria (commensal and pathogenic), has provided us with experience in the use of bacteria to model human gastrointestinal/liver infections. In this project, we will use models suitable to investigate the mechanisms mediating intestinal and liver inflammation in response to bacterial challenges, including the administration of Salmonella typhimurium or Escherichia coli. Thus, we will follow well-established protocols approved in existing PPLs from our colleagues to refine our procedures so we minimise the suffering of mice to reach our scientific objectives.

The dosing, volumes and routes of administration as well as the duration of experiments and sampling procedures used will be refined to cause the minimum pain, distress or lasting harm is a good principle and will be the minimum consistent with the scientific objectives

Imaging of live animals:

The imaging protocols are highly sensitive allowing sequential visualisation of small numbers of animals without having to cull animals at predetermined times post treatment. The timing and number of imaging interventions is minimised through the adoption of established and published protocols using similar assessment and imaging equipment.

In all the procedures detailed in this project, animals will be regularly monitored (with a frequency depending on the techniques performed) and in case any sign of distress appears in the animals, the animals will be humanely killed.

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

The aim of this project is to define the complex interactions among the microbiome, the intestine, the liver and the immune system to preserve health and treat disease. These multi-cellular and multi-organ interactions can only be precisely modelled in live organisms. The strong genetical, protein and immunological resemblance between mice and humans (>85%) makes mice the most ideal model organism to investigate the mechanisms preserving health and to test potential therapeutics based on the modulation of the microbiome.

Our work and others’ showing the resemblance of the mouse and human microbiome during liver disease supports the use of this animal to reach our scientific aims. Mice are the least sentient species that allows genetic manipulation and still resembles human physiology.

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

We don't anticipate deleterious phenotypes for the majority of the genetically altered mouse strains that we will breed. Nonetheless, we will closely monitor these breedings, especially when new strains are generated. If welfare problem is identified, animals will be humanely killed as soon as possible.
We will always use the lowest severity procedure and model to achieve our scientific aims. Most of the treatments/procedures proposed may only cause a transient distress and no lasting pain.

In the case of the procedures involving surgeries, we will follow the LASA guiding principles for preparing for and undertaking aseptic surgery (2010). Thus, we will proceed using the highest standards of aseptic techniques and high quality pre- and post-operative care, including analgesia administration, measurements to avoid dehydration, maintenance of body temperature, wound treatment and ensuring appropriate food intake.

Especially the surgical cholestatic model has generally a moderate severity that can have adverse effects such as weight loss or ascites. Mice will be closely monitored a minimum of once/day for the duration of the experiment. We will provide continuous supportive care measures including additional monitoring, provision of fluids if dehydration is observed, pre- and post-surgical pain relief (e.g. buprenorphine), provision of soaked diet and a warm environment for the duration of the procedure if required. These provisions will be administered based on the clinical assessment of the mice.

Diet modifications are not expected to cause any pain or distress. When inducing obesity with enriched diets, any obese mice developing signs of significant ill-health including

movement and breathing difficulties, inability to feed/drink, hunching, crouching or pilo-erection will be humanely killed.

When testing new compounds/enriched diets, we will closely monitor the food intake within the first days after presenting the new diet to mice. We will ensure that all diets used in this protocol will satisfy the nutritional and palatability requirements of the animals, these will be formulated by competent companies.

Mice will be closely monitored and any mouse showing signs of suffering greater than moderate (e.g. hunched posture, inactivity, unsocial behaviour, abnormal breathing, dehydration, muscle rigidity, twitching/trembling, staring piloerection, and little peer interaction) will be immediately killed.

Induction of inflammation will generally be done by administration of substances, bacteria or related products. We anticipate these will cause a mild transient distress and no lasting pain. Mice will be closely monitored for the duration of the experiment. When the duration of the experiments is longer than 12h mice will be monitored daily. Any animal showing signs of suffering greater than moderate (eg hunched posture, inactivity, unsocial behaviour, abnormal breathing, dehydration, muscle rigidity, twitching/trembling, staring piloerection, and little peer interaction) or weight loss reaches 20%, mice will be immediately killed.

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

All our studies follow the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines that will ensure our work reaches the maximum quality and reliability enabling other researchers to evaluate and reproduce or work.

In the case of the procedures involving surgeries, we will follow the Home Office guidelines and the LASA guiding principles (2017) for preparing for and undertaking aseptic surgery .
How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

All researchers involved in this project will be encouraged to regularly attend NC3R webinars and online workshops, as well as to subscribe to the NC3R newsletter. This will allow our researchers to stay informed and updated on the latest guidelines relating animal handling and experimental design.

A retrospective assessment of refinement will be due by 19 January 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. Developing novel therapies for rare genetic diseases

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

Key words

Gene Therapy, Rare genetic diseases of the Neuromuscular Junction, Rare genetic diseases of protein glycosylation, Fast Channel Syndrome, Small Molecule Therapy

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

The aim of this project is to develop novel effective therapies to treat Congenital Myasthenic Syndromes and Congenital Diseases of Glycosylation.

A retrospective assessment of these aims will be due by 21 January 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?

Only symptomatic treatments are currently available for Congenital Myasthenic Syndromes (CMS) and most Congenital Diseases of Glycosylation (CDG) patients, leaving both sets of patients living with life- long disabilities, which are potentially life-threatening. Therefore, effective disease targeted therapies are urgently needed to help these patients, to improve their quality of life, and reduce the decades-long burden of care on patient families and society.

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

The outputs from this project will be new information on the potential of different therapeutic strategies for treating CMS and CDG. All the work conducted on this project will be published in peer review journals and free online preprint databases. If the therapeutics tested can successfully improve disease phenotype, then we would seek to conduct rigorous pre-clinical testing before initiating clinical trials, which if also successful, may lead to development into commercial products.

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

The main beneficiaries from this research would be patients suffering from CMS and CDG, as the end goal of all the research in this project is to develop life changing disease specific treatments for these patients. The data generated from this project on the novel approaches we take, and the technologies developed could benefit other scientists and doctors developing therapeutics for related diseases such as other neuromuscular diseases, or other metabolic diseases. Therefore, our work could help patients that suffer from those diseases as well.

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

Our group works as part of an international collaborations network, and works closely with other academics, clinicians, industrial partners as well as charities representing the patient groups. The new knowledge acquired from this research will not only be disseminated through scientific publications, but also through presentations at international conferences, and charity patient days. We have regular meetings with our clinical and industrial partners, where we share new findings directly.

Species and numbers of animals expected to be used

- Mice: 11200

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 mice as mouse neuromuscular junctions (NMJ) are developmentally, structurally and functionally very similar to humans. Therefore, mice are good models of human diseases associated with the NMJ such as CMS. Mice are also developmentally, physiologically and genetically similar to humans. CMS and CDG are developmental disorders caused by rare genetic changes. It has been demonstrated that genetic changes homologous to those that cause CMS and CDG in patients produce similar clinical symptoms in mice. As in patients, these symptoms typically arise in neonates or juveniles, and last throughout their lives. This is why treatments need to be tested at these stages of development.

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

Genetically Altered Animals will be produced by conventional methods and genotyped to identify model animals of the rare genetic diseases of interest. Experimental treatments, or SHAM treatments, will be administered to the animals and subsequently assessed using phenotypic screens such as daily weighing and weekly strength tests as well as 2 electromyography assessments. Typically, a single treatment will be applied, and the animals are monitored for 2-6 months before they are killed by a schedule 1 method.

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

All model animals of CMS will start to exhibit fatigable muscle weakness as neonates or juveniles, which is the main hallmark of disease. In addition some strains such as Dok7 deficient mice also suffer from reduced weight gain, and vitality. If untreated, Dok7 mice will be euthanized before day P10 to avoid spontaneous deaths.

After treatment, pilot studies have shown that the DOK7 mice can live full lives without muscle weakness. However, they are still smaller than WT littermates even after treatment, and may take longer to be weaned.

Mouse models of CDG develop abnormally, typically resulting in reduced weight gain and vitality. As glycosylation is an intrinsically variable process, animals may also suffer from a variety of other symptoms including increased curvature of the spine, myopathy, and an inability to open their eyes. These symptoms would typically be long lasting, but due to the reduced vitality of animals, they will typically be euthanized by 3 months of age.

Treatments may also cause adverse effects. Gene therapies using adeno associated viruses (AAV) may cause spontaneous tumorigenesis, particularly in the liver.

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)?

3% Severe, 20%

Moderate, 2%

Mild, 75%

Subthreshold

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

Killed

A retrospective assessment of these predicted harms will be due by 21 January 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?

As the aim of this project is to develop effective new therapies for 2 groups of rare genetic diseases, we need to know that the therapies we develop are safe and effective. To do so, it is necessary to test the new therapies on experimental models of disease that are physiologically similar to humans, and develop similar clinical symptoms to those found in patients. Mice are ideal for this, as they are genetically and physiologically similar to humans. When genetic changes homologous to those found in CMS and CDG patients are introduced to mice, they develop similar symptoms to those found in patients, at similar stages of life.

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

We considered computer based methods to design drugs.

We also considered testing the drugs in biochemical and cell culture based models of disease.

Why were they not suitable?

Although computer-based methods are very useful for the initial design of therapeutic reagents for doing a very specific purpose, they cannot currently model the complexity of whole organisms, or how different tissues and organs would react to a drug.

Similarly, although biochemical and cell culture models are very useful to test whether a drug works in the way it is intended to, there are no cell culture based models that can tell us whether enough of a drug will reach the place it is intended to, and what adverse reactions the drug might cause in different parts of the body.

A retrospective assessment of replacement will be due by 21 January 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 total number of animals used is estimated from the total number of animals used in the 2 breeding and maintenance protocols, which will produce nearly all the animals used for the experimental protocols, plus an extra 200 WT animals that will likely be bought from trusted suppliers for certain control experiments.

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

For efficacy studies, POWER calculations were conducted using software such as the NC3R Experimental Design Assistant, or GPower. For more complex studies POWER calculations were conducted in consultation with professional statisticians.

For late stage preclinical trials, the regulators were consulted to ensure our experimental design will produce the necessary data package for the regulators to consider clinical studies.

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

Computer modelling will be used for initial drug design. Biochemical and cell culture models will be used to obtain pharmacology and toxicity data, which will inform the dose ranges that should be used.

Small pilot studies will be conducted on all new treatments using a small number of animals to give indications of efficacious dose and toxicity.

A retrospective assessment of reduction will be due by 21 January 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 be using mouse models of CMS and CDG, created by genetic alterations homologous to those found in patients. This includes mouse models with genetic changes in one of their muscle acetyl choline receptor genes, or Dok7, or Pmm2. The clinical signs observed in these mouse models reflect the symptoms suffered by patients, and are necessary to model human disease. They do not cause visible distress or pain to the animals, and symptomatic animals will only be kept for the minimum length of time needed to conduct the experiment before they are euthanized.

CMS

The main hallmark of CMS is reduced neuromuscular transmission, causing fatigable muscle weakness. To assess the clinical phenotype of CMS model mice, and whether treatments improve their clinical symptoms, it is necessary to assess their neurophysiology using electromyography (EMG), and their strength endurance using strength tests such as hang tests on an inverted screen.

CDG

As CDG are metabolic disorders that affect multiple tissues and organs all over the body, it has been shown that measuring animal growth through weight measurements is the most reliable way of assessing the clinical phenotype.

Procedures

All of the procedures carried out on the animals in this project, such as administration of drugs, or phenotypic tests such as strength tests or electromyography, should only cause momentary distress or pain. The animals will be monitored carefully after a procedure is carried out for signs of continued discomfort. If any lasting suffering or discomfort is observed, then the animals will be euthanized.

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

Mice are the lowest vertebrate group in which effective animal models of CMS have been generated. The molecular structure of muscles and neuromuscular junctions of lower order animals are too different from humans to effectively model disease.

One of the main aims of this project is to test gene therapies for CMS and CDG. The best established vectors for delivering gene therapies are viral vectors, which have different tropism for different tissues and different organisms. Therefore, to conduct pre-clinical trials on viral vector delivered gene therapies, we need to use animal models which have been shown to have a similar range of transduction efficiencies across different target tissues to that observed in humans. The lowest order animal that enables this is mice.

Clinical trials on gene therapies, particularly those on spinal muscular atrophy (SMA) patients, have shown that better clinical outcomes are often produced when patients are treated as early as possible. Therefore, on this project treatments will be administered to test animals as early as practically possible after clinical symptoms are presented. This is to reflect the earliest possible time of clinical intervention in patients after they are observed and diagnosed in clinic. Usually this means animals are treated as neonates or juveniles.

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

We will closely monitor our mouse strains with reduced vitality, visually assessing them at least once a day in the first week of life when they are most vulnerable. We will also monitor them at least once a day in the week immediately after administration of a treatment, to observe whether the treatment has any adverse effects.

The only procedures proposed to be used in this project that require general anaesthesia are electromyography (EMG) and insertion of an osmotic mini pump. Animals will be closely monitored after the procedure for signs of pain or discomfort, and if any is

observed, analgesics will be applied. If the animals continue to show signs of discomfort or pain, they will be killed.

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

We will follow the most up to date guidance published by the NC3R such as the ARRIVE guidelines to conduct our experiments in the most refined way.

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

We receive termly newsletters from our NTCO with updates on the advances in the 3Rs. We also regularly attend workshops set up by NC3R to be trained in implementing advances in the 3Rs.

A retrospective assessment of refinement will be due by 21 January 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?

9. Neuron-glia interactions in health and disease

Project duration

5 years 0 months

Project purpose

- Basic research

Key words

Alzheimer's disease, Dementia, Parkinson's disease, Immune system, Aging brain

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

To dissect glia and neuroimmune mechanisms of brain homeostasis, health and function

A retrospective assessment of these aims will be due by 05 January 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 common hallmark across multiple brain diseases, including neurodegenerative diseases such as Alzheimer's disease and neurodevelopmental diseases such as autism, is the dysfunction and loss of neuronal synapses, which are the basic communication bridges of nervous cells in our brain.

Importantly, the loss of the synapses occurs in a brain region-specific manner during early stages of the disease pathogenesis. Thus, a longstanding question in neuroscience has been to understand what underlies this region-specific vulnerability of neuronal synapses to dysfunction and loss.

Recently, genetic studies in Alzheimer's disease patients have enabled our focus on potential mechanisms underlying disease. These studies implicate immune mechanisms as drivers of disease pathogenesis. Furthermore, research in mouse models strongly suggests that microglia, which are the major resident immune cells of the brain, play important roles in Alzheimer's disease and many other neurologic diseases. Functional studies in animals from multiple laboratories around the world further suggest that the immune system involving microglia and other immune cells are critical for proper brain wiring, homeostasis and function in both developing and diseased brains. However, we still lack the critical insights into whether and how immune system confers brain function, and importantly, how these neuroimmune pathways are regulated. Mechanistic insights into these interactions will be critical to understand how neurons become dysfunctional and lost in disease.

Therefore, we aim to decipher key neuron-glial interactions important for proper synaptic health and function. This knowledge will be key in revealing fundamental insight into how we can therapeutically intervene in the disease. Our work here will contribute to identifying novel biomarkers to assess disease severity and points of intervention as well as ideal drug targets to slow down disease pathogenesis. Using a combination of molecular, pharmacological, genetic and biochemical approaches, we aim to decipher immune mechanisms of region-specific synapse loss and dysfunction that underlie devastating neurologic diseases, such as Alzheimer's and Parkinson's diseases. Our work will also have important relevance for understanding neurodevelopmental and neuropsychiatric diseases, where immune pathways are also being suggested to play key roles in disease pathogenesis.

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

The primary objectives of this project are to:

● Understand how our immune system contributes to region-specific synaptic health and function and to related behaviour, such as learning and memory;

● Assess how the immune system confers region-specific synapse loss in neurologic diseases, such as Alzheimer's and Parkinson's diseases;

● Examine how we can rescue major disease phenotypes, such as impairment in learning and memory in dementia-causing animal models, by targeting immune and other glial pathways.

Understanding how neurons and glial cells interact with each other to execute neural function and how these interactions break down in disease models will be critical. Scientifically, they will fill in the current gaps in our knowledge of what makes certain synapses vulnerable to dysfunction and loss, a hallmark across multiple neurologic diseases spanning autism, schizophrenia, Huntington's, Amyolateral sclerosis (ALS), Parkinson's and Alzheimer's diseases. They will also help us to develop a more targeted and refined approach to developing therapeutic tools in dementia, which is a global socioeconomic problem. Altogether, insights into these neuroimmune pathways will lead to development of targeted therapy as well as novel biomarker tools, benefitting the worldwide ageing population that suffer from dementia and other age-related neurologic diseases. Our project will yield multiple publications in peer-reviewed journals as well as presentations in local and international scientific meetings. We will also communicate our findings to the public, as we strongly believe that it is a key responsibility and privilege of scientists to communicate with the public to raise awareness regarding the state-of-the-art on dementia research.

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

The academic sector will be impacted by my proposal in the short term. Specifically, the field that we will impact will be neuroimmunology, neurobiology, and/or neurodegenerative disease-focused biomedical research. As our project directly aims to understand novel mechanisms behind neurodegeneration, the translational aspect of biomedical research will also be impacted by our research in the short term. We will also communicate with the public to raise awareness regarding the state-of-the-art on dementia research. In the long term, as dementia is a global socioeconomic problem, outputs of our project will benefit the ageing population around the world and their families and carers.

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

We will disseminate all new (successful and unsuccessful) knowledge internally within our institute, as we pledged, and the university as a whole. We will also present our findings in multiple local, national and international scientific meetings. Our project will yield multiple publications in peer-reviewed journals where data will be accessible worldwide. We will also regularly communicate our findings to the public, as we strongly believe that it is our responsibility and privilege as scientists to communicate with the public to raise awareness regarding the state-of-the-art on dementia research. Given the nature of our project, we

already do, and will continue to, actively collaborate with other leading researchers around the world studying various aspects of synapse and immune biology, and attend international conferences to further disseminate research findings internationally.

Species and numbers of animals expected to be used

- Mice: 25600
- Rats: 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.

Dementia is caused by diseases of ageing in adults and thus it necessary for us to undertake our research in mature adult animals that have a fully developed central nervous system. Furthermore, it is becoming increasing clear that the immune system plays an important role in the homeostasis, function and health of the central nervous system. In order to understand how immune and nervous systems work together, we need to utilise in vivo animal models of disease where we can modulate synaptic and/or immune pathways. These will allow us to link molecular and cellular changes to specific dementia-relevant phenotypes.

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

Many of the mice on this licence will be used for breeding. This is due to the complex nature of neuroimmune interactions and dementia-causing diseases, and our aim to understand the genes and cells that impact disease. This requires several steps of breeding to get to a cohort with the necessary combination of genes that can be studied.

In order to understand specific neuroimmune pathways implicated in neuronal health and function, some animals will undergo short (5 min-maximum 1 h) procedures where either expression of specific genes and/or proteins will be altered or certain substances that will modulate synaptic and/or immune function will be applied at appropriate life stages of the animal. Anaesthesia and analgesics will be given as appropriate to the procedure, and careful monitoring and humane endpoints will be applied to all animals. A subset of mice will undergo a combination of phenotyping and/or behavioural tests to characterise preclinical models and for hypothesis testing. Each experiment will use a combination of tests over the lifespan of the animals, most of which are non-invasive. Typically mice are then deeply anaesthetised and a terminal bleed or perfusion carried out.

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

Genetic alterations in the mice used in this project may lead to the development of dementia-relevant phenotypes. In mice, these changes may lead to increased aggression/fighting, seizure-like activity, decreased food intake leading to weight loss or altered response to some procedures. For example, impaired wound-healing after surgery because of a compromised immune system may occur in some lines used in this project.

Mice undergoing cognitive testing using a food reward for motivation will have reduced access to food to reduce their body weight to ~90% of their free-feeding weight. Studies have shown that in some mouse models that are genetically modified to have dementia-causing disease, food restriction increases survival, consistent with data for C57BL/6 mice in general. Thus, we do not anticipate adverse welfare outcomes associated with food restriction. To improve the validity of our results we will monitor long-term effects of food restriction by assessment of blood glucose before and after food restriction and body mass composition.

For some tests, mice will need to undergo anaesthesia. All surgery and anaesthetic use in mice carry a risk of mortality and a risk of pain (short and long term), which may differ between genetically altered lines.

Blood sampling requires the use of restraint to ensure safe sampling in a controlled manner. This poses the risk of induction of a stress response. The use of assays which minimise the required sample volume will be used and larger volumes of blood will not be sampled when the mice are on restricted food intake protocols.

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)?

Approximately 30% of mice on the mild breeding protocol will experience mild severity. Almost all animals on this protocol will experience sub-threshold severity. Mice on this protocol will have no procedures other than ear biopsy, and will only be bred on this protocol if previous experience or reasonable assumption shows no evidence of welfare concerns related to the genetic alterations. Mice will only reach a mild severity if multiple ear biopsies are needed for genotyping.

Approximately 40% of mice on the moderate breeding protocol may experience moderate severity. Some knock-in genetic alterations that cause dementia-causing diseases may additionally result in neurodevelopmental alterations that manifest as adverse phenotypes early in life (such as reduced weight gain / body weight, or altered behaviour). Additionally, combinatorial crosses designed to modulate dementia-associated phenotypes in adult mice may result in the production of progeny which have phenotypes that occur before or shortly after weaning. It is expected that a maximum of half of offspring will develop these effects.

Depending on the protocol, <1% to 100% of animals experience moderate severity (details and reasoning on each protocol).

<20% of adult mice on selective subdiaphragmatic vagotomy protocol may experience severe severity, as they will be subjected to either laparotomy and vagotomy, or laparotomy alone (control surgery).

Laparotomy only will constitute a maximum of moderate severity to the animals.

90-100% of adult mice on injection of antisense oligonucleotides (ASO) protocol may experience acute severe adverse effects, as they will be subjected to ASO injection, but >90% of these mice are expected to make a full recovery to normal behavior and wellness within 24 hours.

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 January 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 objectives of this project are to:

Link molecular and cellular changes of dementia-causing diseases to dementia-relevant phenotypes (including changes to cognition, behaviour, metabolism, sensory systems, body fluids, tissues and whole-body physiology).

Understand how environmental changes and pharmacological interventions can modulate dementia- relevant phenotypes.

It is not possible to manipulate the molecular and cellular pathways in people who have dementia in order to determine how these processes impact on cognition, behaviour, metabolism, sensory systems, body fluids, tissues and whole-body physiology. Thus, an animal model must be used.

The wider research community is undertaking significant work analysing clinical and human post- mortem datasets from people who had dementia-causing diseases, as well as preclinical work in cell, organoid and invertebrate model systems of dementia-causing diseases.

This project will link with and draw on these experiments to minimise animal use and maximise translational value of the preclinical mouse work in this project. The work in this project cannot be addressed elsewhere using alternative approaches.

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

Clinical longitudinal studies of people who have dementia (biomarkers including blood, CSF and neuroimaging), genetic and cognitive/behavioural/clinical scoring.

Human post-mortem and brain biopsy studies.

Invertebrate animal models.

Induced pluripotent stem cells (iPSCs) and brain organoids.

Why were they not suitable?

Clinical longitudinal studies of people who have dementia, provide correlative data between an individual's genetics, environment, biomarker (blood or cerebrospinal fluid biochemistry, or brain imaging data) and cognitive and behavioural outcomes. These datasets can be used to generate hypotheses of the molecular and cellular causes of dementia-associated biology but cannot be used to test how specific factors influence dementia outcome.

Human post-mortem and brain biopsy studies comparing people who had dementia (early and late in disease course) with people who did not have dementia, can be used to determine which cellular and molecular changes occurred in the brain. In some cases, clinical and biomarker data from life will also be available for these individuals and this observational data can be used to hypothesise which cellular and molecular changes resulted in clinical disease. However, these samples cannot be used to test all hypotheses.

Invertebrate (fly models) can be used to study the response of neurons to the proteins that misfold and aggregate in dementia-causing diseases and to screen for genetic modifiers of these processes.

However, they do not have complex behavioural and memory biology, thus it is not possible to study the cellular and molecular processes that result in altered executive function or subtypes of memory in dementia. For example, it is not possible to quantify working memory, motivation or apathy in a fly. Flies also lack key brain-resident immune cells (microglia) and a closed circulatory system (veins and arteries), which have key roles

in the development of neurodegenerative disease. These aspects of the biology of dementia cannot be modelled in flies.

Similarly, iPSCs and brain organoids cannot currently be used to study cognition, behaviour, metabolism, sensory systems, body fluids, tissues and whole body physiology.

A retrospective assessment of replacement will be due by 05 January 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?

Current estimates are that we will need a sample size of 10 per sex and genotype for each phenotyping experiment. In next-generation preclinical mouse models of dementia-causing diseases, which will be typically used in this project, some key dementia-relevant phenotypes do not develop until mice are 12- 18 months of age. However, we are primarily focused on analysing the earliest stages of disease progression in order to pinpoint the best preclinical targets for therapeutic intervention. As a result, the mice will not usually progress to ages beyond 12 months of age. At least 2 rounds of breeding will be required to generate the mice required for some experiments and we anticipate providing other researchers with cohorts of mice matched to those studied for collaborative phenotyping projects.

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

Power equation calculator will be used for group size calculations for all experiments plus attrition rate calculation will also be used to ensure sufficient power is maintained until the end of longitudinal studies.

The combination of tests in each experiment will be designed to gather the most meaningful data. Tests which can inform each other will be carried out on the same mouse to remove inter-animal variability and increase the power, thereby decreasing the overall sample size and the scientific utility of generated data. This approach will also provide novel scientific insight into the relationship between dementia-relevant phenotypes and help validate new testing approaches such as home cage assessment.

SOPs have been written and used routinely for previous projects. This standardises the way the data is collected and reduces the variability and therefore the sample size.

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

Efficient breeding and holding lines as frozen-down embryos and sperm will be used to minimise the number of mice being produced for these studies. Genetically modified lines will be sourced from repositories to avoid remaking of lines whenever possible. Any excess stock will be offered to other researchers to minimise wastage. Pilot studies will be undertaken to generate means and standard deviations for work using background strains for which data is not available. Tissues sampled from the animals used in this project will be shared with other researchers and the data produced linked to that generated by the project, to maximise long term utility.

A retrospective assessment of reduction will be due by 05 January 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.

Transgenic models of Alzheimer’s disease (AD)-causal mutations can have, very rarely, sudden death caused by seizures, while some transgenic models of Parkinson’s disease (PD) mutations develop slight motor impairment but which does not deter grooming or feeding behaviour. Here we propose to principally use alternative preclinical models that have a reduced welfare burden and provide a more refined scientific tool. Mice will be generated and bred on co-isogenic inbred lines to remove any confounding factors of genetic background.

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

Here, we will assess how neuroimmune pathways lead to dementia-relevant diseases (including cognitive and behavioural changes); this will focus on the molecular and cellular

biology that underlies these changes to cognition, behaviour, metabolism, sensory systems, body fluids, tissues and whole body physiology.

Invertebrate (fly models) can be used to study the response of neurons to the proteins that misfold and aggregate in dementia causing diseases and to screen for genetic modifiers of these processes.

However, they do not have complex behavioural and memory biology or complex neuroimmune system and thus it is not possible to study the cellular and molecular processes that result in altered executive function or subtypes of memory in dementia, as in this project. For example, it is not possible to quantify working memory, motivation or apathy in a fly. Flies also lack immune brain cells (microglia) which have a key role in the development of neurodegenerative disease and this aspect of cellular biology of dementia can thus not be modelled in flies. The fly cardiovascular system significantly differs from that in mammals (being open without veins or arteries) thus the effect alterations to cardiovascular biology on dementia cannot be readily modelled in a fly. This work needs to be undertaken in an adult model organism with an intact nervous system, blood-brain-barrier and robust immune response without anaesthesia to assess disease-causing pathology in their brains.

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

Here we aim to collect a wide range of phenotyping data to understand neuroimmune mechanisms of dementia-causing diseases. These data will also be used to understand the biology that underpins changes to mouse behaviour and will also be used to monitor and improve animal welfare, drawing on a welfare improvements technology development pipeline. For all behavioural tests, it is important that the animal has no additional stress, therefore mice are handled calmly and habituated to testing rooms as well as arenas if possible. Animals will be preferably held under one protocol and transferring between protocols will be avoided as much as possible.

For all tests, mice are only housed in modified cages or arenas for the minimum time needed to gather meaningful data. Mice undergoing phenotyping tests have increased monitoring and are removed from tests if they appear to be suffering from an adverse stress reaction, or other unexpected adverse effects of the phenotyping tests.

Mice which have undergone anaesthesia have extra monitoring until fully recovered and extra checks when back in the holding rooms. We will carefully monitor the full progress of the mice until they are in full health, and in case complications arise, we will consult with the NVS, When general anaesthetics are necessary, the combinations with least adverse effects will be used. Pain from tail bleeds is reduced by using local anaesthesia.

For more severe procedures, mice will be monitored more frequently and followed up using scoring sheets, in consideration with NVS.

Experiments will be designed to balance the overall experience of the mouse and the number or type of tests undergone by any one animal against the value of a full understanding of the biology of individual animals and why phenotypes are altered in order to maximise utility of the research data obtained.

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

Routes and volumes for administration of substances are taken from Laboratory Animal Science Association good practice administration of substances guidelines 1998 (http://www.procedureswithcare.org.uk/lasa_administration.pdf).

The animal house has full AAALAC and ISO9001-2015 accreditation. To conform to these standards we must ensure a high level of quality control on all fronts including husbandry, phenotyping and administrative processes.

Standard operation procedures for most tests have been generated using data and expertise from multiple animal houses and can be found at https://www.mousephenotype.org/impress.

ARRIVE and PREPARE guidelines will be followed at all times.

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

We (lab and PI) will attend the NC3R annual conference; we will also attend international meetings focusing on animal models of dementia causing disease and webinars run by the MODEL-AD consortium. In addition we will attend local 3Rs seminars and events, as the NC3Rs liaises closely with the Institute and University.

A retrospective assessment of refinement will be due by 05 January 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. Cardiovascular development and regeneration

Project duration

5 years 0 months

Project purpose

- Basic research

Key words

Congenital heart disease, myocardial infarction, aortic disease, cardiac regeneration, vascular protection

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?

The overall aims of this project are to understand the embryonic origins of congenital and adult-onset heart and vascular diseases and to identify novel and improved approaches for myocardial regeneration and vascular protection, based on insights from the developing embryo.

A retrospective assessment of these aims will be due by 21 January 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 diseases remain the primary cause of morbidity and mortality worldwide. Congenital heart disease is the most common type of birth defect and treatment frequently requires multiple surgical interventions throughout childhood. There are no drug treatments to prevent aortic aneurysm or to cure heart failure, leaving millions of patients with debilitating and live-threatening conditions.
Improved therapies are therefore urgently required, which necessitates basic research to understand how the disease arises and identify the best drug targets for treatment.
What outputs do you think you will see at the end of this project?
We will gain new information on how the heart and coronary vessels adapt after a heart attack, in an attempt to repair the damage caused. We will also gain an understanding of the changes that occur within coronary and large arteries, that lead to coronary heart disease and abdominal aortic aneurysm, respectively. Most importantly, from this knowledge, we aim to identify new ways to repair the damaged heart and to protect vessels from disease. We will publish our findings in high impact journals.

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

Damage to blood vessels causes accumulation of cholesterol and leads to destruction of muscle and elastic layers, to weaken the vessel. Rupture of weakened vessels causes life-threatening events (heart attack, stroke or aneurysm – balloon-like swelling of the body's main artery). There is currently no drug treatment for aortic aneurysm, thus high risk surgical intervention is the only option. A better understanding of these pathways will reveal targets for new drugs (or re-purposed existing drugs) to protect patients against these major life-threatening diseases. Time frame: 5-15 years.

After a heart attack, a significant portion of the heart's muscle is irreversibly damaged, leading to the debilitating condition of heart failure in growing population of patients. We are researching novel ways to reactivate 'embryonic' mechanisms in the adult heart for regeneration. Beyond this project (10-15 years from now), this information may be used to develop drugs to repair of the heart by boosting new muscle formation and blood vessel growth. This would provide a powerful treatment for the 900,000 heart failure patients in the UK and millions more worldwide.

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

We collaborate extensively with researchers at our university, across the UK and internationally. Their expertise facilitates aspects of the study that would not otherwise be possible and allows us to achieve our goals more rapidly and maximise outputs, in terms of publications. We also present our work at the major national and international conferences within our field. Whilst it is more difficult to publish unsuccessful studies in scientific journals, it is important for the scientific community to learn from these studies. We would therefore deposit reports of such studies, if appropriate, on preprint repositories, such as Biorxiv.

Species and numbers of animals expected to be used

- Mice: Maximum 22,600

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 investigate heart and blood vessel development in the mouse embryo, because we need to understand how to treat congenital heart disease; also, because knowing how the heart and blood vessels form naturally in the embryo provides important clues to understanding how to develop protective therapies to prevent common vascular disease and therapies to regenerate the heart muscle damaged after a heart attack. In order to test the most relevant pathways, we are modelling heart attack, coronary artery disease and aortic aneurysm in adult mice.

Mice represent an accurate model organism for the study of cardiovascular development and they are essentially the only mammalian model amenable to genetic targeting to assess individual gene function in the context of cardiac growth and regeneration.

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

Most animals will be used for breeding, with no further procedures, or will provide tissues and embryos for our studies.

A smaller number (maximum 20%) will undergo more invasive procedures to model human cardiovascular diseases:

- In some mice (5-10%), we will simulate a heart attack by tying a suture around a major coronary artery.
- Up to 2% will be fed a high fat diet to induce atheroma (cholesterol-rich plaques similar to those that form in human arteries).
- Up to 2.5% will be used to model abdominal aortic aneurysms by infusion of the naturally occurring hormone Angiotensin II.

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

The vast majority of animals will be used for breeding and collection of embryos or tissues post mortem, so there will be little or no suffering.
In some animals, we will administer substances, usually orally but occasionally by injection. This will cause minor discomfort and stress, however this will be of short duration and repeated as few times as possible to achieve the scientific objective. Longer (e.g. MRI imaging) or more invasive (surgical) procedures will be carried out under anaesthesia, with analgesia if appropriate, which also minimises pain and stress.

Adverse effects are only expected in a small proportion of animals:

Modelling a heart attack requires an invasive surgical procedure, which can cause pain, weight loss and occasionally respiratory difficulties (albeit most mice show no clinical symptoms). Pain can last up to 48 hours but is relieved through analgesia. With a suppressed appetite, mice may lose weight over the first 4 post-operative days and usually regain starting weight by 7 days. Normal respiration is usually restored by 24 hours and will not exceed 48 hours, otherwise the animal will be humanely killed.
Modelling abdominal aortic aneurysms: as with humans, the majority of cases will be asymptomatic; however, in <10%, aneurysm rupture may occur and may cause severe pain, however this will be short- lived as animals will become unconscious within seconds and die within minutes.

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; 15% 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 21 January 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 diseases, injury and repair are brought about by a complex interplay between cells of the heart, vessels and the immune system. Thus, despite advances in cell based systems, computer modelling and the benefits of using clinically relevant patient biopsies, none of these methods faithfully models events such as atherosclerotic plaque progression, aortic aneurysm, myocardial infarction or heart failure. Hence, the use of animals is unavoidable if we are to answer important questions about causes of, and identify effective treatments for, cardiovascular diseases.

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

We have developed and validated a range of cell-based systems that allow us to model some aspects of cardiovascular cell behaviour (in particular, the epicardial cells that are central to our project).

Importantly, we can derive human epicardial cells by converting skin or blood cells into a type of stem cell. We can also obtain epicardial cells from patient biopsies. We have used such cells to test the efficacy of small molecule drugs on in a dish and, only once promising compounds with potential for therapeutic use in humans have been identified, would we test the drugs in live mice.

Why were they not suitable?

Cell-based screening is invaluable to predict whether a compound is likely to achieve a beneficial effect if given as a drug. However, the heart is a complex organ which functions through the interaction of various diverse cell types, both within the heart and circulating throughout the bloodstream (immune cells). Such complexity cannot be faithfully reconstructed in a dish, thus the amount of information that can be obtained this way is limited. We need to understand precisely how the heart and blood vessels respond to injury in order to know which cells to target, and the optimal time frame, to predict which types of drugs may have a beneficial effect.

A retrospective assessment of replacement will be due by 21 January 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 have 20 years of experience of designing experiments with animals. Our previous studies and the published literature will guide us to determine the number needed per experiment. I have considered the number of ongoing and planned projects and which experiments are needed for each study.

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

I consulted the following sites:
http://www.3rs-reduction.co.uk/ https://www.nc3rs.org.uk/experimental-design-assistant-eda
I have completed four statistical and experimental design courses, the most recent of these four years ago.

All of my recent funding applications required me to demonstrate how animal numbers were calculated and these have been peer reviewed.

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

Most studies will develop our previous findings, where new and important questions arise. For new areas of investigation, pilot studies will be performed, the results of which will determine the value of pursuing a larger scale experiment.

We work carefully to make sure that we can derive as much useful information as possible from each mouse. For example, we may take the aorta, heart and blood from a single animal which, depending on the treatments given, can be used in multiple projects. From one heart, we can determine expression changes of approximately 35 genes or we can collect around 100 sections of aorta or heart to localise and quantify protein expression within the tissues.

We will manage animal breeding carefully to reduce animal numbers to the minimum required for our experiments and colony maintenance.

Where we can obtain tissues from collaborators, we will do so and, likewise, we will make tissues available to others.

A retrospective assessment of reduction will be due by 21 January 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.

Heart attack and aortic aneurysm are serious, life-threatening conditions in human patients and can only be accurately modelled using severe protocols in mice. Although severe, the protocols have been refined as much as possible to minimise suffering and achieve the scientific objectives. For the protocol to induce a heart attack, the step to insert an intubation tube into the trachea to inflate the lungs has been refined by use of a dedicated intubation platform, which reduces trauma to the trachea and reduces the overall time that the mouse is anaesthetised. Ligation of the coronary artery cannot be achieved without opening of the chest to expose the heart, however we make the smallest possible incision, to minimise pain and reduce respiratory difficulties after recovery.

The surgical procedure to induce aortic aneurysm (implanting a small pump under the skin) does not itself cause more than mild discomfort for up to 24 hours. However, aneurysms develop at a different rate in genetically altered mice and the possibility and timing of rupture is therefore difficult to predict. Our experiments are designed to end before rupture, however, in a small number of mice (10%), rupture may occur before the

endpoint. There is no way to avoid this, and so the most refined approach is to very closely monitor behaviour (daily or more frequently where there is cause for concern) and weight loss to detect the earliest signs of disturbed aortic function.

In both models, pain, suffering and distress is minimised by anaesthesia, analgesia, daily monitoring and use of humane endpoints when necessary.

We model coronary artery disease through cholesterol accumulation by use of a genetic model (loss of ApoE, a protein involved in fat metabolism) and feeding of a high fat diet. As well as being the most widely characterised model scientifically, this is a non-invasive method with limited welfare impact and is, therefore, more refined than alternative surgical models (carotid artery ligation).

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

Mice represent an accurate model organism for the study of cardiovascular development and they are essentially the only mammalian model amenable to genetics to assess individual loss or gain of gene function in the context of cardiac growth and regeneration. Much of the work is performed on early stage embryos, however, the diseases we wish to model are ones which occur in the adult human population, thus adult stages are very relevant. Studying heart function and the changes that occur require the animal to remain alive for several weeks following a simulated heart attack.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?
For all surgical procedures, analgesia will be administered routinely for the control of post-operative pain. We also routinely use heat support, access to water-softened chow, injected fluids and oxygen, as required, after surgery. Sterile techniques will be used to minimise the risk of infection. Animals will be monitored at least daily after severe procedures and additional analgesia provided as needed.

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

We will continue to follow all local guidelines on best practice and routinely consult the following when planning new studies:

https://nc3rs.org.uk/experimental-design
https://arriveguidelines.org/arrive-guidelines (sections on study design, statistical analysis and experimental procedures are particularly helpful for less experienced researchers).
I have found this resource to be incredibly helpful and my team members frequently refer to it: http://www.procedureswithcare.org.uk/

The following provides useful guidance for aseptic surgery:

https://www.lasa.co.uk/PDF/LASA_Guiding_Principles_Aseptic_Surgery_2010.2.pdf
We refer to the LASA guidance on maximum administration volumes (http://www.verutech.com/pdf/lasa_administration.pdf), a summary of which is included below:

Reference on fostering of Caesarean section and fostering of pups:

Cai, Z. et al (2018). Caesarean Sectioning and Cross-Fostering of the Mouse. Bio-101:e3085

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

I read the NC3Rs monthly e-newsletters, as well as 3Rs newsletters distributed to University researchers. I have attended the 3Rs symposium held at the University and encourage my team members attend. We frequently discuss 3Rs at group meetings.
A retrospective assessment of refinement will be due by 21 January 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. Immunopathology of experimental blood-stage malaria

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

malaria, immunopathology, brain, treatment, inflammation

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

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 improve our understanding of the pathways and processes that control the activation of the immune system and cause severe disease during malaria.

A retrospective assessment of these aims will be due by 28 February 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?

It is important to undertake this work as malaria still causes significant illness and death in developing countries throughout the world. Animal models provide critical opportunities to identify and mechanistically test the processes and pathways responsible for promoting severe malarial disease, using procedures that are impossible to perform in humans.

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

The work in this project will lead to significant new information on the pathways and processes that activate and regulate the immune system and which promote pathology during malaria. Direct outputs from the work will be peer-reviewed research articles, dataset resources that will be shared with the research community, and presentations, where we will disseminate our discoveries.

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

The outlined programme of work will provide new insight into the most appropriate molecules and pathways to target as treatments for severe malarial disease, in particular cerebral malaria, which is the most severe complication of malaria that causes brain pathology. This will be of major benefit to researchers working on human malaria and should, in the short and mid-term future, direct clinical trials of therapies for cerebral malaria, which will ultimately be of benefit to millions of individuals in malaria- endemic regions of the world.

In addition, by dissecting the activation and regulation of the immune system during malaria, our work will demonstrate how to therapeutically manipulate the immune response against Plasmodium spp. parasites (the causative agent of malaria), which in the mid-term will have impact for strategies to augment protective memory responses to malaria and improve vaccine designs for malaria.

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

We will publish our results in peer-reviewed journals, in open-access format when possible. We will also initiate new and build upon existing collaborations to enhance the impact of our results. We will disseminate unsuccessful approaches or negative data through specific journals or online forums.

Species and numbers of animals expected to be used

- Mice: 2850

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 most appropriate species for this work as murine malaria infections are the most well- characterised of the various animal models (when using established parasite lines, as will be done in most experiments within this licence), and there is a significant body of literature, including from ourselves, that results obtained in murine malaria studies are relevant for understanding human malaria. We will utilise young adult mice as we require that the immune system is fully formed so we can appropriately translate results from mice to humans.

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

The general project plan will involve infecting mice with different species of Plasmodium parasites that cause specific types of malarial disease - ranging from mild malaria to cerebral malaria (a severe syndrome of malaria that affects the brain). The course of infection will be monitored by following peripheral parasite levels, through obtaining drops of blood from the tail vein. Depending on the question addressed in each experiment, mice may receive injections to modulate the immune system or physiological processes, may undergo surgery to modify tissue function (e.g. removal of the spleen to influence the immune system, or ligation of brain lymphatic vessels to change how cells and molecules drain out of the brain), or may receive anti-malarial drugs to kill parasites. Injections can be by different routes depending on the research question and the nature of reagents administered (i.e. reagents may be injected directly into the brain or provided systemically into the blood). The vast majority of animals will recieve less than 4 injections to manipulate the immune system or physiological processes, by a maximum of two different routes. The experiments will be typically short duration of 7 -14 days when assessing the immune response and level of pathology during primary Plasmodium infections; however some experiments may be > 60 days, when studying the development and activity of memory immune cells (the cells that are maintained post-infection or vaccination to provide protection against subsequent infection). In some experiments, animals may be re-infected with Plasmodium parasites after clearing a previous infection to assess how repeated infection influences parasite control and the development of severe malarial syndromes. Multiple manipulations in a single animal will be avoided, when possible. Cumulative effects (e.g. additive effects) of multiple treatments will be minimised by allowing animals to fully recover from any serious procedure (i.e. surgery, irradiation and reconstitution) before the animals undergo any subsequent treatments.

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

Depending upon the species and strain of Plasmodium parasite and the strain of mice utilised, malaria infection may lead to mild, moderate or potentially severe suffering. Mild suffering occurs due to activation of the immune system and the general feeling of malaise (e.g. lethargy, fever or aches) that is associated with infection. Severe suffering during malaria occurs due to weight loss and loss of circulating red blood cells (anaemia) and / or damage to the tissues in the body (such as the brain during the development of cerebral malaria). In particular, during cerebral malaria the damage to the brain causes the tissue to swell, which causes pain to the animal and may lead to fitting and / or coma. However, of the experiments involving infections that have the potential to cause severe suffering in animals, not all infections will be allowed to progress to the stage where severe suffering occurs (i.e. experiments will be terminated at early stages before severe malaria develops to allow us to define the factors responsible for development of disease, or animals will be treated with anti-malarial drugs to terminate the infection). Most of the procedures performed or the reagents administered should not directly promote animal suffering. Animal suffering will be minimised by closely monitoring all animals in relation to a well-defined grading system and providing analgesia, when required and when possible without negatively impacting the course of the experiment. All administrations will be performed via the most appropriate route through (when applicable) the careful control of injections. Using our well- defined grading systems, of the animals that may experience severe suffering during the course of our experiments, suffering will typically be less than 4 h and not more than 12 h.

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)?

Experimental animals will be routinely monitored during the course of infection and the severity of disease and level of suffering will be graded according to well-defined scoring systems.

In infections that do not cause cerebral malaria, we expect 25% of animals may experience short-term (<24 h) moderate suffering (principally evidenced by lethargy or hyperventilation).

For infections that cause experimental cerebral malaria, the majority (>50%) of mice on this protocol will experience short-term (<12 h) severe levels of suffering (principally evidenced by hunching, respiratory distress and reduced responsiveness to stimulation).

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 28 February 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 can only address the majority of our questions when a complete immune system is present in its normal anatomical and physiological configuration (for example within the spleen, the major site of immune priming and parasite killing during malaria infection), or when parasites and immune cells can interact with the complex architecture of the intact brain (leading to cerebral malaria): the use of animals is, to a significant extent, unavoidable in our experiments.

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

When we have simple and reductionist questions, such as how parasites directly interact with brain endothelial cells, then we can establish in vitro co-culture systems to study this interaction.

Why were they not suitable?

Such in vitro co-culture approaches are suitable for only very specific questions as during the course of a normal infection in vivo, the interaction between parasites and brain endothelial cells is shaped by a myriad of factors, including circulating immune cells and immunological mediators, and the multi- faceted communication with other brain resident cells. Thus, for the majority of our investigations to obtain accurate and physiologically relevant results, we need to study our objectives within intact tissues, in vivo or ex vivo.

A retrospective assessment of replacement will be due by 28 February 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 numbers of animals based upon our previous experience of running similar project licences in the last 15 years. Thus we have accounted for the nature and requirement of the projects we are currently working on, including the numbers of times experiments must be repeated, the numbers of different experimental groups in experiments, and the numbers of mice required in different groups. We have also estimated the number of animals to be used based upon future plans and collaborations.

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

We calculate the required group size using data from previous experience, and published work. This ensures that we have sufficient power to detect a biologically relevant effect using as few animals as possible. We also perform sample size calculations based upon pilot and preliminary experiments to ensure we perform subsequent experiments with the correct number of mice to detect statistically significant results. We also adhere to ARRIVE and PREPARE guidelines for reporting of research involving animals, which outlines appropriate study design (e.g. control groups and sample sizes), how to avoid experimental bias, and the analytical framework for simple and complex 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 will perform pilot experiments when undertaking new experimental approaches so we can discontinue uninformative or inappropriate methodologies and so we can also evaluate the variability and magnitude of experimental effects. This will allow us to accurately assess the numbers of mice to use in future studies. We also consult the literature when we are performing similar approaches as others have previously performed, in malaria or in other models. This allows us to predict the strength of expected effects within our experiments, and therefore, the numbers of mice that need to be used to detect statistically and biologically relevant results. We will carefully manage maintained colonies (i.e. by employing short-term harem breeding) to ensure we have sufficient numbers of mice for planned experiments but ensuring we do not have surplus mice. Any unneeded mice will be shared with researchers, who have authority to receive animals.

A retrospective assessment of reduction will be due by 28 February 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 the most appropriate species for this work as murine malaria infections are the most well- characterised of the various animal models (when using established parasite clones, as will be done in most experiments within this licence), giving us essential background information that is lacking in other systems. For example, in a previous project licence we performed important comparative assessments of the murine cerebral malaria model with human cerebral malaria, to evaluate the relative merits and translational utility of the murine model for studying the pathogenesis of human malaria, and for identifying the events that determine the anti-malarial drug treatment effectiveness of the syndrome.

The only alternative experimental models of mammalian malaria infections are non-human primate models, involving monkeys or apes. Mice are also the animals of choice for immunological investigations as so much is known about their immune systems, different well-characterised inbred strains of mice exist with differing responses to infection, there are a large number of genetically modified murine strains available for use, and all the reagents that we require (such as for modulation of the immune system) are available. Lastly, mice are well-adapted to captive environments.

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

To obtain informative results in this project we need to utilise a warm-blooded mammalian host that can be infected with evolutionary adapted Plasmodium spp. parasites and where the biology of Plasmodium infection is comparable to that in humans. This precludes the use of less sentient Zebrafish or drosophila models. We must also use adult mice with a fully formed and functional immune system. Otherwise, our results would be difficult to translate to the study of human malaria. We will perform certain protocols under terminal anaesthesia but due to the length and course of experimental malarial infections, it is not possible to perform all work under anaesthesia.

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

Due to the overall purpose of this work - to study the factors responsible for development of severe malarial disease - we do need to allow experiments to proceed to the point where animals will experience some suffering, recapitulating the development of severe malaria in humans. However, through using our well-defined grading system, of the animals that may experience severe suffering during the course of our experiments, we will ensure that none of these animals will experience prolonged suffering for more than a few hours (generally less than 4 h and no more than 12 h).

Moreover, animal suffering will be minimised by providing analgesia, when possible and when required. For example, whilst we can provide analgesia following surgery, we are unable to provide analgesia during the course of infection or to mitigate the effects of cerebral malaria, as the analgesia itself will modify animal behaviour and the course of the experiment. Multiple treatments to manipulate the immune system or physiological process within a single animal will be avoided, when possible, with a maximum of two separate approaches applied in any animal.

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

We will follow NC3Rs and LASA guidance and we will continually assess our experimental designs in relation to advances within the relevant malaria and immunology literature.

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

We are on the NC3Rs mailing list where we obtain newsletters with new advice and guidelines and information from other sources (such as LASA) are also communicated to us via email announcements. Standard operating procedures for users working with animals are in place within our institution, the adherence to which is compulsory, which incorporates advances in animal handling and ensures animal welfare.

A retrospective assessment of refinement will be due by 28 February 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. Transcriptional regulation of genes in osteoarthritis

Project duration

5 years 0 months

Project purpose

- Basic research

Key words

osteoarthritis, serpins, gene regulation, extracellular matrix, degradative enzymes

Animal types	Life stages
Mice	embryo, adult, 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?

Osteoarthritis (OA) is a disease of the joints with unmet clinical needs. This programme of work is a continuation of previous PPL 70/9047 in order to characterise new enhancers of genes involved in OA, unravel how such genes exert their action and determine molecular targets for pharmacological intervention.

The specific aims are:

1. Characterise enhancers of genes involved in osteoarthritis.

2. How do genes regulate the development and maintenance of the musculoskeletal system.

3. How metalloproteinases and their activators (the serpins) and their inhibitors (TIMPs) regulate extracellular matrix.

A retrospective assessment of these aims will be due by 10 February 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?

Currently there is no effective treatment for osteoarthritis except for joint replacements of certain joints such as hips and knees. The program proposed will identify target molecules to either enhance cartilage repair or block degradative enzymes as well as determining basic understanding of the musculoskeletal formation through gain and loss of functions of critical genes.

1. We will characterise the cartilage specific enhancer(s) in MMP13 and the transcription factors that control their activities and that of other gene enhancers that we have characterised such as ACAN and CCN2.

2. Elucidate the mechanism of how LRP-1 or HuR loss in skeletal development leads to malformation of joints and bones including signalling molecules such as Wnt and syndecans.

3. Determine which is the critical serpin in activation of MMPs in the regulation of musculoskeletal tissue.

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

In aim 1, we will determine the cartilage-specific enhancer(s) in MMP13, CCN2 and other genes and what factors drive them to regulate their activities in OA.

In aim 2, we will provide possible mechanism(s) as to how the loss of either LRP-1 or HuR in skeletal development leads to malformation of joints and bones.

In aim 3, we will elucidate the critical serpin that activates MMP13 and regulates its activity in joints.

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

The main beneficiaries of this applications are the human and animals that develop OA in ageing or human and animals that will be prone to OA following injury. They will benefit from advances in our knowledge of how to combat this disease and will be the beneficiaries from drugs that are developed to target molecules that we have shown to be critical to the development of the disease. For example, since MMP13 is a major enzyme that breakdown articular cartilage, we will delineate approaches that can halt the activation of the MMP13.

The other beneficiaries are scientists around the world who are working on this and related diseases, including pharma companies developing drugs that can manipulate some of the molecules that are highlighted in this programme of work. For example, if we find an enhancer that is cell specific to chondrocytes, this enhancer can be used to target pharmaceutical agents to these cells alone rather than at random which will be beneficial in terms of lower concentration and toxicity.

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

We will disseminate our results periodically through learned societies and in clinical departments related to these diseases such as rheumatology and orthopaedics. We will publish our data and make them accessible to a wider audience beyond the geographical location of the university.

Species and numbers of animals expected to be used

- Mice: 5500

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 animals used in this project are mainly transgenic animals. Mice are a genetically tractable mammalian system. We need adult mice because osteoarthritis is an ageing disease.

In aim 1, we use ENCODE to test in silico DNA sequences that regulate the expression of a given gene and we test these sequences using transgene readout mainly in embryos to test the line we generate, and in adult mice using OA-induced injury models.

In aim 2, we used the tissue-specific enhancer(s) generated above to drive Cre recombinase for gain and loss of function of genes that are involved in the development and maintenance of the musculoskeletal system .

In aim 3, single and double transgenic mice carrying either a transgene or over expressing genes or deleting genes such as MMPs and/or serpin genes will be used in OA models of

injury to track their expression and evaluate their contribution to articular cartilage maintenance.

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

In aim 1, day 1 fertilised eggs will be genetically modified, placed back in foster mothers under anaesthesia, in some instances embryos will be removed to investigate the pattern of expression in development. In other times, the mice are allowed to be born, genotyped and undergo surgery to induce OA or loading regime to accelerate ageing in joints (up to 12 weeks after injury, or up to one year without injury).

In aim 2, double transgenic mice (floxed gene X Cre recombinase) will either be induced by tamoxifen or doxycycline to delete or over express a gene involved in skeletal development and observe the consequences in embryos or adults (up to one year except if it is severe).

In aim 3, some of these mice will also undergo surgery to induce OA or loading to accelerate ageing in joints (up to 12 weeks after surgery or up to one year without surgery).

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

This project contains experiments that span three different severity categories and therefore, have a mild to severe impact and /or adverse effects for the animals depending on the experiments or breeding undertaken. In aim 1, most of the single transgenic mice that we generate or use fall under mild category because we express a transgene that does not cause harm. In aim 2, we will analyse loss and gain of function of specific genes and we already know that the loss of LRP-1 and HuR in early skeletal development is detrimental to the integrity of the skeleton and therefore we have placed them in "severe" category. Despite their anatomical abnormalities, the impact on their welfare is not detrimental because they grow in size and do not show behavioural akin to animals in distress. We are also planning to delete new genes as as serpins and ligands that bind to LRP-1. We do not know what the impact or adverse effects of these proposed animals.

We have a number of mice that we have generated or acquired that we will be using such as STR/ort mice that have spontaneous OA. These mice show a premature ageing and osteoarthritis that is perhaps associated with alteration of gait but this is variable and affect males more than females.

Syndican 3 knock out and TIMP3 knock out have weaker bones and by µCT analysis, both of these mouse lines show lower trabecular and cortical bone. However, we have not seen increased fracture in these lines. These mice are classed as "moderate" severity.

Mice that are NOT in "severe" category may undergo one of the two OA injury model and as these models accelerate ageing will show erosion in ONE of their knee's articular

cartilage. This may alter their gait, and as destabilisation of the medial meniscal ligament (DMM) injury involves a surgery, they are classed as "moderate". However, we only keep them up to 12 weeks after injury to minimise the adverse effects.

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)?

In aim 1, most of the transgenic mice are expressing a transgene which will be mild as the transgene does not interfere in endogenous gene. The proportion of mice in this category is 60%

In aim 2, we know from previous experience that HuR loss and LRP-1 loss in early skeletal development (using prx1 Cre, active at E9.5) have major skeletal defect and are considered "severe". The proportion of mice in this category is 4% of the total animal number. We also have animals carrying floxed genes that when crossed with a cre expressed in cartilage do not show skeletal deformities during development or any alterations from wild type mice. These mice are classed as mild. There are a number of genes, such as the serpins in which we do not know thus far the severity associated with their conditional loss in cartilage.

In aim 3, mice from aim 1 and animals that are not considered severe from aim 2 (for example serpin loss not showing skeletal defect in development) will undergo DMM surgery or loading regime and they are classified as moderate.The proportion of mice in this category is around 36%

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

- Killed

A retrospective assessment of these predicted harms will be due by 10 February 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?

Osteoarthritis is multifactorial and involves several cells such as bone, synovial fibroblasts, tendon fibroblast, articular cartilage chondrocytes, each of which responds to insult and stimulus in a different way. Therefore using one cell type will not provide us with a complete picture to understand the pathophysiology of OA. Although ex-vivo cartilage is used to inform us about chondrocytes behaviour it can not be used as a replacement for in vivo animal model. Therefore, to understand the complex interaction between molecules in the extracellular matrix, and their signalling to the chondrocytes in the articular cartilage, in the presence of synovial filtration of nutrients in an avascular and aneural system, we need an animal model and mice are the smallest animals that have joints similar in compositions to human. Osteoarthritis is a disease of ageing and in some mouse lines we will maintain mice until 1 year of age and in some cases, we will accelerate ageing using OA injury models or cartilage loading.

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

We test many of our interventions using ex-vivo material, such as porcine or other cartilage, before we test them on animals. In the majority of cases, we test all the constructs for promoter activity in transient expression in appropriate cells and only those that show responses are taken further to embryo injections.

In dealing with transcriptional regulation of gene, we have used in silico ENCODE with functional regulatory regions before we test them in culture and before we generate transgenic mice.

Why were they not suitable?

The OA injury models that we use are the most trusted and allow us to compare our results with other laboratories using the same method.

The cre-lox system is the best in vivo system to study gene function by either deleting or overexpressing a gene in an animal model.

The characterisation of gene enhancers through the generation of transgenic animals is the most accurate way to show tissue or cell specific activity that can be visualised and monitored throughout development and adulthood.

A retrospective assessment of replacement will be due by 10 February 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 the last 7 years we have used past results that we have published in peer-reviewed papers to establish power calculations in order to obtain the minimal number of mice that allow us to show differences between test and control groups.

In aim 1, using enhancer driving transgene expression in embryos. We look for 3-5 embryos expressing in the same manner (out of 20 transgenic mice), which indicates to us that irrespective of integration site, the sequence can express in a similar pattern. We take also into consideration the ratio of expressors vs transgenic to inform us of how penetrant is the enhancer.

In aim 2, the number of mice in gain or loss of function of genes in transgenic mice depends on the gene in question and whether it is critical during development or in adulthood. In some cases, the phenotype is only observed following injury such as in OA injury models .

In aim 3, the OA injury model differs between cartilage loading (milder outcome over longer period of time) compared with DMM (more cartilage degradation). Based on this the number of animals used is significantly different (12 vs 6, respectively).

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

We have been generating genetically modified animals for over 20 years and have developed experience in this type of experiments as illustrated by our publications. However, when we embark on identification of a new enhancer for a particular gene, we rely on our extensive experience until we establish a measurable and distinctive sets of numbers. This is because each enhancer works differently. In these cases, we do not have a power calculation. In other experiments, we have provided a power calculation for protocols 6 and 7 based on published results. For example, the LRP-1 x Prx1 Cre, the phenotype was obvious, we only needed three mice to verify the phenotype. The steps we have taken to reduce the number of animals is to follow the protocol generated by the experimental design without any deviations, everytime an experiment is conducted.

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

In aim 1, we use our extensive experience in determining what an enhancer is based on in silico using ENCODE and expression pattern. An enhancer in principle is able to express irrespective of location or orientation of DNA, but it is copy number dependent. Injection of naked plasmid DNA into a fertilised egg is random. In other experiments, we have

provided power calculations in order to obtain the minimal number of mice that allow us to show differences between test and control groups.

A retrospective assessment of reduction will be due by 10 February 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 do use embryos to identify enhancer expression during development and in specific lines where the expression is tissue or cell specific, we generate adult mice to verify that the enhancer remains active in adulthood. In some cases, such as in animals expressing MMP13 or other enzymes that degrade the matrix, animals are subjected to OA injury models as described below.

Since OA is an ageing disease, we have adopted the loading of joints as an accelerated ageing to minimise the long term suffering of the mice through their life course. In addition, recent experiment had shown that injury and inflammation may ultimately lead to OA pathology in 50% of those who are injured (Watt et al ARTHRITIS & RHEUMATOLOGYVol. 68, No. 9, September 2016, pp 2129–2140).

Therefore the destabilisation of medial meniscal ligament (DMM) model has been adopted to answer this type of OA. In both models, we have also added a digigait which allows us to remotely monitor the mice and pick up on lack or altered movement in mice that have undergone wither of the injuries.

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

Animals that are less sentients do not have joints similar to human. In addition, we need adult mice because we need to replicate osteoarthritis which only occur late in life.

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

We will always try to provide alternatives in route of administration of substances: for example, through food or drinking water rather than injection. Provide analgesic following surgery and increased monitoring and weight measurement to ensure animals are recovering. Terminate experiments when new unexpected phenotype occurs or consult local vet in case of scientific importance.

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

In the scientific community that work on animals , we all follow as the Animals (Scientific Procedures) Act 1986 (ASPA) and guidance. New development that focuses on "lessons learnt" disseminated by the NC3R website, LASA recommended maximum volume of administered substances and guidelines for blood removal as well as intervals between imaging sessions (LASA appendices A-C).

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

As an animal user, I currently receive updates from the NC3R through emails and the website and through the meetings held locally by the NC3R regional programme manager.

A retrospective assessment of refinement will be due by 10 February 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. Neuroinflammatory diseases in the central nervous system

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

Neuroinflammatory Disease, Multiple sclerosis, Autoimmunity, Neurodegeneration, Genetic susceptibility

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

The aim of this project is to identify and understand the role of genes and disease pathways that contribute to the risk of developing neuroinflammatory diseases, such as

multiple sclerosis. Importantly, our research aims to understand the pathways that lead to progressive disease and how this can be inhibited or slowed using novel, safer treatments.

A retrospective assessment of these aims will be due by 04 February 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?

Developing new treatments for progresssive neuroinflammatory diseases are hampered by a lack of understanding of those genes and pathways that cause and drive disease. These studies aim to untangle the complexity of such pathways in a manner that cannot be recapitulated by either in vitro or patient-based research, alone.

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

The considerable delay in diagnosing and beginning treatment for patients with neuroinflammatory diseases, including multiple sclerosis (MS), is caused by the fact that patients present with a wide range of symptoms and degrees of severity at onset. As such, a conservative, reactive, treatment approach is taken so that potent, toxic drugs are reserved for later in the disease when symptoms have worsened. Unfortunately, this strategy, while considered the safer option at present, means that large numbers of patients develop severe disability, quickly, with current treatments unable to stop its progression. Growing evidence suggests that early treatments are the most likely strategy in which to prevent neurodegeneration, but without better tools to diagnose disease and understand causal pathways that may be targeted therapeutically at disease onset, current treatment strategies and the range of drugs available will remain limited.

This study aims to unlock some of these unknown causal pathways that are present at disease onset that initiate the cascade of events that lead to neuroinflammation and neurodegeneration. By using genetic insight and data from patient studies, we will test whether genetic modifications or drugs that aim to prevent or inhibit these identified pathways or cell types/subsets can slow or stop progressive disease. Targeting specific pathways or cell subsets using new treatments aims to provide safer and less immunotoxic options for patients at the beginning of the disease and thereby inhibit neurodegeneration before it becomes untreatable.

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

Short-term benefits: Having identified and characterised the role of a protective MS gene polymorphism in our previous project licence, which is also protective against developing at least nine other autoimmune diseases, we now aim to test whether a small molecule inhibitor that mimics this genetic modification (developed by our industrial collaborator), is protective in an MS mouse model.

Our aim is use these new findings to establish a basis on which to take this forwards into patients. The drug, which was developed based on our previous research, has already been trialled in psoriasis patients with huge success. We are therefore confident that our data will provide valuable insight into its potential use for MS patients in the immediate future.

Long-term benefits: As exemplified above, understanding genetic risk factors that predispose to disease creates new insight and strategies of how to treat patients with more specific and effective treatments that may help to prevent neurodegeneration in the longer term. Having developed in vitro models in which to identity causal cell subsets and pathways using human samples by integrating data from genetics studies, we now aim to test how these pathways function and drive disease using our in vivo models. These findings will complement data that we obtain from patient samples (peripheral blood, cerebrospinal fluid (CSF) and post-mortem brain tissue, thereby providing a comprehensive understanding of potential new options for treating patients in the future.

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

Outputs of this work will be published in peer reviewed journals. This work will also be presented at local and international conferences as well as with our academic and industrial collaborators. Our aim is to translate this work into new treatment options for MS patients, while providing new insight and data repositioning to support the treatments for other neuroinflammatoy diseases.

Species and numbers of animals expected to be used

- Mice: 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.

Neuroinflammatory diseases are hugely complex, involving both peripheral immune responses, which travel into the brain and spinal cord, as well as involving changes that occur within discrete structures of the central nervous system. As such, mice provide a valuable model in which to understand how all of these processes are connected and how one destructive event may lead to another. As our neuroinflammatory disease model will

aim to mimic MS (as well as neuromyelitis optica (NMO), to a degree) which typically develop in young adults, our study will use adult mice. Ageing mice or infant mice will not be required for this research.

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

Genetically modified animals will be bred and maintained under a mild breeding protocol. Unless moved to protocol 2, all animals under protocol 1 will be mild or sub-threshold.

Genetically modified mice or wild-type animals, will be immunised to developed experimental autoimmune encephalomyelitis (EAE) under Protocol 2. Induction will be performed under short-term general anaesthesia (typcially <5 min) and will be injected by the subcutaneous and intravenous routes. Animals will develop a progressively disabling disease, experiencing complete hind limb paralysis, while retaining use of the forelimbs. Movement around the cage to access food and water will be possible at all times. Animals will be treated at symptom onset with drugs designed to reduce disease symptoms (or with a placebo) either once or on a once-daily basis. The drug will be administered orally in most cases. Animals will typically be monitored for up to 30 days. Full hind-limb paralysis is likely to last approximately 4 days with up to 20% weight loss experienced. Animals will be killed at the end of the experiment, with full recovery from symptoms within the experimental period unexpected in the placebo group.

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

Most mice will be bred and maintained under a mild protocol and therefore are expected to develop no adverse symptoms. Mice which are allocated towards experiments where they develop MS-like disease will become partially disabled, typically experiencing complete loss of the ability to use their hind legs for an average of 4 days. In some cases, animals may experience weight loss of up to 20% within this time. In a small number of cases (<10%), mice will have relapsing-remitting, full or partial hind leg paralysis for longer periods of time (up to a maximum of 140 days, with no longer than 5 consecutive days of full hind-limb paralysis at any one time) in order to investigate the progressive, chronic stages of disease. Most experiments will last no more than 30 days (with an upper limit of 140 days when using a relapsing-remitting model; in this instance, animals also experience phases or 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)?

Under protocol 1 - 100% of animals will have a mild severity (or sub-threshold). Under protocol 2 -

90% of animals immunised will be on the C57Bl/6 background and have a disease duration of approximately 30 days. 50% of these animals will experience full hind limb paralysis (severe category) and 50% will be immunsed with a control antigen and not develop disease (moderate severity).

10% of animals immunised will be on the Biozzi background and have a disease duration for up to 140 days. Again, 50% of these animals will experience full hind limb paralysis, as well as experiencing disease relapses (severe severity) and 50% of these animals will be immunsed with a control antigen and not develop disease (moderate severity).

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

Killed

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

Neuroinflammatory diseases are hugely complex, involving both peripheral immune responses, which travel into the brain and spinal cord, as well as involving changes that occur within discrete structures of the central nervous system. As such, mice provide a valuable model in which to understand how all of these processes are connected and how one event may lead to another. The research conducted under this licence aims to complement the wealth of data that we are collecting from multiple patient cohorts, for example, patient samples from those experiencing early neuroinflammatory symptoms (eg, optic neuritis), MS and NMO patient samples undergoing different treatments, post-mortem brain tissue and control human samples with specific genotypes. No singular experimental model can complete our understanding of complex neuroinflammatory diseases in isolation and, at present, the animal models used here form an integral part of our effort to answer questions that cannot be answered through other resources.

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

We have established a number of in vitro assays to recapitulate a number of experiments that will no longer need to be performed in animals, including understanding gene functions using CRISPR-editing and through the recruitment of donors by their specific

genotypes. We have also established a number of resources in which to obtain patient samples in which to obtain data. The development and inclusion of new technologies, such as single-cell analysis, spatial transcriptomics and CRISPR has allowed for many animal experiments to be replaced with other methods and we will continue to strive towards replacing the use of animals even further, as soon as it becomes possible.

Why were they not suitable?

Access and interrogation of longitudinal brain and spinal cord tissue, which is reacting to a peripheral attack by the immune system cannot be modelled by any other method, at present. Thus, a void in our understanding of critical interconnecting pathways would be lost without the use of animals for this research.

A retrospective assessment of replacement will be due by 04 February 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 protocol 1, our breeding strategy will be in line with standard breeding protocols for maintaining inbred strains, with routine backcrossing performed to maintain integrity of the genetic background. The numbers of animals estimated are based on breeding 2 different transgenic lines in parallel.

For protocol 2, our use of each EAE model will be determined by prior results obtained from patients or control studies, that generate a specific hypothesis in which to test. On prior experience, we know that disease incidence will be achieved in 90% of animals and 1 in 10 animals will likely need to be culled before the experiment end, due to either exceeding weight loss limits or due to developing symptoms beyond the limits of the licence. To monitor the effectiveness of a new drug, we will use knowledge from our prior studies to determine the number of animals required for each group, considering percentage disease incidence and likely termination rates, that we have collated over many years.

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

We have extensive experience using EAE models to test both drug efficacy as well as the impact of specific genetic variants. We will use this prior experience to determine the likely success of subsequent experiments under this licence that along similar lines, thereby increasing our likelihood of a successful outcome. In addition, some of our research will be performed on a collaborative basis with an industrial partner who will be performing separate, complementary experiments to those performed under this licence. Therefore, we will eliminate the need to replicate experiments in multiple sites and will regularly share our results with our collaborators so that experimental designs are as streamlined as possible.

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 will be achieved by using a rodent tracking database for all animals bred and maintained under this licence.

We will share tissue from our experiments with collaborators, and vice versa, to reduce total animal usage as much as possible. When more than one experimental drug requires testing for treatment efficacy, for example, we will aim to perform this as one experiment, such that the same control group can be used.

Transgenic lines that are new will first be analysed in pilot studies, where 3 animals per group will be used for standard phenotyping. For EAE studies, this will be increased to 4 animals per group, so that if only 90% disease incidence is achieved, we still achieve 3 animal data points to determine experimental efficacy.

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

Short-term experiments (~30 days) using the C57BL/6, EAE model will be performed preferentially to long-term experiments (~60-140 days) using the Biozzi ABH, EAE model, for the majority of experiments. Our aim is to ensure that the cumulative suffering of any animal is as minimal as possible in order to sufficiently test the experimental question.

Induction of EAE will be performed under general anaesthesia (lasting ~ 5 min) to ensure accurate and aseptic subcutaneous placement of reagents, thereby reducing the risk of ulceration at the injection site.

All animals that experience complete hind limb paralysis (usually for no more than for 4 consecutive days), will retain continuous locomotive capabilities and housing will be adapted to ensure continuous access to food and water; additional food and water supplements will be placed in the cage throughout the experiment. Animals will be monitored twice daily during this period and weighed once daily throughout the experiment.

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

Modelling neuroinflammatory and neurodegenerative processes to understand causal pathways and develop new treatments for patients requires an immune system and central nervous system that closely reflects that of humans. Physical assessment of mice that are affected by central nervous system damage is possible by visible changes in their gait and progressive disability that mirror those symptoms seen in patients. A lower sentient animal will not exhibit such features, nor will its immune response and subsequent impact on the central nervous system provide the clinical insight needed in order to translate this into the clinic.

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

Male mice typically experience a more progressive disease than females and, furthermore, incidence of fighting post immunisation is also common, requiring individual housing, in most cases. Therefore, for the majority (>90%) of experiments, only female mice will be used to reduce overall disease severity and to ensure that animals can be housed together in small numbers (typically 4 animals per cage).

However, for final, conclusive experiments. For example, when determining the efficacy of a new drug for use in clinical trials, an equal number of male and female animals will be used in the final analysis. This will ensure that we can detect any gender bias in our results, and explore/consider these where necessary, before publishing and/or moving into clinical trials.

Access to supplementary food and water, which is unfamiliar to them, will be added at the start of the experiment. Our previous experience has shown that this is more effective at reducing excessive weight loss in comparison to only giving this to when it is necessary, assumingly as it gives them time for it to become part of their routine diet.

In the last 3 years we have ordered our EAE immunisation reagents pre-mixed from a commercial source, which achieves a more uniform disease across animals compared to being made in-house. Reducing the standard deviation of clinical symtpoms within each experimental group increases reduces the number of total animals required per group; this will therefore be our prefered option, whenever possible.

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

General guidance will be sought from published documents by the Laboratory Animal Science Association (LASA), the NC3R's and by following the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines.

Information about about further developments when using our research models will also be sought from peer-reviewed journals, close collaborations with local and international groups who use these models and working closely with companies who manufacture the reagents required to induce disease.

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

Our establishment has a local 3Rs committee who report new developments on a termly basis. Collaborations with local and international partners who also use this model will allow for progressive changes that reduce the cost to the animal, whenever possible.

A retrospective assessment of refinement will be due by 04 February 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. Quality control testing of clinical products

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

Regulatory requirement, pharmaceutical, medicinal products, safety assessment

Animal types	Life stages
Mice	adult
Guinea pigs	adult
Rabbits	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 demonstrate safety and potency of pharmaceutical products and vaccines manufactured and tested to comply with regulatory requirements.

A retrospective assessment of these aims will be due by 09 February 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 project relates to the quality, safety assessment and potency testing of the medicinal products. These products are manufactured to prevent the onset of severe infectious diseases by either producing an immune response to vaccination or by passive immunity by transferring antibodies from a pool of human sera.

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

We provide a service to test specialist clinical products made to prevent life threatening human infections. This project ensures that pharmaceutical products requiring highly specialised facilities for regulatory and developmental animal testing are available in the United Kingdom. We provide a service to test vaccines and antitoxins made to prevent life threatening human infections to ensure they are safe for people to use and that they work.

The testing proposed in this licence application is to release new batches of product and check that the product works correctly during its shelf life. Animals are only used to test pharmaceutical products where there is no alternative test which meets regulatory requirements.

We also intend to validate an alternative potency assay which uses antibodies raised to the vaccine and the antibodies are then used in a cell culture to neutralise the disease toxins.

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

Specialist vaccines and specialist biological products are tested for both safety and potency. The public health benefits of these vaccines are substantial, providing protection from infection and outbreaks of lethal pathogens. Validating the alternative potency assay will allow this new test to replace the existing potency assay which is the one authorised by the regulatory authority. The alternative assay uses mice instead of guinea pigs, reduces the severity to mild and reduces the numbers of animals required.

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

All work performed under this licence under regulatory control. All testing performed is for the Quality, Safety and Efficacy of pharmaceutical products for human use. The testing proposed in this licence application is to release new batches of product and check that the product works correctly during its shelf life. Animals are only used to test

pharmaceutical products where there is no alternative test which meets regulatory requirements.

Species and numbers of animals expected to be used

- Mice: 10,450
- Guinea pigs: 12,380
- Rabbits: 200
- 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.

All work performed under this licence under regulatory control. The choice of species is described within the required Authority which states precisely how the pharmaceutical products are tested. These are robust tests which provide reliable results enabling the manufacture of safe and effective pharmaceutical products. The numbers of animals used are proportional to the number of batches which require testing, although more testing may be necessary if a product becomes in demand for public health needs.

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

Injections of various dose levels of samples (vaccines):

Protocol 1 - One injection, monitored for 14 days

Protocol 2 - One injection, monitored for 10 days

Protocol 3 - Five injections over 10 days. One injection on Day 17, monitored for additional 10 days (28 days total)

Protocol 4 - Eight injections, 24-hour test

Protocol 5 - One injection, monitored for 7 days

Protocol 6 - One injection, monitored for 7 days

Protocol 7 - One injection, monitored for 7 days

Protocol 8 - Six injections, monitored for 48 hours

Protocol 9 - Two injections over 14 days (Day 0 and Day 14), monitored for further 14 days (28 days total)

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

Surviving animals will be euthanised at the end of the test before safe destruction at the facility. These are hazardous infectious agents and are therefore controlled to prevent exposure or release.

During welfare checks indicators such as appearance (e.g. unkempt fur/coat, orbital tightening, discharge), behaviour (e.g. isolation/withdrawn, posture and mobility), body functions (e.g. respiration accelerated/laboured) and environment (e.g. presence of blood, are animals using enrichment items/material/nesting) are monitored. The main harms identified in clinical signs for this infectious disease are ruffled fur, eye watering, fever and abscess which is not known to cause discomfort. These are serious infectious diseases where some animals may not survive, but rigorous procedures are in place to prevent suffering. Accordingly, a severe severity limit applies to this type of work. The humane end point is applied where any animals are found to be showing significant adverse effects - persistent immobility - of the infection by using the above indicators and will be culled by appropriate Schedule one method.

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 animals are administered with human pharmaceutical products to determine that they are safe and potent. The safety protocols aim to confirm the product is safe with a representative dose. Some of the protocols determining potency use toxin producing bacteria. There is a rare chance that there could be a complication with toxin neutralisation and precautions are taken to ensure this does not occur. Some animals may develop clinical signs which indicate that they will not survive. We will always try to humanely euthanise these animals before natural death occurs and the criterion used is described in this licence. These are serious infectious diseases where some animals may not survive, but rigorous procedures are in place to prevent suffering. Accordingly, a severe severity limit applies to this type of work.

Protocol 1 - Mice - Severe category - 40% expected to develop severe clinical signs per test. The humane end point is defined as any animal not able to turn itself back onto its feet when inverted on a firm surface.

Protocol 2 - Guinea Pigs - Severe category - 65% expected to develop severe clinical signs per test. The humane end point is defined as any animal not able to turn itself back onto its feet when inverted on a firm surface.

Protocol 3 - Guinea Pigs - Severe category - 65% expected to develop severe clinical signs per test. The humane end point is defined as any animal not able to turn itself back onto its feet when inverted on a firm surface.

Protocol 4 - Rabbits - Mild category - 100% per procedure. No adverse effects are expected. Protocol 5 - Mice - Mild category - 100% per procedure. No adverse effects are expected.

Protocol 6 - Guinea Pigs - Mild category - 100% per procedure. No adverse effects are expected.

Protocol 7 - Mice - Moderate category - This procedure has a moderate severity limit as this test will only be performed following a potential safety issue with an Protocol 5 and/or 6 - 40% per procedure. No adverse effects are expected.

Protocol 8 - Guinea Pigs - Mild category - 100% per procedure. No adverse effects are expected. Protocol 9 - Mice - Mild category - 100% per procedure. No adverse effects are expected.

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

Killed

A retrospective assessment of these predicted harms will be due by 09 February 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?

Animal use is for releasing new batches of product and testing product already released to the market when there is no alternative currently available. The tests are prescribed by the required Authority for these products.

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

We have been able to remove all animal tests for two of our products during the current licence and will continue the application of the 3Rs principles wherever possible as new techniques become available. There are no regulatory/approved non-animal alternative methods that can be used in this project.

There is good communication between regulatory bodies, such that any changes to regulations would be discussed and implemented.

Why were they not suitable?

The tests are prescribed by the required Authority for these products.

A retrospective assessment of replacement will be due by 09 February 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 provided are based on previous years' numbers and taking into account the potential for increased testing.

The numbers of animals used are proportional to the number of batches which require testing, although more testing may be necessary if a product becomes in demand for public health needs. If product comes under greater demand, the final release testing with increase and additional batches will undergo stability testing therefore increasing the number of animals required.

Completing the validation for the alternative assay (Protocol 9) may require more animals in the short term if the additional 5 studies are required, however there will be fewer required in the medium and long term if this new assay is successful.

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

The choice of species is described within the required Authority which states precisely how the pharmaceutical products are tested.

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 used for each test is defined by regulations and, where possible, testing is grouped to minimise the number of control or standard animals used. If the alternative toxin neutralisation potency assay is successful fewer animals will be required in the future.

A retrospective assessment of reduction will be due by 09 February 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 choice of species for animal testing is dictated by the required Authority. These are robust tests which provide reliable results enabling the manufacture of safe and effective pharmaceutical products.

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

The choice of species for animal testing is dictated by the required Authority for these products. The disease progression is relatively predictable and enables us to determine clinical signs as an end point to reduce severity, enabling the humane euthanasia of individual animals once a terminal decline has been recognised.

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

The species of animals and the methods to be used in regulatory tests are stated in the relevant documentation therefore must be adhered to.

Relevant monitoring of procedures and appropriate euthanasia will ensure that suffering is kept to a minimum.

Wherever possible, animals are housed in compatible social groups and given environmental enrichment appropriate for the species.

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

The tests are prescribed by the required Authority for these products. These are robust tests which provide reliable results enabling the manufacture of safe and effective pharmaceutical products. We will continue the 3Rs principles wherever possible as new techniques become available.

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

We are in the process of replacing a potency assay which if successful will reduce the severity from severe to mild and fewer animals will be used.

The NC3Rs, ECVAM and FRAME websites are regularly monitored and as potential alternatives/advances become available they are investigated to determine if they are suitable as replacement tests for specific products which would be acceptable to the licensing authorities.

A retrospective assessment of refinement will be due by 09 February 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 in vitro medical diagnostic reagents

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

Sterile Blood, agar plates, vaccine production

Animal types	Life stages
Horses	adult
Sheep	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?

The aim is to produce high quality blood products for diagnostic, clinical and research use.

A retrospective assessment of these aims will be due by 05 February 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?

Animal blood provides the growth factors for a wide range of bacteria and is therefore vital for rapid diagnosis of infection in humans and animals.

Horse serum is a necessary component in the production of some vaccines.

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

Listed below are the benefits from each of the products produced.

Several hundred hospitals, universities and research institutes and large plate manufacturers throughout the UK, Europe, Middle East, Africa and Asia are supplied with their requirement for blood and serum products for use in culture media and vaccine manufacture to benefit human and animal health.

Plates facilitate rapid diagnosis and therefore rapid treatment in both the medical and veterinary fields. Patients, clinicians, health and veterinary authorities will benefit from this work. These benefits will be achieved almost immediately and throughout the life of the project licence and for the foreseeable future.

Horse blood free of Transmissible spongiform encephalopathies (TSE) is essential for some vaccine production and has been validated by the National Health Service (NHS) and other European Union (EU) medical services for use primarily in the United Kingdom (UK) for microbiological diagnosis. There are at this time no viable alternatives for this essential product.

1. Horse and sheep blood is used daily for growing bacteria for diagnostic purposes in microbiology laboratories.

2. Blood which has been heat-treated is an essential supplement for media used for the cultures of many nutritionally fastidious bacteria such as Neisseria meningitidis, Haemophilus influenza, Bordetella species and Neisseria gonorrhoeae which are responsible for serious conditions such as meningitis, sepsis, whooping cough and sexually transmitted diseases.

3. Horse serum - donor serum, a product of sterile blood, is also required by pharmaceutical companies for the manufacture of a wide variety of vaccines for human and animal health. Its freedom from viral and bacterial contamination makes it essential in some vaccines. Serum from Specific Pathogen Free animals is being recommended by the EMEA (European Agency for the Evaluation of Medicinal Products) for use in vaccine production, as the problems with newly discovered pathogenic viruses become apparent to

the Regulatory Authorities. Donor Horse Serum, free of Bovine Spongiform Encephalopathy (BSE), for instance has become essential in some vaccine production. Cattle could not be considered for this purpose due to the UK's potential risk from BSE.

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

Wherever in the world clinical microbiological investigations are carried out, the characteristic appearance of bacterial colonies on agar plates, supplemented with horse or sheep blood is a universally accepted and recognised first stage of the isolation and identification process.

Several hundred hospitals, universities and large plate manufacturers throughout the UK, Europe, Middle East, Africa and Asia are supplied with their requirement for blood and serum products for use in culture media and vaccine manufacture to benefit human health. These products can also be used to develop techniques for the analysis of blood in both human and animal health including disease investigation.

The great majority of the blood is used for agar plate production but horse serum, a bi-product of horse blood production, is also supplied for clinical applications and vaccine production. Horse serum is a component of many clinical test systems and is used in microbiology laboratories as a culture media supplement for otherwise difficult to grow bacteria.

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

The farm strives to provide a sterile product of the very highest quality.

Species and numbers of animals expected to be used

- Sheep: 3500
- Horses: 420

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.

Wherever in the world clinical microbiological investigations are carried out, the characteristic appearance of bacterial colonies on agar plates, supplemented with horse or sheep blood is a universally accepted and recognised first stage of the isolation and identification process.

Animal blood provides the growth factors for a wide range of bacteria and is therefore vital for diagnosis and the need for absolute sterility, blood of the highest quality, for diagnostic

purposes makes the choice of large healthy donor animals essential. It is difficult to obtain sterile blood from abattoirs.

Horses have a close relationship with humans which limits any stress during the process. Also being large not so many are needed to produce the required product. Historically laboratory technicians in the UK and Northern Europe have always used horse blood as a nutritional supplement in microbiological culture media and the characteristic appearance of the bacterial colonies on horse blood agar plates is a universally accepted and recognised first stage of the isolation and identification process. Because horse blood is more nutritious than sheep blood it can isolate fastidious organisms that blood from other species cannot. Horse blood allows detection of haemolytic reactions and supplies both the X factor (heme) and the V factor (nicotinamide adenine dinucleotide, NAD) necessary for the growth of many bacterial species including Haemophilus influenza, which requires both the X and V factors. On horse blood plates, colonies tend to be larger and growth is more luxuriant than on media containing other blood types making diagnosis of clinical specimens more reliable and assured. It should be noted that beta-haemolytic reactions depend on the type of blood added, as an example enterococci which only very rarely haemolyse sheep blood, will produce a well visible beta haemolysis on horse blood.

Staphylococcus aureus however, which is usually beta haemolytic on sheep blood, will often be non- haemolytic on horse blood. Specific organisms have highly specific nutritive requirements.

Horse blood free of TSEs is essential for some vaccine production and has been validated by the NHS and other EU medical services for use primarily in the UK for microbiological diagnosis. Cattle cannot be considered due to the potential risk of carrying BSE, therefore there is no viable alternative for this essential product.

The haemolytic reactions of horse and sheep blood are not identical. Blood agar media specifically designed for horse blood may not be satisfactory with sheep blood and vice versa.

Typically horses aged 5+ and sheep aged 2+ are considered as adult stage and have been used to significant handling and human interaction. This interaction reduces the levels of stress incurred in handling and during blood donation and therefore makes them an ideal choice for this project.

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

Aseptic withdrawal by venepuncture of horse blood up to a maximum of 8 litres or 12% of blood volume is taken twice monthly. A continued monitoring of the red blood cell count (PCV) shows that this volume and frequency has no detrimental effect on the health and wellbeing of the horse. The sheep at three week intervals, although giving a greater volume up to a maximum of 1.5 litres or 15% of blood volume, benefit from less handling with the extended interval between blood donation. A continued monitoring of red blood cell count (PCV) shows us that this volume has no detrimental effect on the health and

general wellbeing of the sheep. The animals can continue for reuse at the same frequency until, due to old age and/or loss of condition, they are no longer suitable. This decision is made after consultation between the NACWO and the NVS.

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

Experience of 55 years has shown that horses and sheep rarely have any ill effects or secondary injuries from the above procedure. However, possible side effects could be haemorrhage or infection at the site of the needle insertion but in practice this is extremely rare and would be treated accordingly.

This is prevented by appropriate preparation of the site (clipping, cleaning and disinfecting) and the use of sterile single use hypodermic needles.

A slight lowering of the red blood cell count could be considered an adverse effect on the horses and sheep but this is continually monitored throughout the protocol. Rest periods are implemented if this occurs. This only happens very occasionally, at the end of the long winter period when animals are housed and therefore restricted in their exercise and have had a long period without fresh grass.

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 procedures are categorised as mild.

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

- Killed
- Kept alive

A retrospective assessment of these predicted harms will be due by 05 February 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?

1. Horse and sheep blood is used daily for growing bacteria for diagnostic purposes in microbiology laboratories.

Animal blood is used as an alternative to human blood in biological and haematological investigations.

Horse blood free of Transmissible spongiform encephalopathies (TSEs) is essential for some vaccine production and has been validated by the National Health Service (NHS) and other European Union (EU) medical services for use primarily in the United Kingdom (UK) for microbiological diagnosis. There are at this time no viable alternatives for this essential product.

2. Blood which has been heat-treated is an essential supplement for media used for the cultures of many nutritionally fastidious bacteria such as Neisseria meningitidis, Haemophilus influenza, Bordetella species and Neisseria gonorrhoeae, responsible for serious conditions such as meningitis, sepsis, whooping cough and sexually transmitted diseases.

3. Horse serum, a product of sterile blood, is also required by pharmaceutical companies for the manufacture of a wide variety of vaccines for human and animal health. Its freedom from viral and bacterial contamination makes it essential in some vaccines. Serum from Specific Pathogen Free animals is being recommended by the EMEA (European Agency for the Evaluation of Medicinal Products) for use in vaccine production, as the problems with newly discovered pathogenic viruses become apparent to the Regulatory Authorities. Donor Horse Serum, free of Bovine Spongiform Encephalopathy (BSE), for instance has become essential in some vaccine production.

There is no viable alternative because cattle cannot be considered due to the potential risk of carrying BSE.

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

There is no viable alternative.

Why were they not suitable?

The difficulty of replacing animal blood with a synthetic source is that whilst the growth support of bacteria might be replaced chemically, the reaction of the cell envelope in haemolysis is vital in diagnostics. Therefore, although artificial blood is being developed, it does not carry the required growth factor for a wide range of bacteria and in addition would not define bacteria as haemolytic or otherwise.

A retrospective assessment of replacement will be due by 05 February 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 farm works closely in conjunction with its customers to ensure that annual forward forecasting of blood requirements is as accurate as possible. This helps us to maintain the correct number of animals on the farm, allowing for fluctuations in orders and periods of rest and recuperation as required by the individual animal. The minimum number of animals for 2020 was 400 horses and 3000 sheep but the current requirement for 420 horses and 3500 sheep ensures a much better level of care, refinement and general wellbeing than if we were to reduce these numbers to the bare minimum.

Six herds of horses and six flocks of sheep are re-used in rotation and with this familiarisation of the process they become increasingly relaxed the longer they stay on the farm. The extended rest periods limit any stress to the animals that might be caused by more frequent blood withdrawals and this stress free environment is good for the welfare of the animals and the essential high quality of the product.

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

Animal blood provides the growth factors for a wide range of bacteria and is therefore vital for diagnosis and the need for absolute sterility, blood of the highest quality, for diagnostic purposes makes the choice of large healthy donor animals essential. It is difficult to obtain sterile blood from abattoirs.

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

The minimum number of animals for 2020 was 400 horses and 3000 sheep but the current requirement for 420 horses and 3500 sheep allows for suitable rest periods, sickness and unforeseen spikes in weekly blood volume requirements which will ensure a much better level of care, refinement and general wellbeing than if we were to reduce these numbers to the bare minimum.

A retrospective assessment of reduction will be due by 05 February 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.

Horses are herd animals, interaction within a herd is very important for minimisation of stress. On arrival on the farm horses are quarantined in smaller social groups where they will form their close knit friendships which in many cases will continue throughout their life on the farm. The introduction into herds of these small groups of new horses always takes place just after Spring turn out giving maximum time for these new horses to become established and to settle within the larger group. The herd into which these horses are introduced will remain their herd for life. This is very important for their hierarchy within the herd. During this time in the fields, they learn the herd routine and the hierarchy system. In Winter all herds are housed in barns with large open yards. They have extensive areas where they are fed and can exercise including sand areas where they love to roll. The yards and barns are fed and cleaned daily without the necessity of ever restricting the access to these large areas.

Should a horse need to be isolated for individual treatment as a result of illness or injury, the treatment bays are adjacent to the herd and large enough to include their closest friend. We aim never to have a horse isolated and unable to have social interaction with another. This is very important for a herd animal and minimises stress associated with individual stabling.

Horses have a close relationship with humans which limits any stress during the process. Also being large not so many are needed to produce the required product.

The farm employs a dedicated shepherdess. Sheep are quarantined in small social groups before being introduced to the flocks. This helps them to form a friendship bond and minimises stress. Sheep flocks have a pattern of grazing the same pastures to enable them to become familiar with their surroundings which also reduces stress. This routine also applies to their winter housing which is in large ventilated barns with open air exercise yards.

Experience of 55 years has shown that horses and sheep will have no ill effects from the frequency and quantity of blood volumes withdrawn. This is further substantiated by the continuous monitoring and testing of the red blood cell count.

Horse blood is required for use in the northern part of Europe whilst in southern Europe sheep blood is used. It is important that laboratories use the same species of animal blood as identification of some bacterial colonies varies with the species of blood used. The haemolytic reactions of horse and sheep blood are not identical and blood agar media designed for horse blood may not be satisfactory with sheep blood and vice versa.

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

The need for absolute sterility in the use of blood for diagnostic purposes makes the choice of large healthy donor animals essential. In comparison with small animals, blood donation subjects horses and sheep to minimum stress and reduces the number of donors involved.

Horses have a close relationship with humans and, therefore, those that have been previously used in recreation are generally well handled and used to standing quietly during the donation process. This makes them an ideal species to use for quick aseptic blood withdrawal to prevent clotting of the blood.

Horse blood free of TSEs is essential for some vaccine production and has been validated by the NHS and other EU medical services for use primarily in the UK for microbiological diagnosis. Cattle could not be considered for this purpose due to the UK's potential risk from BSE. There are at this time no viable alternatives for this essential product.

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

Horses and sheep stay on the farm for many years and the 485 hectares of extensive grazing and housing allow for natural and stress free herd/flock environment and behaviour.

On arrival, new animals are quarantined in small groups, for approximately two weeks, prior to their release in the same small groups into the herd/flock system when they will become part of the donor rotation. These friendship groups often remain intact through the animal's whole life on the farm and make a big difference to the stress free environment that the farm is promoting. The welfare of the animals, their quality of life and our animal husbandry are the top priority. The herd/flock system remains intact throughout the winter period, which is November to April, when the animals are housed in big barns and fed in surrounding large open yards. Animals have complete freedom of movement between the barns and the yards where food is continually available. All winter food and bedding is home produced (grass silage, whole crop silage, oats and hay). This helps to maintain a healthy diet at all times.

Our experience of 55 years and our monitoring and recording of the PCV of each animal after donation has proved/shown that the minimum interval between donations (two weeks for horse and three weeks for sheep) has no detrimental effect. This interval is often much longer as we always keep an excess pool of both species so that donations can be tailored to the individual and therefore extra rest periods can be given if necessary.

We operate a continual monitoring policy before, during and after the procedure throughout summer and winter housing to ensure that all animals demonstrate natural behavioural traits and are fit and healthy. Any animal showing adverse effects or signs of ill health will be isolated within their immediate friendship group, to be monitored and cared for as is appropriate. The named animal care and welfare officer (NACWO) and named veterinary surgeon (NVS) will be involved with this process.

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

Home Office Code of Practice for the Housing and Care of Animals Bred, Supplied or Used for Scientific Purposes.

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

We review and discuss all documents published by AsPEL. The named persons meet regularly to review and discuss published policy and changes to legislation. We regularly attend farming conferences and shows to further our knowledge of cropping, grazing, housing and handling equipment. We also subscribe to farming and animal health publications to keep abreast of any developments in areas of interest. Proposed refinements are highlighted and any training requirements will then be implemented at the establishment.

A retrospective assessment of refinement will be due by 05 February 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. Treatments and interventions for heart disease and ischaemic-reperfusion 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

Key words

Heart disease, stents, coronary heart disease, heart valves, Ischemic-reperfusion injury

Animal types	Life stages
Sheep	juvenile, adult
Pigs	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?

To develop and evaluate treatments and interventions for heart disease and cardiac ischemic- reperfusion injury.

A retrospective assessment of these aims will be due by 09 February 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?

Heart disease is the most common cause of failing health and death in people living in the developed world. In the UK an estimated 7.6 million people suffer from the disease and over 160,000 die annually as a direct consequence.

A wide range of intravascular implants, such as stents, valves and cardiac assist devices, are used in the treatment of heart disease. It is well recognised that, whilst initially highly effective, many of the currently available devices have either: a limited life expectancy, necessitating periodic replacement, or require the patient to take long term medication or are prone to evoking localised tissue reactions that can compromise their effectiveness. In an endeavour to overcome these limitations, medical companies are developing new devices based on modified or novel designs. Prior to introduction into human clinical practice the safety and effectiveness of such devices has to be demonstrated in a representative animal model. A primary purpose of the outlined studies is to facilitate this evaluation process.

Foremost amongst the causes of heart failure is coronary heart disease. This condition is characterised by the progressive blockage of arteries supplying blood to the heart, starving its tissues of oxygen (ischaemia) and predisposing to the development of lesions within the heart muscle, i.e. myocardial infarctions, and heart attacks. The treatment of severe coronary heart disease is based upon improving blood flow to the heart muscle, using both medical and surgical interventions however, the loss of functional heart muscle caused by a myocardial infarction permanently impairs cardiac function and results in a shortened life expectancy. As a consequence, there is an urgent unmet clinical need to find ways to restore normal heart function following myocardial infarction.

Ischaemic reperfusion injury to the heart occurs commonly during cardioplegic arrest. This technique is used during open heart surgery to stop and protect the heart to enable a surgical repair to be undertaken safely. In the UK approximately 50,000 children and adult patients undergo this procedure each year. Whilst the heart is stopped, the circulation of blood to the patient's vital organs is maintained using a cardiopulmonary bypass machine however, the blood supply to the heart is shut off. Although measures are taken to protect the tissues of the heart during cardioplegic arrest, these are at best only partially effective at preventing ischemic-reperfusion injury. A primary purpose of the outlined studies will be to evaluate drugs and interventions aimed at preventing ischaemic-reperfusion injury during cardioplegic arrest.

Whilst the basic stages in cardiac injury induced by ischaemic and reperfusion are known, many of the underlying biochemical pathways have yet to be fully elucidated. Further work is needed to determine the contribution made by a number of biochemical pathways to the pathological process of ischaemia. A secondary purpose of the outlined studies is to provide tissue samples needed by scientist investigating the contribution of specific biochemical pathways to cell injury during ischaemia and reperfusion.

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

The outlined studies will assess the safety and effectiveness of novel devices, drugs and interventions intended for used in the treatment of heart disease and generate the data needed to determine their suitability for progression into human clinical trials.

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

The main long-term beneficiaries of the work will be patients suffering from heart disease. This will be achieved through the introduction, into routine clinical practice, of more effective devices, drugs and interventions developed and assessed in the outlined studies. In the short term the work will be of benefit to medical companies and scientist developing equipment and drugs for use in the treatment of heart disease. In the medium term the publication of the research findings generated will benefit the career development of the scientists involved in the studies.

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

The output of the studies will be maximised by working in close collaboration with NHS clinicians, scientist and engineers based in academia, medical equipment manufacturers and pharmaceutical companies.

Species and numbers of animals expected to be used

- Sheep: 109
- Pigs: 298

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 studies will be undertaken using juvenile and adult pigs and sheep as their cardiovascular system closely resembles that of humans in both structure and size. Consequently, they provide models that enable the effectiveness and safety of drugs, treatments, devices and interventions, intended for used in human patients suffering from heart disease, to be determined.

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

The animals used for these studies will be obtained from commercial suppliers at least one week in advance of surgery. All animals will be group housed throughout the study. During the first week the animals will be habituated to human contact and trained to enter the weighing crate and transport trolly in order to minimise handling stress. On the day of surgery, the animal will be moved to the surgical suite in the transport trolly and will remain in the trolly during anaesthetic induction. The animal will be intubated and mechanical ventilation established before moving to the operating theatre. The animal will be mechanically ventilated and maintained at a deep plane of general anaesthesia throughout the procedure. Animals may be imaged non-invasively before, during and after the surgical procedure.

Surgery will be performed by specialist cardiac surgeons supported by specialist in all relevant clinical areas including perfusionists, veterinary anaesthetists, radiographers and theatre nurses. During surgery, animals may be implanted with intravascular devices or given substances to assess their safety and efficacy prior to entering human clinical trials. Alternatively, a small ischaemic heart lesion may be induced by the temporary occlusion of a coronary artery to replicate a typical cardiac infarction. These animals may be given treatments aimed at restoring normal cardiac function, either before surgery or at the time of injury or subsequent to it. Animals that are allowed to recover following surgery will be given pain control comparable to that given in a clinical setting to human patients and will be cared for by animal technicians experienced in post-operative care. Following surgery, all animals are expected to make an uneventful recovery and to resume normal behaviour within 24 hours. All animals are expected to continue to grow and behave normally throughout the duration of the study. Animals may be allowed to remain alive for up to one year during which time they will be periodically imaged under general anaesthesia. At the end of the study period the animals will be terminally anaesthetised, imaged, and have tissue samples collected before killing.

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

All of the models used in this study require the animals to undergo surgical/interventional procedures. In some cases, the interventions are major, replicating complex cardiac surgical procedures. In addition, some procedures have the potential to impair cardiac function to minic human clinical disease conditions and thereby impact on the long-term wellbeing of the animals. In all cases the procedures undertaken have been extensively refined to ensure that the adverse effects are minimal. All surgical procedure will be performed by specialist cardiac surgeons supported by specialist in all relevant

clinical areas including perfusionists, veterinary anaesthetists, radiographers and theatre nurses. The procedures are undertaken whilst the animal is maintained under a deep surgical plane of general anaesthesia. Following recovery from the anaesthetic all animals are expected to experience some degree of post-surgical pain. To mitigate this, all animals

will be given pain control comparable to that given clinically to human patients. Animals are expected to recover uneventfully from the procedure and to resume normal behaviour within 24 hours. Thereafter, the animals are expected to continue to grow and behave normally. In a small number of cases, animals may undergo two surgical procedures, separated by up to two months, in order to replicate a common clinical scenario however, recovery from the second procedure is not expected to differ from that of animals undergoing the latter procedure alone. Recovered animals will usually undergo imaging under general anaesthesia at several time point, however recovery from these is expected to be uneventful and animals are expected to resume normal behaviour within a few hours.

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 for these procedures is moderate. All animals will undergo a surgical procedure and can therefore be expected to experience some level of pain and suffering during the postoperative period.

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

- Killed

A retrospective assessment of these predicted harms will be due by 09 February 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 outlined studies are translational and aim to provide the data required to progress effective treatments and intervention for heart disease and ischaemic-reperfusion injury into clinical trials. It is not possible to achieve this objective without using animals as only data generated using a representative animal model will suffice to meet the criteria needed to obtain approval for progression into clinical trials.

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

Non-animal models are not appropriate for these studies as only data generated using a representative animal model will suffice to meet the requirements of the regulators responsible for approving clinical trials however, it is likely that non-animal studies will have been used during the development of some of the agents that will be tested.

Why were they not suitable?

Non-animal models are not appropriate for these studies as only data generated using a representative animal model will suffice to meet the requirements of the regulators responsible for approving clinical trials.

A retrospective assessment of replacement will be due by 09 February 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 estimated number of animals is based upon the throughput of work undertaken over the previous five years and the current available funding for exisiting projects.

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

The outlined animal models were developed by my research group and have been carefully refined to minimise the associated adverse effects whilst still yielding the required data. As a result, my research group has considerable experience in designing studies to obtain the required data using the minimum number 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?

Pilot studies will be used to determine the group size needed for treatments or intervention for which no relevant data set exists.

A retrospective assessment of reduction will be due by 09 February 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 purpose of these studies is to evaluate the safety and efficacy of new interventions, drugs or devices for use in the treatment of heart disease.

This will be achieved using three animal models.

1) To evaluate the safety and efficacy of intravascular implants including, stents, valves and cardiac assist devices. The animal will be implanted with the device in a procedure that replicates that used for human patients and its performance assessed using non-invasive imaging. Implants will be inserted by specialist NHS cardiac surgeons and catheterologists in a state-of-the-art hybrid operating theatre using aseptic precautions in-line with best NHS practice. Anaesthesia and pain control will be provided by specialist veterinary anaesthetists and will be in-line with that given to human patients undergoing similar procedures. Animals are expected to make an uneventful recovery following the procedure and to resume normal behaviour within 12 hours and thereafter to continue to grow and develop normally. Animals may continue on study for up to one year, during which period they may undergo imaging at predetermined time points to assess the performance of the implant. At the end of the study the animals will be anaesthetised, imaged, killed and the heart taken for detailed histological examination.

2) To evaluate novel treatments aimed at restoring normal heart function following a myocardial infarction, animals may undergo a procedure to induce a small infarct. This model was developed and refined under a previous PPL and involves a minimally invasive procedure performed under general anaesthesia using a balloon catheter inserted into a peripheral blood vessel and guided into a coronary artery using non-invasive imaging. Pigs undergoing this procedure make an uneventful recovery, immediately resume normal behaviour and continue to grow and develop normally. Thereafter, either at the time of infarction, or in a subsequent procedure, animals may receive treatment aimed at restoring normal cardiac function. All surgical procedures will be performed by specialist NHS cardiac surgeons in a state of the art hybrid operating theatre. The treatments given will assess novel devises and interventions intended for translation into clinical practice. The procedure undertaken will replicate the way in which the device or intervention would be

delivered to human patients and will use aseptic precautions in-line with best NHS practice. Anaesthesia and pain control will be provided by specialist veterinary anaesthetist and will be in-line with that given to human patients undergoing similar procedures. Animals are expected to recover uneventfully from the procedure and to resume normal behaviour within 12 hours of and thereafter to continue to grow and develop normally. Animals may continue on study for up to one year post treatment, during which period they may undergo non- invasive imaging to assess cardiac performance at predetermined timepoints. At the end of the study the animals will be anaesthetised, imaged, killed and the heart taken for detailed histological examination.

3) To evaluate drugs and interventions aimed at mitigating ischaemic-reperfusion injury during cardioplegic arrest, animals may undergo cardioplegic arrest in a procedure that replicates that used for human patients. This model was developed and refined under a previous PPL. During the procedure animals may be given treatment aimed at mitigating the adverse effects of ischaemia and reperfusion. All surgical procedures will be performed by specialist NHS cardiac surgeons in a state-of-the-art hybrid operating theatre. The treatments given will assess novel devises and interventions intended for translation into clinical practice. The procedure undertaken will replicate the way in which the device or intervention would be delivered to human patients and will use aseptic precautions in-line with best NHS practice. Anaesthesia and pain control will be provided by specialist veterinary anaesthetist and will be in-line with that given to human patients undergoing similar procedures. Animals are expected to recover uneventfully from the procedure and to resume normal behaviour within 12 hours of and thereafter to continue to grow and develop normally. Animals may continue on study for up to one year post treatment, during which period they may undergo non-invasive imaging to assess cardiac performance at predetermined time points. At the end of the study the animals will be anaesthetised, imaged, killed and the heart taken for detailed histological examination.

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

It is not possible to perform these studies in less sentient species as only larger mammals have the anatomy, physiology and size required to undertake the procedures and to provide representative models that meets the requirements of the regulators responsible for approving the translation of treatments and interventions into human clinical trials.

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

The models used have been developed and extensively refined by my research group to have the minimum impact on the wellbeing of the animals. It is essential to the work that the animals make a rapid recovery post-induction and remain healthy throughout the duration of the study, which often extend over several months. Throughout the surgical procedure and subsequent recovery phase, the animals will receive a comparable standard of treatment to that provided to patients within the NHS.

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

All studies will be undertaken at GLP comprable standards. All surgical procedures will be performed to NHS standards, which either meet or exceed those recommended by LASA. All studies will be designed with reference to NC3Rs recommendations.

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

Staying current with developments in the field is an essential part of all research programmes, which includes 3Rs advances. My institute is strongly committed to the 3Rs and has its own NC3Rs representative who actively promotes the uptake of 3Rs initiatives. All studies are extensively discussed at the pre-study stage, which are attended by representatives from the surgical, anaesthetic and animals care teams, and every opportunity is taken to incorporate improvements that will benefit the welfare of the animals.

A retrospective assessment of refinement will be due by 09 February 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. Calcium-permeable channels and mechanobiology in health, disease and therapeutic development

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

Heart disease, Vascular disease, Liver disease, Exercise, Therapy

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

The aim is to advance understanding of calcium-permeable channels, associated calcium-regulated mechanisms and other related mechanobiology in health and disease and to investigate ways in which this information might be used to improve the lives of people who have conditions caused by defective calcium-permeable channels, associated calcium-regulated mechanisms or other related mechanobiology manifesting in heart and cardiovascular system diseases.

A retrospective assessment of these aims will be due by 04 February 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?

It is important because it has possibilities to help humanity address common problems such as heart and blood vessel diseases (cardiovascular disease) and associated conditions such as diabetes and liver disease. These problems are major reasons for premature death, reduced quality of life, costs to health services and reduced work efficiency and work lifetime. Globally, cardiovascular disease is the most common cause of death. Physical inactivity, which is a failure to sufficiently stimulate normal mechanobiology mechanisms, is the 4th leading cause of mortality globally (World Health Organisation) and responsible for 1 in 6 deaths in the UK (UK Government). It often leads to cardiovascular disease and related conditions such as diabetes.

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

Our ultimate goal is to provide health service personnel new therapeutics for treating and managing cardiovascular diseases. We expect numerous original high-quality research discovery and translational papers to be published in competitive peer-review journals under open access licence agreements, open access review articles on this topic in peer-review journals, advancement of new scientific careers and new fruitful clinical research partnerships and international research collaborations. We expect to see new small-molecule modulators of these mechanisms (i.e., new drug- like molecules) with demonstrated effectiveness that enter licence agreements with pharmaceutical and other commercial partners so that they can be developed as new therapeutic drugs.

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

During the period of the licence and beyond, beneficiaries will be other scientists, research funding agencies including charities, our own establishment, other universities, university undergraduate and postgraduate students, postdoctoral researchers and research technicians. There will similarly be benefit to scientific knowledge and therefore to deep knowledge available to humanity for improvements to and protection of life. Potential beneficiaries in the period of the licence and beyond will be commercial partners and associated investors working with us to develop new therapeutic agents. There may be benefit to patients in the life-time of this licence but it is more likely that benefits of this type

will be evident in the longer term, such as 10 years; because sufficient time needs to be allowed for drug safety and efficacy trials in large patient cohorts.

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

We will look to maximise outputs through collaboration with basic science and clinical partners at our own establishment and partner establishments.

We will look to publish all findings, including unsuccessful approaches, wherever possible. We will deposit complex data sets such as 'omic' data (e.g., transcriptome results from RNAseq studies) in public repositories.

Species and numbers of animals expected to be used

- Mice: We estimate using 12,500 mice in this project (i.e., 2,500 per year), with about 3,000 of these mice used for experiments (i.e., 600 per year).

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 have been selected because they have established suitability for gene modification experiments and subsequent detailed investigation of in vivo and ex vivo phenotypes. Many of the modified mice we need have already been engineered by us or are available from repositories. Mice are the smallest mammal in which it is currently possible to combine studies of gene modification and invasive approaches for measurement of the relevant biology, including cardiovascular, metabolic, hepatic functions and exercise responses. The breeding cycle is relatively rapid and the lifespan short, enabling studies of the impact of gene modification through to old age within a 5-year programme of research. The widespread use of mice as a model of humans will facilitate comparisons of our data with data arising from other research groups and enable independent cross-validation of our findings (e.g., by sharing genetically modified mice and studying them using common analysis systems). While all mammals have differences (e.g., mice are much smaller and shorter-lived than humans and have a faster heartbeat), mice are widely accepted as a suitable first step for exploring the in vivo relevance and therapeutic potential of mechanisms. Like humans, mice have strong desire and capability for voluntary long-distance running, which is important for our research proposals to transform understanding of the health benefits of exercise through determination of the molecular detectors of the mechanical forces of exercise; importantly, prior studies show that exercise is protective against disease in mice and that they are a good model for many fundamental aspects of the benefits of exercise seen in humans.

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

The first action will be to breed existing genetically modified mice or introduce new genetic modifications aimed at subtle gene changes that do not threaten life but alter physiological responses and may cause a disease-like phenotype. A typical aim is to disrupt a specific gene in a specific cell type to determine the functional role of this gene/ cell in whole animal (mouse) physiology. We will use standard techniques that usually achieve conditional disruptions in the genome, so that the mice develop to adult stage normally and then the gene is modified; e.g., by tamoxifen injection to induce cre-mediated disruption. Such an approach minimizes unwanted effects that would otherwise be a risk to embryonic development, resulting either in embryonic or neonatal death. Another typical genetic aim is to recapitulate in mice a mutation identified in humans through association with disease. We identify such mutations, for example, in collaboration with research partners and in our UK Biobank (https://www.ukbiobank.ac.uk/) data analysis, as shown by our recent study of COVID-19 fatality. The murine version of the mutation is then generated in a new mouse line using CRISPR/Cas9 technology. We did this recently for a mutation that causes mild anaemia associated with malarial resistance. We showed excellent recapitulation of the human disease, previously unrecognized mechanisms underlying this disease and a novel therapeutic strategy. In a paper in preparation for publication we identified cardiometabolic abnormalities in these mice that we anticipate will usefully inform the medical care of people with such mutations, which have been estimated to occur in about half of people of African descent.

The next step is phenotyping of the genetically-modified mice and their matched control (unmodified) mice. We do this by observing the physiology of the mice, for example by collecting and analyzing blood from the mice at 8-22 weeks of age, by remote telemetric observation of blood pressure in conscious mice during physical exercise at 14-18 weeks old, measurement of heart anatomy and function by non-invasive echocardiography in mice at 12-16 weeks of age or by phenotyping in specialized metabolic cages for indirect calorimetry. In some experiments we seek to further improve the relevance to people who have cardiovascular disease by increasing the fat content of the diet and allowing the mice to grow old (2 years), at which point we would, for example, collect blood to determine concentrations of lipids such as cholesterol.

A next step may involve surgical intervention in the mice to induce a condition that models human disease states such as abdominal aortic aneurysms. Risk factors for this life threatening condition are well documented to be high blood pressure, high blood cholesterol and chronic obstructive pulmonary disease (COPD).

A next step may involve administration of a small-molecule (drug-like substance) that is commercially- supplied or synthesized as part of our medicinal chemistry drug discovery programme. Such molecules would be inhibitors or activators of the mechanisms under investigation (e.g., of a specific type of calcium-permeable channel).

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

Most often the impact on the mice will be no more than the captivity and associated experience that may be compared with mice kept as pets in peoples' homes but most likely under more consistently high quality conditions. This is classed as subthreshold.

When impact is more than subthreshold, it will most often be mild impact. A typical expected effect is reduced exercise performance on a running wheel (e.g., 50% fewer rotations of the running wheel during observation for 4 days in mice at 20 weeks of age). Another expected effect is reduced blood pressure elevation during running wheel exercise in mice at 14-18 weeks of age. These are not behavioural changes as far as we can tell, although we plan to study the changes in more detail using remote observation methods to determine any psychological effects. We think instead that they are mild physical effects that are comparable to a person being relatively physically unfit. From some mice we will collect blood and in some mice we will inject substances (e.g., tamoxifen) and this impact can be considered similar to that experienced by a person whose blood is collected or who has a substance injected.

In some instances we will perform recovery surgery, for example to insert a telemetry probe to measure blood pressure, or to implant a device either under the skin or in the perineal cavity of the animal. These devices enable us to constantly administer substances relevant to our research aims, thus eliminating need for frequent handling and repeated injections to animals and are viable for up to one month. In such cases there is suffering that may be compared to what a person experiences when undergoing surgery for a moderately severe condition, although it is not possible to explain the situation to the mouse or justify the intervention in the interests of the mouse's health. These surgical protocols are completed under balanced general anaesthesia, including pain-relief procedures to minimize suffering prior to, during and after the surgery, and sufficient time is allowed for recovery prior to any measurements from the mice.

We do not expect unusual pain or weight loss, tumours, abnormal behaviour or any other such concerning effects in our genetically modified mice or in the mice during any of our experimental protocols.

In some instances, a genetic mutation we engineer in the mice will be expected to cause disease similar to that seen in some people with a similar mutation. We will, however, select mutations that are likely to cause only mild disease in order to minimize suffering. However, if we observe mice with genetic modifications showing more than expected adverse effects, manifested in neonatal mortality or poor growth rate, we will take necessary steps to minimize suffering.

One of our protocols creates aneurysms in mice that have a risk of rupturing and causing sudden death, as in people who have such a condition. We take special precautions with these experiments and only use this approach when necessary.

Very occasionally a mouse may die unexpectedly from unknown causes that seem unrelated to any of our actions. This is a severe event, but we think such events are rare

natural events and we will make effort to minimize the risks of such occurrences. Any such events are reported under a PPL Standard Condition 18 Notification.

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, Mild, Moderate and Severe.

Based on experience under my current licence, I could expect there to be 26.5% Subthreshold, 65% Mild, 8.2% Moderate and 0.3% Severe. I anticipate, however, that the proportions will be left-shifted (i.e., less severe) because of 3R implementations described in this proposal.

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 04 February 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 need to use mice to advance understanding of calcium-permeable channels, associated mechanisms and other related mechanobiology mechanisms that contribute to natural physiological processes in whole animals such as mice and humans. The mechanisms are important for body homeostasis in physiology that is often disturbed in disease and modulated by physical exercise. If we want to understand animal biology and treat diseases in people and animals, we need to advance knowledge in part through whole animal studies. The homeostasis (steady-state of the living system) and its disturbances are whole body interorgan system effects that cannot be reproduced in cell culture environments or artificial or stored organs in the laboratory; this is because animal physiology and diseases depend on integrations across cell and organ types in the living, moving body as a whole; i.e., a human heart is not a human. Other non-animal

approaches are not currently available or possible for generating insight into such whole animal systems. For example, how could we study the health benefits of physical exercise (a key topic of this research) in cells or model organs cultured in the lab when such entities do not run around or exercise in any way that relates to our whole body exercise? In vitro data have already been generated and more will be generated to justify and optimize steps in the in vivo animal studies. Such data help us design the best possible animal studies and minimize risk of wasted life or unnecessary suffering. Moreover, a key technical approach for determining the role of a gene is to modify the gene so that its function is decreased or increased. We cannot do this in humans, although we can observe what happens when genes are modified naturally in people: and we do use such information to guide our mouse studies; it helps us to know what to target and to predict what might happen.

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

We have considered humans, human tissues, human cells and human induced pluripotent stem cell systems to recreate parts of organs (e.g., the heart) in tissue culture. We have considered ‘lower’ species such as flies and worms.

Why were they not suitable?

We will study humans, human tissues and human cells and such studies will be used in preference to animals whenever possible. However, the animal studies are still essential because in mice we can: (1) accurately compare control (non-genetically modified) and test (genetically modified) mice at the same age and in the same sex, matched living conditions and sufficient numbers to enable statistical comparison; and (2) accurately phenotype the mice using a range of powerful techniques, the application of which to humans would almost certainly be impractical and prohibitively expensive and may be unethical.

We are working with collaborators to establish and study human induced pluripotent stem cell systems to recreate parts of organs (e.g., the heart) in tissue culture. The primary motivation here is to create models systems that can inform drug discovery programmes. It is doubtful that they recapitulate normal physiology however, so the arising data are of questionable value for understanding physiology. No systems currently exist for recreating whole animals using such technology, so complex interorgan systems biology and exercise biology cannot be studied using such methods.

We have considered ‘lower’ species such as flies and worms but concluded that their biology is too distant from the biology we want to understand and too remote as a basis for translation to problems of disease in people, such as those caused by insufficient physical activity and calorie-rich diet.

A retrospective assessment of replacement will be due by 04 February 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 numbers based on experience in studies of this type under our current licence, which is similar in many ways (but updated, improved and simplified). We will use related approaches in the proposed new licence and are working to achieve similar investments in our research (i.e., volume of peer-reviewed grant funding).

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

We are working to reduce the number of animals being used by initiating new human cell, human tissue and patient data analysis studies to answer questions. We are doing this by establishing new partnerships with clinicians in the NHS and by studying the UK Biobank and other databases. Such research is the basis of grant proposals we have submitted or are in the process of submitting to the Medical Research Council, British Heart Foundation and other such agencies; these proposals do not include requests for animal studies.

We are working to reduce the number of animals being used by initiating computational projects and collaborations that model calcium-permeable channels and mechanobiology. Such models are increasingly sophisticated. We are using these models to understand human mechanisms and working towards predicting the effect of human disease genetic mutations on the mechanisms and their downstream consequences for cell and organ function. These are ambitious projects but we hope that they will eventually avoid or further reduce the need for animal studies.

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

Our institute employs an expert technologist trained to PhD level in genetics to oversee and optimise our animal breeding programmes to maximise efficiency, maximise value for the science and minimise use of animals. This individual supports our animal studies and those of other investigators in the institute who work on other but related disease challenges. This support system provides a consistent approach and encourages and facilitates sharing and teamwork to maximize scientific benefit from each animal.

We will work with a professional biostatistics collaborator to maximise the quality of experimental design and maximize the value of the arising data. Two statistical approaches will be used as we previously described in work with our statistics collaborator.

We will use small-scale pilot studies to indicate if a particular experiment is promising in terms of the value of the outcome, if the overall experimental design can be improved (e.g., are there any unnecessary steps, are we missing any opportunities to collect important information?) and if the estimate of variance can be improved to inform the power calculation. Only then will the full study be conducted using blinded and randomized experimental processes in the protocol.

We will use telemetric techniques that are available for sequential measurement of blood pressure, cardiac electrical activity and breathing in conscious animals. In addition, use of imaging modalities now available to us can also pick up progression of pathological changes in longitudinal study of one animal. Both these techniques produce reliable data and help reduce the number of animals used.

We are seeking external funding for a new remote phenotyping system that we hope will enable us to further improve the quality of data collection and thereby reduce variance in the data collected from the animals and justify use of fewer animals. We already submitted a grant proposal for this equipment and it has been short-listed for funding (equipment cost £828,745). The system is the TSE-Systems PhenoWorld Multi-Arena for quantification of the physical activity of the mice and matched exercise in knockout and wild-type groups over long periods of time; high-quality standardized physiological phenotyping of multiple parameters including behavioural and cognitive parameters in social groups with minimum human interference; continuous assessments and the potential for interim evaluation of data during long protocols. The equipment is for remote metabolic, physiological, cognitive and behavioural phenotyping of individual mice in social groups of 10 with total capacity for 60 mice to enable sufficient statistical power. In this system, each mouse is tagged, recognized at strategic positions for data acquisition and monitored for controlled access and evaluation in tunnels and compartments including for indirect calorimetry, monitoring of feeding/ drinking and excretion, cognitive assessment and in-cage voluntary running wheel analysis with workload control and motor skill testing. There is detailed tracking of movement for maximum detail on total physical activity and telemetry for remote monitoring of parameters such as blood pressure. This is part of our long-term vision to implement increasingly sophisticated phenotyping and genetic approaches and offer training, standardization and collaborative opportunities worldwide. Nevertheless, even if we obtain funding for this new phenotyping system, there will still be substantial breeding of new genetically modified mice because our plans include increasingly sophisticated genetic approaches to generate more informative insight and less overall modification to the animal (e.g., by disrupting a gene in endothelium of only one organ, rather than throughout the body as we and others do currently).

A retrospective assessment of reduction will be due by 04 February 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 have well-established suitability for gene-modification experiments and are the smallest mammal in which it is possible to combine studies of gene modification and invasive approaches for measurement and induction of disease. Mice are used as a model of the human because they are mammals and fundamental mechanisms are likely to be similar across the mammals. While all mammals have differences, mice are widely accepted as a suitable first step for exploring the in vivo relevance and potential of mechanisms. Progress cannot currently be made directly from in vitro ‘test tube’ studies to human in vivo studies.

Suffering will be minimized through use of inducible gene-modification systems as described in more detail in other sections.

Suffering will also be minimized by use of the appropriate doses of chemicals for observation of effects. Before testing such modulators in vivo we will test them for toxicity in cell viability assays in vitro to anticipate if toxicity might arise in vivo and at what dose. For modulators which remain potentially interesting, we will test them in vivo in mice. We will start by administering the modulator by osmotic mini-pump or i.p. injection at a dose expected to be sufficient to modulate the target and with the Named Veterinary Surgeon (NVS) known to be available for consultation. Where repeated administration of substances is required by subcutaneous or intra-peritoneal (i.p.) injections to achieve the desired effect, we will instead use mini-osmotic pumps to deliver continuous infusion if possible. These pumps are implanted either in subcutaneous or intraperitoneal space under balanced general anaesthesia using aseptic surgical techniques and are well tolerated by mice.

To induce exercise related cardiometabolic effects we will provide free access to a running wheel as part of the home cage environment.

We use acclimatization to phenotype monitoring systems to reduce stress and improve the quality of the data collected.

Sequential imaging of animals in longitudinal studies can pick up pathological changes at an early stage, thus implementation of humane end-points can be implemented more accurately and result in refinement of experiments.

For aneurysm studies we have already refined the dose of the agent used to induce aneurysms and therefore minimize the risk of aneurysm rupture (i.e., to minimize the risk of a lethal event) and have increasingly moved towards use of models that have a very low risk of rupture (especially peri- adventitial elastase application to the aorta) combined with mutations pertinent to the human disease and calcium channel mechanisms we study. We only use the angiotensin II (AngII) infusion model (with rupture risk) to confirm findings from the refined model.

Throughout, a single-use needle policy will apply to all injections.

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

We are working to understand animal physiology and the responses of this physiology to external factors such as diet and exercise and to injury events such as damage to the vascular wall (e.g., leading to aneurysm formation). We are working to develop novel agents that can hopefully be taken forward to achieve new medicines to treat disease problems in people (usually adult people). In order to do this, we need to study animals that are as similar as possible to the human situation. This means using an adult mammal when it isn’t possible or appropriate to advance the research using humans or cell culture systems alone. We need to understand whole body physiology in the living moving state, for example to understand exercise responses, which require whole body movement. We cannot do such research using immature life stages, species that are less sentient or animals under anaesthesia, although we will use terminal anaesthesia strategies where appropriate.

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

As mentioned elsewhere, we are seeking external funding for a custom TSE-Systems PhenoWorld Multi-Arena. In addition to scientific advantages, such systems should substantially improve animal welfare by enabling more remote observation of mouse phenotypes without human interference.

Importantly, the system enables study of individual mice in social groups of 10 in enriched environments with natural-type tunnel systems and free voluntary access to running wheels. We think this will greatly improve the living environment for the mice and thereby further improve animal welfare.

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

https://www.nc3rs.org.uk/ https://www.lasa.co.uk/

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

We are part of an emerging mouse genetics and phenotyping consortium with a group at another university to refine mouse study approaches. A major purpose of this consortium is to share best- practice and work together to optimise and improve methodologies, and thereby refine.

We are part of the National Centre for the Replacement, Refinement and Reduction of Animals in Research mailing list and receive regular Newsletters and updates on the 3Rs initiative.

We are working increasingly closely with clinical research groups and learning about databases for research such as UK Biobank and to find ways to replace animals in research (e.g., machine organ perfusion systems for studies of human organs such as liver and using observations of human genetic variation to understand gene function in the natural environment).

We are working with other groups to collaborate in the development of 2D and 3D human induced pluripotent stem cell approaches and thereby potentially reduce and replace animal usage.

A retrospective assessment of refinement will be due by 04 February 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. Regulatory ecotoxicology

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

Key words

Ecotoxicology, Chemicals, Pharmaceuticals, Agrochemicals, Regulatory

Animal types	Life stages
Zebra fish (Danio rerio)	juvenile
Rainbow Trout	juvenile, embryo, neonate, 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 overall aim of the project is to determine the short-term and long-term toxicity of industrial chemicals, agrochemicals, pharmaceuticals, biocides and microbial pesticides in fish.

We also will assess the hazardous properties of these substances with respect to the endocrine systems in fish, to support the ecotoxicological (potential to cause damage or harm) assessment of substances in the environment.

These properties are a fundamental requirement of the risk assessment process for such substances, and to make sure that humans or animals are safe if exposed

A retrospective assessment of these aims will be due by 11 February 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 need for these studies is driven by regulatory requirements (tests required by governments or international bodies) to provide data on the effect of the test chemical on fish, with the study data being used to support the registration of the new chemical or preparation, as required by the prevailing notification/authorisation scheme in the countries where it is to be manufactured or used.

In other cases, the study data generated on this project may be used for hazard communication purposes without the initial need for submission to a regulatory authority (this identifies that the particular chemical is hazardous in the natural environment).

Whatever the intended use of the data, the studies are conducted in accordance with scientifically relevant and internationally approved test methods and regulatory guidelines so that the data will be acceptable for registration or notification of the chemical or preparation if this eventually becomes necessary.

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

The assessment of the risk posed by new and existing chemicals and waste materials (industrial chemicals, agrochemicals, microbial pesticides and biocides) to the natural environment continues to be an important international issue for industry, governments and the public alike. Synthetic chemical substances will inevitably enter the natural environment as a result of their use and disposal in industrial and domestic environments, and ecotoxicology studies are designed to assess their likely impact on natural populations of plants and animals, and to identify ecologically benign concentrations.

This will allow regulatory authorities (organisations independent of governments who assess the safety of drugs and chemicals) to classify and label these substances, recommend safe handling procedures, and impose risk reduction measures if required such that the benefits provided by the substances can be safely achieved.

Specifically, this project will assess the ecotoxicological effects (effects on the environment) of these substances to fish following a single (acute) or series (chronic) of exposures.

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

The data obtained in these tests are submitted to regulatory authorities to inform decision-making processes and, where appropriate, satisfy the governmental regulatory requirements that are necessary to gain product registration or to assess the risk and impact posed to the natural environment or human health by the use of chemicals, agrochemicals or pharmaceuticals.

Ecotoxicology studies in general are designed to assess the likely impact on populations of plants and animals, and to identify ecologically benign concentrations.

This will benefit the environment, the public, wild animals and the people who produce these chemicals (as it will enable them to be registered and sold to the public or industry).

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

All of the studies conducted in this facility with aquatic species are bound by confidentiality agreements and unless contracted to provide support through the registration process, the testing facility does not normally receive information regarding the progression of a substance through to marketing authorization. It is not possible therefore to identify the number of substances tested in the facility that have gained marketing authorization or product registrations. Success of the programme of work is the provision of fit for purpose and through studies, providing the toxicological data, the sponsor/regulator to enable risk assessment & reduction, and toxicity mitigation strategies

Species and numbers of animals expected to be used

- Zebra fish (Danio rerio): 900 Rainbow Trout: 13400
- Common Carp: 3800
- Bluegill Sunfish: 3500
- Fathead Minnow: 82720
- Japanese Medaka: 400
- Turbot: 450
- Sheepshead Minnow: 1200

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.

All of the studies conducted on this licence will be performed to fulfil a regulatory requirement, to make sure that the environment is protected from potentially harmful chemicals. This means that the way we perform the studies are done to a standard set of conditions (regulatory guidelines) to get the data the regulators require. This includes the type of fish species we use and their stage of development.

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

The individual studies undertaken involve exposure of groups of fish to varying concentrations of the chemical (via the test water) to assess the effect that the chemical may have on survival and/or growth of the fish and any interaction the chemical may have with the endocrine system.

Sometimes food is withdrawn from fish for short periods of time, as this can interfere with the objectives of the study. This is only done to fulfil the regulatory guidelines we are using.

Occasionally, fish may also be anaesthetised for a short period, as we need to examine them, measure them or weigh them as part of the study procedures. This procedure of anaesthetizing should help to reduce stress levels in the fish (which will benefit their welfare) and lead to more accurate data collection.

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

Adverse effects ranging from mild discomfort through to death are expected during the course of this project. However, in the majority of exposed fish adverse effects will only be mild. The programme of work will be designed in accordance with the principles of the 3Rs (Replacing animals with non-animal alternatives, Reducing the number of animals we use, and refining our procedures to minimise any harms animals may suffer) in order to minimise animal use and severity of procedures.

Tiered testing strategies will be implemented, so that the results of one study can be used to refine the remaining studies in the programme thus minimising the severity of any adverse effects. All fish that are exhibiting significant toxic effects, and those surviving to the end of each test, will be humanely killed as soon as possible to avoid unnecessary suffering.

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)?

On the last project, about 90% of animals were classified as having displayed mild or moderate severity. Around 10% displayed severe symptoms.

It's impossible to predict the proportion of severities expected on a service licence, as this will be dependent on what study types we are asked to perform.

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

Killed

A retrospective assessment of these predicted harms will be due by 11 February 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?

At present there are presently no accepted alternative methods to the use of in vivo studies required by this programme. The use of fish cell lines has been investigated as a replacement for acute fish testing (CEFIC-LRI; ECO8-CEllSens project), however, ECVAM have recommended that further evaluation (ongoing) of reproducibility, predicative capacity and applicability domain is required before its use as a replacement can be confirmed.

The zebrafish embryo toxicity test (ZFET) which has been published as OECD TG 236 “Fish Embryo acute toxicity test” also has the potential to be used as a replacement for acute toxicity testing, however, a recent report commissioned by the European Chemicals Agency (ECHA) again recommends that additional scientific investigations into its applicability domain are undertaken before its use to replace short-term fish toxicity studies could be accepted. In the absence of suitable non- animal alternatives, wherever possible studies with invertebrates, e.g. Acute Toxicity to Daphnia magna (OECD 202) or Daphnia magna Reproduction Test (OECD 211), will be conducted in place of animal tests

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

The use of fish cell lines has been investigated as a replacement for acute fish testing (CEFIC-LRI; ECO8-CEllSens project), however, ECVAM have recommended that further evaluation (ongoing) of reproducibility, predicative capacity and applicability domain is required before its use as a replacement can be confirmed. The zebrafish embryo toxicity test (ZFET) which has been published as OECD TG 236 “Fish Embryo acute toxicity test” also has the potential to be used as a replacement for acute toxicity testing, however, a recent report commissioned by the European Chemicals Agency (ECHA) again

recommends that additional scientific investigations into its applicability domain are undertaken before its use to replace short-term fish toxicity studies could be accepted.

Why were they not suitable?

There are no adequate models to replace the whole animal experimental model, as the complexity of fish and their internal physiology and development cannot be fully replicated in a test tube.

A retrospective assessment of replacement will be due by 11 February 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 we have used are based on figures of previous usage from previous projects, or a projection thereof (based on estimated incidence) based on requests received from customers in the past. It is, however, impossible to accurately predict the number of studies that may be performed, in the circumstances.

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

The number of animals, and the number of groups of animals to be used in a particular test are usually defined within the regulatory guideline used for that test. No more animals than the numbers outlined in these guidelines will be used. The use of the specified numbers of animals ensures that the data generated will be acceptable to regulatory authorities and hence will minimise the need for subsequent duplication or supplementary testing.

Whenever possible, common control groups will be used in order to minimise the numbers of groups used.

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

Some of the studies run in this project may use data from previous experiments to inform exposure concentration levels for future studies. Therefore, it will not be necessary to run another study, using more animals, when we already have that information. Similarly, if we have that data from other sources, we will not need to run these preliminary range finding studies.

Where possible the results of computer predictions, physico-chemical testing and non-animal tests will be used to aid in the prediction of toxicity, hence reducing the number of animals required to satisfy the regulatory requirement.

We will try and get as many outputs as we can from a single animal where possible, without adversely affecting its welfare. So if we need to get a blood sample, or if we need to measure or weigh a fish, for example, we will often do that in the same animal, rather than use separate ones, when possible.

A retrospective assessment of reduction will be due by 11 February 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.

Most of our models involve exposing fish to test chemicals, either over a short period (a few days) or longer (a month or so) and observing them for signs of toxicity or changes in their sexual physiology.

Many of the outputs we use are taken after the animals have been humanely killed. This is generally the least invasive set of procedures that can be done to give meaningful outputs to make scientific decisions about further tests.

Occasionally we may need to anaesthetise fish and remove them from their tank to weigh and measure them or check on their development. We make sure the fish are unconscious whilst we do this and afterwards, let them recover in a tank of fresh water until they are fully recovered.

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

The fish species used have been selected in accordance with the relevant Test Guidelines and the age ranges of the fish are such that they are of the lowest neurophysiological sensitivity that will allow evaluation of the specific outputs.

The species selected are representative of wild species. The data generated is therefore designed to protect these representative species in the environment thereby minimising larger scale environmental effects of tested chemicals.

Any fish that are showing a significant departure from the animal's normal state of health or well-being will be identified and humanely killed.

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

When we identify animals that are showing adverse clinical signs, the tank will be identified for close monitoring and anddtional observations performed (all fish are observed at least once or twice daily), depending on the intensity of these signs. We have a list of potential adverse clinical signs within the licence which give examples of the type of things to look for, that would suggest a fish is potentially unwell.

When we see that a fish is unwell, and is unlikely to recover, we humanely kill the fish to prevent any further suffering.

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

OECD Guideline No 203 (2019) - Fish, Acute Toxicity Test.

OECD Guideline No 210 (2013) – Fish, Early Life Stage Toxicity Test.

OECD Guideline No 212 (1992) – Fish, Short Term Toxicity test on Embryo and Sac-fry stages. OPPTS 885.4200 Freshwater Fish Testing, Tier I.

Notification No. 9-Seisan-5090 Test Guideline Guidelines for safety evaluation of microbial pesticides. OECD Guideline No. 215 (2000) Fish, Juvenile Growth Test.

OECD Guideline No. 229 (2012) Fish, Short term Reproduction Assay.

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

This will be achieved by regular discussions with our Named Information Officer, colleagues in Animals Technology and by attending appropriate training courses and conferences, or getting feedback from such events.

A retrospective assessment of refinement will be due by 11 February 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. Training in advanced or complex procedures or devices

Project duration

5 years 0 months

Project purpose

- Higher education and training

Key words
Training, Endoscopy, Laparoscopy, Robotic, Endovascular

Animal types	Life stages
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.

- Education and training 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?

Education and training of practising surgeons in specific, novel/complex techniques, procedures and devices.

A retrospective assessment of these aims will be due by 20 February 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 a recent report by the UK Shape of Training steering group (https://www.gmc-

uk.org/-/media/documents/report-from-the-uk-shape-of-training-steering-group_pdf-79105880.pdf) they acknowledge that:

"...such is the rate of change that elements of the information gained during undergraduate training have been superseded by the time doctors commence work..."

and that "...In the future doctors must be able to adapt to this rate of change by having the flexibility to acquire new skills, change careers and participate in career long learning..."

Also, the second key point in the recent GMC 'shape of training' review states that "We will continue to need doctors who are trained in more specialised areas to meet local patient and workforce needs."

This is particularly true with the continuing development of increasingly complex surgical devices in both the laparoscopic and endoscopic fields and the expansion of the use of robotics in surgical procedures. Given this complexity there is the need for high fidelity training which is currently not possible by simulation alone hence the need for live training prior to use in patients.

How will course attendees use their knowledge or skills in their future careers?

Course attendees will all be currently practising surgeons with an interest in using the devices/ techniques. Upon completion of the course they should be immediately able to transfer the skills gained into human clinical practice.

What are the principal learning outcomes from the course?

To understand how to safely and effectively use novel endoscopic and/or laparoscopic devices and novel methods of energy delivery for cutting, coagulation (stopping bleeding) and/or ablation (destruction of tissue e.g. tumours).

To understand how to safely and effectively use novel endovascular devices (devices designed to navigate and treat the vascular system).

How are these learning outcomes important to the people on the course?

The course will enable the attendees to safely and effectively utilise the new devices/techniques and provide the confidence required for incorporation into their current

clinical practice. This in turn could lead to significant cost savings as well as allowing more procedures being carried out minimally invasively and safely.

Who or what will benefit from the transfer of knowledge, or acquisition of skills that this course will deliver?

The skills developed through these training courses should directly benefit patients through improved surgical outcomes, reduced surgery times, reduced recovery times and, in some cases, allow the use of new, less invasive, treatments that may not have been available before.

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

This training programme in the UK are part of the company's developing global training programme. Training under this licence will also form part of the internal 'train the trainers' programme. Training locations at other sites around the world will benefit from refinements and improvements made in UK.

Species and numbers of animals expected to be used

- Pigs: 380

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 pig stomach, colon and abdomen, in terms of the anatomy and size and because of their omnivorous nature, is closest to that of human, making the pig the most appropriate animal to use for laparoscopy and endoscopy.

Whilst the adult pig vasculature is the model of choice for central, abdominal, thoracic and neuro endovascular training.

The pig is also easily induced to, and maintained in, deep general anaesthesia for long durations.

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

The animals will undergo deep anaesthesia from which the animal is not allowed to recover and will remain insentient throughout.

Then some, or all, of the following techniques will be taught during the live phase of the course, as these have been identified as the most common areas where complications occur during human surgery.

For each animal this will involve inserting instruments through one, or more, of the appropriate routes (e.g. through the abdominal wall, via the anus or the mouth, or through the skin directly into the vascular system.)

For laparoscopic courses this may include stomach, kidney, liver and/or bowel surgery, gall bladder removal, hysterectomy or similar procedures.

For colorectal endoscopic courses, procedures may include a range of new surgical bowel procedures.

For upper GI endoscopic courses procedures may include tonsillectomy, oesophageal, stomach, duodenal or pancreatic surgery, or other new, appropriate procedures.

For Endovascular procedures, vascular access will be gained and a port placed, students will then practice a variety of procedures within the vasculature.

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

All procedures under this licence are non-recovery (i.e. the pig is anaesthetised and is not allowed to recover). Therefore, no other impacts or adverse effects are anticipated. In addition, throughout the procedure animals will be provided with fluids (I.V.) and their temperature monitored with warming or cooling applied if/when required.

Where possible/appropriate, procedures will be carried out in accordance with the LASA Guiding principles for preparing for and undertaking aseptic surgery. However, it is not possible to carry out the majority of endoscopic procedures aseptically.

Another potential adverse effect that can occur during endoscopy training is increased intra-abdominal pressure but this can usually be controlled using needle decompression, combined with endoscopic suction.

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)?

- Pigs- non-recovery - 100%

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

- Killed

A retrospective assessment of these predicted harms will be due by 20 February 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?

Whilst there have been many improvements in non-animal models and simulations for basic endoscopy and laparoscopy they are still not similar enough to the real experience (working with live tissue). For this reason, training in advanced techniques is especially important when using some of the advanced devices for cutting, removing tissue and stopping bleeding. Temperature, blood flow, tissue reaction and movement are all significant factors that can affect how advanced devices, especially those using energy (e.g. Microwave, radio frequency), are used. None of these factors are currently sufficiently, reproducible in an non-living/simulated model, therefore it is not possible to only use cadavers (either human or animal) for training with such devices.

It is not currently possible to simulate a complete vascular system with enough accuracy to carry out endovascular training.

Why can’t your aim be met by observing or by participating in ongoing research or clinical procedures?

Current clinical/ surgical training is moving away from the current standard of see one, do one, teach one. This is especially true for advanced procedures, where there is still no substitute for actually performing the procedure multiple times under supervision. Also, this is rarely possible in a clinical setting and even when it is, is not often advisable, on the grounds of patient safety.

A retrospective assessment of replacement will be due by 20 February 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 estimate is based on one 2-day course per month using 4-6 animals per course (depending upon the number of students) for 5 years for the endoscopy/laparoscopy.

Plus a series of four, 1-day courses per year using 2 animals per course (depending upon the number of students) for 4 years for the endovascular courses. With the option for more similar courses from other companies as requested.

What in silico or ex vivo techniques will you use during training?

Theory training should be carried out before delegates attend this course. However, there are also endoscopic training boxes/simulators available on the training days that allow individual students, and/or their assistants, to practise on non-living tissue. These can also be used for demonstration purposes.

Will these techniques reduce animal numbers? If so, how?

The training boxes will be an adjunct to the primary, endoscopic training but are not sufficient to replace live animal training and therefore are unlikely to reduce the total number of animals used, however multiple students/ surgical teams use the same animal in order to reduce total animals used.

What other measures will you use to minimise the number of animals you plan to use in your project?

Where possible we will use both the upper and lower gastro-intestinal tract (Colon, Stomach and Oesophagus) and on occasion the abdominal cavity, and maximise the number of procedures carried out in each animal. By doing so, it should reduce overall animal usage.

For endovascular training, by starting at the periphery (i.e. further from the centre where the vessels are smaller), where possible and working back to the central vessels enables more of the vasculature to be used. Also, by getting clinicians to work together: one acting as primary surgeon and another as assistant, with others (if present) observing, and then alternating these roles, students get maximum time on the animal and gain maximum exposure, from different perspectives, of the procedure as a whole.

A retrospective assessment of reduction will be due by 20 February 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.

All training will be carried out as a non-recovery procedure under deep terminal anaesthesia and, as such, beyond the initial induction of anaesthesia the animal should not experience any suffering or distress.

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

The pig has been chosen for these courses because surgeons will need to work on an animal that is the same size and general anatomy, and has the same reaction to surgery, as humans and there are no less sentient animals of the same size. However, as the animals are terminally anaesthetised for the duration of the procedure and do not regain consciousness afterwards, their awareness/suffering is reduced as low as possible.

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

All procedures carried out under this licence are non-recovery.

Ensuring good induction, monitoring and maintenance of deep anaesthesia, reducing, as much as possible, any stress prior to the procedure (through acclimatisation, training, etc.), administering fluids (I.V.) and temperature monitoring with warming or cooling applied if/when required, will all help to minimise the suffering the animal is exposed to. Also, any increased intra-abdominal pressure resulting from insufflation can usually be controlled using needle decompression/ venting, combined with endoscopic suction.

At the end of the training the animals will be killed without regaining consciousness.

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

Reviews of the current literature and any revisions to the relevant guidelines.

By maintaining contacts with other training centres nationally and internationally and incorporating any appropriate improvements they make to these and similar courses.

I have also been referred to standard, established, well regarded reference books, for up to date anaesthesia advice/techniques.

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

We will review the current literature. We will have discussions with and input from the local Named Information Officer (NIO), Named Animal Care Welfare Officer (NACWO), Named Veterinary Surgeon (NVS) and other local animal care staff. We will check the Norecopa, NC3Rs and LASA (Laboratory Animal Science Association) and similar animal research and welfare websites. Also by maintaining contacts with other training centres, nationally and internationally, and incorporating any appropriate improvements they make to these and similar courses.

A retrospective assessment of refinement will be due by 20 February 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. Education in experimental physiology

Project duration

1 years 0 months

Project purpose

- Higher education and training

Key words

Cardiovascular, Blood pressure, Cannulation, Stereotaxic surgery, Behavioural tests

Animal types	Life stages
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.

Education and training 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?

This Project Licence will enable undergraduate students in the biomedical sciences to be taught the fundamental physiological and pharmacological principles as they relate to the rat cardiovascular system, and provide a unique opportunity to interpret complex and often unpredictable responses to drugs within whole integrated physiological systems, which cannot be achieved using other methods.

Retrospective assessment

Published: 03 February 2023

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

No

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

The licence was only granted for one year and during that period a video was produced showing the rat arterial and venous cannulations. This will be used in a revised undergraduate course where students will learn the principles of in vivo surgery but without the use of terminally anaesthetised rats.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?

Future scientists require a sound knowledge and understanding of the normal physiological processes involved in the maintenance of normal cardiovascular physiology and function to enable them to design studies aimed at the expansion of physiological knowledge or the identification of novel drugs or therapeutic intervention pathways. For example, a drug administered for one purpose can often have other important effects in non-target tissues, which cannot be observed in non-sentient samples. A further benefit of the studies proposed under this licence is that the students will develop an understanding of the strengths and weaknesses of biological experimentation. This will allow them to understand and appreciate the limitations of pharmacological research and development, and the validity of drug discovery. Students will also observe first-hand the variability

inherent in biological measurements obtained from live animals in experimental settings. Students who have undergone this or similar training should be able more quickly and actively to participate in ongoing research programmes and many of the students do go on to pursue PhDs and other research degrees. The importance of integrative mammalian biology teaching and research in the UK has been recognised and supported by a wide range of bodies including the Higher Education Funding and Research Councils, the pharmaceutical industry and The Physiology Society. The Pharmacological Society in partnership with the Physiological Society have launched their "Undergraduate in vivo curriculum".

More details can be found here:

https://www.bps.ac.uk/education-engagement/research-animals/curriculum-for-the-use-of-research- animals/about-the-curriculum-for-the-use-of-research-animal

The experimental work detailed in this licence will allow a group of undergraduate students to study biological responses as well as being taught about good experimental design, animal handling and welfare, statistical analysis, ethics and how to properly report in vivo data using the ARRIVE guidelines.

Students who have taken this module have been successful in attaining positions within research labs, postgraduate research opportunities and internships in the pharmaceutical industry.

How will course attendees use their knowledge or skills in their future careers?

Prior to the course all participants will have to obtain a Home Office personal licence and undertake approved modular training. Although their licences will be revoked at the end of the course, their training will remain valid and they can use it to apply for a personal licence at another establishment within the UK or further afield. Many graduates who have taken the course have gone on to PhD or Masters degrees by research or have started a job in the pharmaceutical industry and have been able to use their training and skills from the course. The skills they are taught in experimental design, data analysis and reporting and legislation around animal research are also valuable for any career in research or development.

What are the principal learning outcomes from the course?

The intended learning outcomes are as follows:

Develop an appreciation of the importance of animal welfare and the 3Rs (replacement, reduction, refinement);

Develop an appreciation of the issues required to successfully undertake well controlled in vivo experiments, including ethics and legislative requirements;

Facilitate the development of the critical interpretation of the integrated physiological responses arising from specific interventions;

Expansion of knowledge and understanding of the physiological and pathophysiological integrated responses in the cardiovascular system;

Employ the rat brain atlas and use stereotaxic surgery to target brain regions for dye injection in cadavers;

Analyse data from behavioural experiments using techniques such as the tapered beam test; Prepare and present results of experimental work.

How are these learning outcomes important to the people on the course?

Many of these are transferable skills and will particularly assist those students who wish to go on to a career in research in either academia or the pharmaceutical industry. Even if what they do in future does not directly involve in vivo work, many of the principles of good experimental design, data analysis and reporting, ethics and good laboratory practice also apply in most other areas of laboratory work or research.

Who or what will benefit from the transfer of knowledge, or acquisition of skills that this course will deliver?

Students will acquire improved understanding of the complex processes involved in cardiac and vascular physiology and the modulatory effects of known drugs.

Provision of bioscience graduates with a thorough appreciation of how to design and execute in vivo experiments. Also how to treat the data obtained to reach meaningful conclusions.

Students will gain experience of due ethical consideration of the live animals used in scientific studies, and in the application of the 3Rs (refinement, reduction and replacement).

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

Resources will be developed under the authority of this licence which can assist in students acquiring these skills in future without the use of animals. For example, audio-visual resources which will demonstrate the integration of the rat cardiovascular system and the effects of drugs.

Any resources which arise from the educational course will be made available to the wider academic community for use on other undergraduate courses.

Species and numbers of animals expected to be used

- Rats: 200

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.

For the experiments we are performing we need to use adult rats as their cardiovascular systems are fully developed. In order to cannulate blood vessels the vessel has to be of a sufficient size to allow access with the cannula and so the carotid or femoral artery is normally chosen. For veins we would typically use the jugular vein or, on occasion, the femoral vein.

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

All animals will be anaesthetised to measure blood pressure, heart rate and, optionally, a recording known as the electrocardiogram (ECG). The effect of treating them acutely with known drugs will also be studied and in some animals, the effect of stimulating a nerve in

the neck called the vagus nerve will be studied. At the end of the procedure the animal will not be allowed to recover and instead will be killed while still under anaesthetic.

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

All procedures are non-recovery and since the depth of anaesthesia is closely monitored during the procedure, animals should not experience any adverse effects.

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 protocol is non-recovery. All rats would experience this severity.

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

- Killed

Retrospective assessment

Published: 03 February 2023

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

One rat was used and the surgery was conducted under terminal anaesthesia by two experienced personal licence holders. Anaesthesia was induced smoothly with a volatile agent and the animal did not regain consciousness during the procedure. Depth of anaesthesia was closely monitored during the procedure.

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 complexity of the in vivo systems and integrated responses to drugs being measured in the proposed project cannot currently be replicated in a non-animal alternative. The purpose of this educational licence is to allow undergraduates to be taught the fundamental physiological and pharmacological principles as they relate to the rat cardiovascular system and how to interpret these responses and for that reason therefore the work has to involve the use of animals. Alternatives have not been rejected but instead form an integral part of the course preceding the in vivo studies described in this Project Licence.

In addition, under this licence we will create a high-definition video recording of these experiments and this will become a replacement for future students.

Why can't your aim be met by observing or by participating in ongoing research or clinical procedures?

We currently do use other resources to teach students (a video showing the cannulation procedure, cadavers). Under the proposed licence we will create a high-quality audio-visual resource showing the entire procedure and the effects of drugs on the physiological measurements which will ultimately

Replace the need to use non-recovery animals. This will still allow the students to observe the physiological and pharmacological principals we wish to teach them.

Retrospective assessment

Published: 03 February 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?

The purpose of the one procedure conducted under the licence was to produce a video which will replace use of terminally anaesthetised animals in undergraduate education. In that regard, the whole purpose was replacement.

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?

Students will work in small groups (sharing an animal) rather than on individual animals. The design of the experiments has been based on studies published in peer reviewed scientific journals and a professional biostatistician in our University has been consulted to minimise animal numbers.

Based on the number of students participating in the course under the previous educational licence and on the number of animals used under that licence, we can anticipate the course running annually for a maximum of 18 students. Animal numbers used will be reviewed as part of our Ethical Review Process.

What in silico or ex vivo techniques will you use during training?

A demonstration video is used to show the cannulation procedure and we have plans to produce a more immersive version filmed with 360o cameras which may assist students more in seeing the procedure better. We also use recordings of behavioural experiments conducted by one of the staff who runs the course so that students can observe this experiment and analyse the data without using animals.

Will these techniques reduce animal numbers? If so, how?

Yes, the behavioural experiment recordings can be used each time the course runs. Working in groups also reduces numbers but still allows all participants, over the course of several experiments, to experience all parts of the procedure.

What other measures will you use to minimise the number of animals you plan to use in your project?

On each experimental day, groups of students are allocated two rats with one reserved and only used if required; for example if students fail to successfully measure blood pressure from the artery. Any stock animals left at the end of the course can be allocated to other projects or used for breeding. We minimise the number of animals we specifically order for this course by making use of ex-breeding animals which would otherwise by culled.

Retrospective assessment

Published: 03 February 2023

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

Only one animal was used and this was the minimum that was required to produced the video. This video will now replace use of animals in the undergraduate course.

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.

Only rats will be used in this project as they are a suitable species for studying integrated physiological responses and the effect of drug administration. Also their blood vessels are

of a suitable size for cannulation with a good chance of the procedure being successful. All procedures are non-recovery and performed under carefully controlled anaesthesia which minimises stress, pain and suffering for the animals.

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

We are investigating the cardiovascular system and so the best models of cardiovascular disease are in mammals and this is why we use rats. They are also of a suitable size for blood vessel cannulations. In ALL experiments we are using terminally anaesthetised animals and depth of anaesthesia is carefully monitored.

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

In all experiments, suffering to the animals is minimised by careful experimental technique and monitoring of the depth of anaesthesia. Close supervision by experienced staff of the students as they perform the experiment means that they can step-in if required.

Once terminally anaesthetised the harms are minimised. We also have refined the animal handling procedure during induction of anaesthesia to minimise stress by adopting the tunnel handling technique and we demonstrate this to the students: https://www.nc3rs.org.uk/handling-and-restraint

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

We design and report all our studies according to the ARRIVE guidelines which are widely accepted and endorsed by the scientific community:

https://www.nc3rs.org.uk/arrive-guidelines

Students are taught the principles of ARRIVE and when writing their laboratory reports they follow these guidelines.

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

There are several sources of information available including the NC3Rs website: https://www.nc3rs.org.uk/

I also regularly correspond with the NACWOs (animal house staff) and the NVS where any problems arise and to get the most up-to-date information on best use of anaesthetic agents or any other information relevant to the procedures performed under this protocol.

Retrospective assessment

Published: 03 February 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 rat was the most appropriate animal for education and learning about the function of the cardiovascular system. As a terminal procedure, post-operative care was not relevant but good handling, smooth induction of anaesthesia and close monitoring of depth of anaesthesia ensured that the animal's wellbeing was being considered during the procedure.

21. Skin cancer survival in the ageing population

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

Skin Cancer: melanoma and non-melanoma, Tumour Microenvironment, Sex bias, Damage accumulation, Aging

Animal types	Life stages
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?

This project will dissect the biological mechanisms explaining poor survival in elderly patients with melanoma and epithelial cancer, and may provide new rationales for adjuvant therapy. Briefly, we aim to discover why old people develop more of these cancers, why it is more aggressive and why they die more than similar patients, with equal disease stage, who are younger.

The overarching aims of this project are:

Aim 1: To study how the aged host across different skin types is more vulnerable to melanoma and epithelial cancer development and progression, or presents an aberrant response to ultraviolet radiation (UVR), the main risk factor for melanoma.

Aim 2: To determine if melanoma and epithelial cancer from older and male has distinct biology that leads to poor outcome.

Aim 3: To determine the differences in skin and oral/respiratory epithelia by age that change homeostasis, cancer onset and progression, which encompass the microenvironment, tumour biology and therapy.

Aim 4: To determine how adipocytes affect homeostasis, disease, and therapy by age.

Aim 5: To determine how damage accumulates in tissue and leads to tissue failure in different organs, the heterogeneous consequences of damage to tissues in individual hosts, and how this links to cancer risk, onset and progression.

A retrospective assessment of these aims will be due by 15 March 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 aged population and their disease represent a top NHS priority, as the population ages and suffers from comorbidities. The majority of cancer deaths occur in the aged population, with a male bias, and this is in part due to the accumulation of damage to tissue over a lifetime. For example, 70% of skin cancer deaths occur in those aged 65 and older, and most skin cancer new diagnoses (>80%) occur in the same cohort. This is similar to most cancers driven by environmental damage (UV light, tobacco compounds), which present a strong age and sex bias. Although cancer is a disease of the elderly, the majority of programmatic cancer research does not stratify care by host age, sex and amount of damage accumulated as we age. Thus, the question I wish to address will impact patient care directly. If these studies reveal that certain adjuvant therapies may benefit specific strata of the patient population, I am in the ideal position to translate these findings directly into clinic, and the long-term goal is to set up adjuvant clinical trials to care for elderly patients with skin cancer.

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

The primary output of this project is to prevent age-related cancer onset and death. We will additionally develop new methods to advance our knowledge acquisition in the ageing and cancer field. We will study mechanisms of: 1. How tissue ages and responds to damage accumulated through life; 2. How tumour cells interact with aged / young host cells of the microenvironment cells from males and females; 3. How this affects tumour onset, progression and response to therapy; 4. Can we find ways of mitigating the original ageing process to avoid age-related tissue failure and cancer. Our findings will be made available to the broader public and other scientists through publication in peer-reviewed journals, presentations at scientific conferences and meetings and outreach.

The expected benefits of the work can be summarised as follows:

1) Knowledge of the impact of age and sex on ageing tissues.

2) Knowledge of the role of damage accumulation in ageing tissues (microenvironment) and in initiating, driving cancer forward and therapy response.

3) Development of more representative models of ageing and cancer.

5) Identification of key therapeutic targets for future hypothesis driven therapeutic intervention to mitigate the effect of age on cancer risk.

6) Test of novel therapeutic agents currently investigated in clinical trials and their potential mechanism of resistance specifically by age and sex.

7) Publication in peer-reviewed journals and presentation at conferences to share the work with the wider scientific community.

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

We shall publish our work in peer-reviewed journals, thus sharing our findings with the scientific community.

For this, we will:

1. Study how aged people/hosts and aged skin is more vulnerable to skin cancer in order to better advise the at-risk population and compare how damage accumulation due to UV affects tissue function and cancer onset, progression and death. We will obtain and breed mice with a genetic propensity to develop ageing in a similar pattern to humans, so we expect to fully accomplish these aims within the next 4 years.

2. Compare our findings to other ageing organs exposed to environmental damage with a predisposition to develop cancer, particularly in the aged population. These experiments will require breeding of animals, exposure to carcinogens, and time to develop changes in keeping with ageing, preneoplasia and neoplasia. These experiments are long term, we aim for 5 years.

3. Investigate if age and UV damage background to the host affects prevention, adjuvant and late stage therapies. These experiments will require developing the ageing phenotype with exposure to damaging agents and then therapeutic cycles of prevention or treatment. We aim to start delivering in 3 years, as the main lab goal.

4. Conduct studies in mice to address how UV damage accumulates depending on the pigment background. Once we obtain the correct pigment background animals, we hope to run experiments in the next 2 years.

Our data may truly change clinical care of patients at-risk of suffering from skin cancer. We will complement mouse studies with human tissue analysis. These human experiments will run in parallel and will be finalised within similar timeframe.

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

Our findings will be made available to other scientists through collaborations, publication in peer- reviewed journals and presentations at scientific conferences and meetings. The Establishment has a policy of ensuring that all publications from Establishment scientists are available on free access to all.

Species and numbers of animals expected to be used

- Mice: 1250

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 have demonstrated, in my previous work and seminal work from other groups, and validated peer review publications, that they present highly comparable pathophysiology to human ageing and disease. They offer a highly reproducible system, higher levels of conservation in nucleotide and amino acid sequences, with greater finesse and human overlap than less sentient model systems (fish,invertebrates) in their pathophysiology. This is important as we intend to use reagents such as small molecule inhibitors and antibodies that have been developed to target human proteins.

Adult mice will be used and the development of the ageing phenotypes, disease and pathophysiology closely resemble the human condition. Specifically, the skin and epithelial structures of non-protected species and less sentient species do not sufficiently resemble the human organs, so we would be unable to use them for animal models of ageing cancer due to environmental toxics. We cannot model age in embryos.

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

Some mice (bred under other Project Licences) harbour genetic modifications similar to the human disease, which pre-dispose them to the development of cancers when exposed to an appropriate agent such as UV light or chemical carcinogens that are inhaled due to pollution and smoking, such as benzopyrene. In other model mouse system approaches, tumours / cells will be implanted under the skin of the mouse for ease of monitoring, or into the mammary fat pads or the blood stream to study cancer spread (metastasis). Mouse cancer cell lines can be transplanted into mice sharing the same genetic background (so called syngeneic mice) without rejection, which enables the study of the immune system in disease development. This also works when mice and cell lines are congenic, with a minimal genetic variation between the host and the tumour/cell implanted. Skin cancers and most epithelial cancers, which are frequent in ageing humans, may be induced by either chemical or environmental agents, exposure to ultraviolet light to accelerate the disease development (and possibly progression).

Tumour growth is generally not associated with pain during the period in which we conduct our observations. However, on occasions some of the tumour growths may become ulcerated. Under these circumstances we monitor their progression very carefully and manage their treatment to minimise the potential pain or discomfort, taking advice from the NVS and NACWOs. Tumour growth will be monitored regularly by either use of callipers for superficial tumours, or by imaging methods such as ultrasound and other imaging methods for internal tumours. For some procedures that involve surgery under general anaesthesia, such as implanting tumour fragments or removing a primary tumour in order for secondary tumour to grow, we will administer pain relief and monitor the mice closely during recovery.

Some mice may have either potential novel therapeutic agents, existing clinical agents or placebo administered by a variety of routes, but usually either orally, or by injection either under the skin or into the abdomen to study the effects on tumour growth and / or tumour composition. The mice will also have blood samples taken either from the tail vein whilst conscious or by sampling from a heart chamber under terminal anaesthesia (in which case the animal does not regain consciousness before humane termination).

Mice may be studied for up to their 24 month of age to study the effectiveness of treatments on tumour growth. Fast growing tumours will be monitored daily.

Mice will be group housed in ventilated cages which have their environment enhanced with items such as tunnels, houses, nesting material and gnawing blocks.

At the end of any protocol mice will be killed humanely.

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

The impact of the gene modifications is not expected to cause any adverse effects per se other than, in the case of the tumorigenic mutations, promoting the propensity to produce tumours. One difference is if we use UV to induce tumour formation or tumour progression,

we may see different UV sensitivity depending on the animal strain. We have experience with this and we know to start any UV regime with very low doses (equivalent of 5 minutes UVA / UVB in North West England in June, 6 Standard erythema doses (SED)) until animals progressively adapt to higher doses and we confirm tolerance.

We do not administer more than 16 SED. It is also possible that the tumour growth might affect normal physiological functions (such as eating, locomotion or breathing) however, mice will be observed daily and if they develop any side effect that cannot be managed satisfactorily they will be killed humanely.

Injections would only cause very transient pain. Tumour growth is generally not associated with pain during the period in which we conduct our observations. However, on occasions some of the growths may become ulcerated or interfere with bodily functions. Metastatic tumour effects are anticipated to be sub-clinical but mice will be monitored carefully for signs of pain and discomfort for up to 18 months.

Signs of metastasis usually are shortness of breath (lung metastases), general malaise (liver, kidney) and alterations in gait (brain).

After surgical procedures we will monitor mice for signs of pain and administer effective pain relief for as long as it is required.

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 vast majority of mice are only expected to experience the mildest clinical symptoms due to tumour growth before they are humanely killed. A number will experience ulceration of the tumour growth. The number of animals that experience ulceration varies depending on strain. The resilient strains to be used in this work are the ones most commonly used and present approximately 20% superficial ulceration that can be managed with topical care alone. We have extensive experience in our center and on our previous licence. The most sensitive strains are animals carrying defects in pigmentation (MC1R red hair, albino white coat) and those with defects in damage repair genes (XPC). To this date, ulceration has not led to termination of experimental animals. Some mice will also experience the discomfort of repeated (daily) injections of therapeutic agents or oral delivery with a specialist tube.

Some will experience mild sunburn (mild redness of the skin followed by skin blistering). We will aim to utilise the least stressful dose and route of drug administration wherever possible.

Mice that undergo surgery will be anaesthetised for the operation and receive pain relief peri- operatively until pain subsides. Some mice will also have repeated anaesthesia for

the purposes of imaging the internal tumours. Whilst loss of consciousness may be distressing this is not painful. We do not predict other unexplained adverse events in these models.

Our anticipated severity proportions for this project are:

5-10% subthreshold (mice used for breeding only) 60% mild (mice undergoing simple non-surgical subcutaneous implantation of tumours that do not grow beyond a certain size and do not ulcerate) 35% moderate (mice bearing more invasive tumours that may ulcerate and undergo surgical procedures).

We aim to minimise the appearance of severe endpoints with careful monitoring, surveillance and care.

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

Killed

A retrospective assessment of these predicted harms will be due by 15 March 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 will only use animals where there is no suitable alternative. Modelling ageing and cancer is complex and involves a number of different tissues and cell types e.g. epithelial cells, fibroblasts, immune cells, blood vessels, and as yet no in vitro cell culture system exists that faithfully phenocopies the aged microenvironment in different organs. In addition, epithelial and skin cancers are characterised by a particular pattern of extracellular matrix (ECM) remodelling and damage during ageing, as a function of environmental toxicity. The infiltrating cells and ECM deposition, collectively known as the tumour microenvironment, plays a major role in age-related loss of tissue function, tumour initiation, progression and metastasis. Moreover, drug efficacy is can be affected by the properties of the aged ECM and tumour microenvironment.

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

My laboratory invests strongly to establish better in vitro models that reflect the complex in vivo environment. We use a large proportion of 3D models from non-cancer and cancer

cell lines established from aged human donors. For example, we mimic 3D skin by combining all the cells, and then add cancer cells to test how tumours behave. We have published these methods. We also are developing tissue explants to model disease onset and progression. We also collaborate with the local School of Mechanical, Aerospace and Civil Engineering, to develop 3D Bioprinting to synthesise and print human skin grafts using patient material. We conduct a great deal of research in these systems already and use these to inform our in vivo experiments such that the number of animals used can be limited. Moreover, we are continuously assessing new ex vivo model systems to compliment the in vitro co-culture models. We will continue to test and develop these in vitro and ex vivo systems over the next few years to address how well they model human tumours.

Why were they not suitable?

Whilst enormous progress has been made in the field of cancer research using in vitro models, there are a number of questions that can only be addressed using animal models of disease. For example: heterogeneity of ageing and disease onset within an animal and within a population (inter- and intra- heterogeneity), investigation of disease progression and therapeutic response, resistances and interactions with the immune system is most faithfully addressed using 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 15 March 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 to be used is estimated as follows:

- We currently have 5 projects which directly interrogate the interactions between skin tumour cells, aging of skin, environmental damage and the microenvironment. Of these, all have planned in vivo studies. We have two projects which aim to optimise and

refine models of metastasis. In addition, several projects in my laboratory rely on in vivo models to test specific hypotheses generated from in vitro studies.

- A few studies are anticipated to start and develop under the next 5 years which will require extensive analysis of tumour / stromal cell interdependencies and effect of therapeutic intervention

- The experience from last 5 years and recent increase in the number of animal models that have been used in the lab.

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

Experiments are designed using the principles outlined in the experimental design tool on https://eda.nc3rs.org.uk/ and reported following the ARRIVE guidelines.

Power analyses will be performed to ensure that we use the minimum number of mice to generate significant results. In cases where power calculations are not feasible, group size will be estimated using the relevant statistical tests. Moreover, we involve those with statistical expertise to ensure that we are using optimum groups sizes, and hence minimum number of mice, in our experiments, we use optimal procedures to reduce the number of mice. For each implantation experiment with a new tumour cell line, a small number of animals may be used to determine their tumorigenic potential and the required number of cells necessary to establish tumours so that the smallest number of animals can be used for the experiment itself. Similarly, for implantation experiments involving a new stromal cell line or subsets, a small number of animals may be used to determine their biological impact on tumour growth and the required number of cells necessary to establish an effect so that the smallest number of animals can be used for the experiment itself.

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

Pilot experiments (using up to 4 animals) may be conducted to estimate the biological effect of an intervention (such as a drug or exposure to a carcinogen) when not known or available in the literature.

We use optimal breeding strategies to reduce the number of genetically engineered animals.

We take care to ensure that each experiment is appropriately analysed and that the maximum amount of information is gathered thus reducing the need for experiments to be repeated. For example, at the end of each experiment, data is compared to previous studies by appropriate statistical methods (e.g. Kaplan-Meier plots of age at endpoint, Mann Whitney analyses using a one tailed distribution to reduce mouse numbers). We are keen to run experiments in parallel that can share a single control arm, which can

sometimes represent a 25% reduction of animals within a given experiment. Where cell transplantation models are no longer needed, cells and / or tissue will be frozen to avoid unnecessary propagation of the model. In vivo experiments are preceded by relevant in vitro experiments.

One approach in our lab is to generate carcinogen-damaged cell lines from the stroma (fibroblasts) of in vivo animals from pilot experiments, then use the cell lines to establish further in vitro evidence, missing with tumour cell lines, enriching and confirming in vitro approaches, before re starting experiments in vivo in larger numbers. This allows for significant planning and refinement.

A retrospective assessment of reduction will be due by 15 March 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 skin, oral and lung squamous cell carcinoma (luSCC) models of cancer which can be driven by environmental exposure and/or genetically modified backgrounds. The aim is to compare how environmental damage, which is linked to tissue ageing, then tissue loss of function, then cancer incidence and possibly cancer progression compares across organs with the ultimate goal to understand why it happens, why the rate differs in different organs (maybe by sex), how to improve it, how to stop cancer. For the early damage acquisition models, we will expose animals to low dose of carcinogen (I.e UV, benzopyrene, acrolein), mimicking human exposure, to drive the ideal tumour microenvironment (TME) upon which later skin, oral SCC and luSCC arise. For the cancer onset models, we will use the same approach, increasing the amount of exposure to carcinogens to drive premalignant, early skin, oral SCC and luSCC, then the full malignancy. We may compare this pathophysiological mechanism of disease to the established GEMM models for each organ, but only if necessary as our focus is in environmentally driven tissue damage, ageing and cancer. We will induce these changes directly in the organs by exposing them to the relevant damaging agents, which include but may not be limited to: skin: UV light, UVA, UVB or a mixture of both (UV6); DMBA/TPA;

oralSCC: i.e benzopyrene, acrolein; luSCC: benzopyrene, tobacco extracts. For the skin, the skin of animals will be exposed to different regimes of damage under general anesthesia (for UV) or without anesthesia (DMBA/TPA), with veterinary advice if aiming to reduce the amount of interventions to an animal. For oral SCC the animals will be exposed to the carcinogen swabbed within the oral mucosa under general anesthetic vs in drinking water. For luSCC, the animals will be exposed to inhaled carcinogen under general anesthesia. For studies looking at stroma tumour microenvironment, we may use the original milieu (skin) or mammary fat pad injection for adipocyte-specific studies. We will also use intradermal or subcutaneous orthotopic models with cancer cell lines.

Some of the models may lead to acceleration of growth and tumour ulceration, the most likely adverse side effect we presume will arise, based on our experience. We will manage ulceration as follows:

Some cancer models are aggressive and ulceration is a common feature of the normal history of cancers nearing a body surface or lumen in humans, and since we aim to develop mouse models that reflect the human condition, we expect ulceration in the tumours in our mice, even at early stages of growth, especially when implanted in the mammary fat pad, intradermally or subcutaneously.

Assessing the impact of ulceration on quality of life in a mouse is subject to great inter-observer variability. There is clearly a need to define clear, replicable assessment criteria to guide our practice. We propose to use our scale to assess ulceration, from perfect, unblemished skin to skin deficits that are larger than 0.5cm and very symptomatic. Briefly, for very mild loss of the upper layers of the skin that have no symptoms, we will observe, moisturize and clean. If the wound is slightly more deep, and the wound is wet, we will treat with antiseptic. Anything of larger size to 0.5 cm that causes distress to the animal due to pain or superficial infection will require that the mouse is humanely killed.

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

Less sentient animals do not exhibit a similar microenvironment reaction and histopathological features as ageing humans. Mouse is far more similar to humans than other animals and this is critical both for using reagents like drugs developed for human targets and for translating findings to the clinic. Cancers develop over many weeks to months, so use of terminally anaesthetised animals or immature animals is not possible.

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

Animal suffering will be minimised by making every effort to keep the tumour models employed at the subclinical levels. Wherever possible, this will be achieved by using non-invasive imaging modalities to monitor tumour growth and the development of metastatic disease. In addition, as detailed in the individual protocols, steps will be taken to minimise the severity of the procedures. Finally, we will ensure that all animals receive the highest

standard of care, and preventative medicine (including anaesthesia and analgaesia where required) will be used.

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

Relevant published literature will be used as template for experimental design and decision making (Workman et al., 2010. Guidelines for the welfare and use of animals in cancer research. BJC, 102, 1555-1577).

We will follow guidelines of good practice [Morton et al., Lab Animals, 35(1): 1-41 (2001); Workman P, el al. British Journal of Cancer, 102:1555-77 (2010)] administration of substances will be undertaken using a combination of volumes, routes and frequencies that themselves will result in no more than transient discomfort and no lasting harm.

Guidelines for Body condition score. [Ullman-Cullere, Lab Anim Sci. 1999 Jun;49(3):319-23]

Aging mice will be monitored and managed according to Wilkinson et al (2020) Laboratory Animals: 54(3): 225 – 238.

We will consult the NC3Rs guidelines and monitor refinement where such practices are published (NC3Rs website and elsewhere).

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

By reading 3Rs literature and participating in 3Rs workshops locally and nationally. Through discussing refinements with our NACWO, NVS and HO inspectorate. We have regular Establishment updates such as Annual licence holder meetings.

A retrospective assessment of refinement will be due by 15 March 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. Developmental dynamics of tissue formation

Project duration

5 years 0 months

Project purpose

- Basic research

Key words

developmental biology, spinal cord, central nervous system, vertebrate embryology

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?

How are the right types of cells produced in the right place, at the right time, in the right numbers in a developing embryo? We study these questions in the central nervous system (CNS). Despite its complexity, the CNS is assembled in a remarkably precise and reliable manner. This precision is necessary for the wiring of nerves into the functional neural circuits that gives the CNS its function. Our research focuses on the spinal cord, which is the part of the CNS that allows us to sense our environment and respond to it by moving muscles. Our goal is to understand how the spinal cord forms during embryonic development by determining the mechanisms that produce and organise the cells involved.

A retrospective assessment of these aims will be due by 23 March 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?

Understanding the embryonic development of the spinal cord will shed light on the formation and function of the adult spinal cord. Understanding the molecular and cellular processes of spinal cord development is important for developing stem cell based methods for the generation of artificial spinal tissue for use in regenerative medicine and disease modelling applications. Moreover, knowledge of normal spinal cord development will provide insight into the diseased and damaged nervous systems. Neurodegenerative diseases such as motor neuron disease, tumours such as paediatric glioblastomas, and congenital disorders such as spina bifidia all involve the spinal cord. Understanding how the spinal cord forms in embryos will help in the development of therapies and treatments for these severe conditions.

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

The main goal of this work is to advance our biological understanding of embryonic development and the outputs from the programme will include new knowledge and publications in peer-reviewed journals.

Expected outputs include:

1. A better understanding of how the actions of a protein, Shh, organises the pattern of gene expression in the spinal cord.

2. Knowledge of how gene regulation in individual cells allocates developing progenitors to one of several possible fates.

3. Insight into how gene activity is controlled by the actions of specialised regions of the genome.

4. Genetically altered lines of mice that contain reporters for specific molecular activities or specific mutations in defined genes. These may be of broad benefit to the research community as they can be used in numerous other projects.

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

Knowledge of the basic mechanisms of embryonic development will be of interest to scientists studying development and stem cell biology and is essential to understand the causes of dysfunction in disease states. The availability of this knowledge will lead to a better understanding of tissue function and is likely to be employed and extended by other researchers. These intellectual impacts have practical significance. First, it is now well established that, if deregulated, basic developmental processes can result in disease states that range from neurological disease, such as autism, neuromuscular diseases, to cancers such as gliomas. This will be of interest to clinical researchers studying these diseases.

Second, the ability to direct the differentiation of stem cells to specific cell types will be a major impact of this project. Given their unique properties, stem cells are promising candidates for tissue engineering, cellular therapies and drug screening. A significant problem, however, is generating populations of desired cell types from initially pluripotent stem cells. We anticipate that based on new knowledge of the basic mechanisms of development uncovered in this project, improvements in the current state of the art approaches for stem cells will be forthcoming.

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

We will maximise the outputs of our work by the timely publication of primary research papers and the presentation of the work at both big international meetings and smaller workshops. Not only will the positive results be communicated, but where it is useful for other researchers, unsuccessful approaches will also be highlighted.

We collaborate widely, both internally and externally and this provides another avenue to share details of approaches that were ultimately sub-optimal and how methods were improved.

Where will release pre-peer reviewed versions of our work on bioRxiv to ensure its rapid dissemination.

Species and numbers of animals expected to be used

- MICE: 8000

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 aim is to unravel the cellular, molecular and genetic mechanisms of embryonic development of a vertebrate tissue. Use of animals and tissue derived from animals is essential for this. In particular, the generation and analysis of genetically altered mouse

embryos is necessary to test the function of specific genes and this requires the breeding of genetically altered mouse lines. The similarity between the human and mouse nervous system means that it is necessary to work with mice. Mice offer the unique advantage of offering sophisticated genetic tools that allow precise functional experiments and the ability to construct quantitative reporters of gene activity.

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

The vast majority of our regulated procedures involve the breeding of genetically altered animals or minor interventions such as injections, with minimal effects. Typically several hundred matings of genetically altered mice will be performed each year. These will be used either to maintain specific genetic lines or to produce embryos with specific genetic make up in order to study gene activity.

Injections will be used to introduce substances that alter gene activity or label replicating DNA, these substances typically have little if any noticeable effect on the animals. Some of the genetic mutations we use may directly lead to mild effects on the animals, such as mice having more than the usual number of toes. Some procedures involve surgery. These surgeries are necessary for the creation of new genetically altered animals and involve transferring embryos into uterus of female adult mice. Such animals will be closely monitored, and anaesthetics, analgesics and/or other ameliorative procedures will be used as appropriate. In all cases, animals will be humanely killed if there are signs of pain, distress or suffering above agreed limits. We are careful about group sizes, using the minimum numbers of control and experimental animals compatible with robust conclusions, making use of statistics when appropriate.

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

For the majority of experiments we do not expect any adverse effects as we will be mainly working with genetically altered animals that have little if any noticeable negative effects on the animals carrying the genetic alterations. Some the genetic mutations we use to study the spinal cord may also lead to mild effects on the animals, such as mice having more than the normal number of toes or small eyes. These malformations are lifelong but do not cause distress or suffering. Some of our procedures involve surgery, these are carried out under appropriate anaesthesia. Post-operative pain is managed with analgesics and full recovery is usual within one or two 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)?

Moderate severity is expected for about 5% of mice. For particular genetically altered strains, between 5-10% of genetically altered mice die suddenly, without showing any prior

health concerns. As the reason for death is unknown, we cannot exclude any suffering prior to the event, those mice will be deemed to have had a severe experience. The rest of the mice will reach a maximum of mild severity.

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

- Killed

A retrospective assessment of these predicted harms will be due by 23 March 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 have been studying vertebrate embryonic development for 25 years and have gained tremendous insights into the mechanisms of tissue formation and the molecular and genetic control of cell behaviour from working in model tissue culture systems. However, in order to be able to investigate tissue formation in embryos, we need to perform experiments in animals.

We use mice because, as a mammal, their embryonic development closely resembles that of humans. Moreover, sophisticated genetic and transgenic tools are available that make it possible to generate mutant or transgenic lines in a highly precise and efficient manner. To minimise the number of procedures performed on animals deemed to be sentient, we do most of our work with embryos before they have reached 2/3 of their gestation time. The only work that will be done on adult animals is biopsying for genotyping, administration of substances to control gene expression and surgical procedures necessary in the generation of new transgenic lines.

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

We complement our in vivo analyses with the use of tissue culture models and organoids. Our studies are likely to further validate these in vitro systems and promote their use with other researchers.

Nevertheless, in the development and validation phase it will be necessary to carefully compare and benchmark these methods with normal embryonic development.

Why were they not suitable?

We gain a certain amount of information from in vitro systems, but there are limitations. It is not currently possible to replicate the complexity and precision of mammalian tissue development in culture models. The complexity of embryonic development, which arises from multiple interactions between different cell types, involving short and long range signalling molecules, and complex morphogenetic events over time, requires in vivo analyses

A retrospective assessment of replacement will be due by 23 March 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 calculate the numbers of animals required based on the numbers of mutant and transgenic lines that we are currently maintaining, plus those that we need to generate to be able to achieve the aims of the project. We carefully design experiments to be sure that we use the minimum number of animals required to give clear scientific answers. We also make extensive use of in vitro assay, in particular cell culture, and in silico mathematical modelling and simulation. This greatly helps experimental design and reduces our use of animals. We also use several hundred chick embryos every year, from embyonated eggs before 2/3 of the gestation period. The accessibility of chick embryos allows us to do experiments that would otherwise have to be performed in mouse embryos and require the termination of the pregnant female. This results in a substantial reduction in the number of animals we use.

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

We regularly review our mutant and transgenic stocks and cull any that are no longer required. We freeze sperm and/or embryos to archive the line. Thus, we will only maintain breeding lines that we are actually using in on-going experiments. Through exchanges with other labs, in the UK and elsewhere, we are able to minimise the number of mutant and genetically modified strains that we keep. Moreover, the stocks of adult animals that

we keep are mostly heterozygotes carrying recessive mutations and are phenotypically normal. In addition, because we share and exchange mouse lines with a number of other labs, we ensure we decrease the number of animals in use.

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 aim to keep as few mice as possible by careful monitoring our mouse colony and good practice. To minimise breeding, lines under sporadic use are maintained at lower levels, and frozen whenever practicable. Lines will be maintained in collaboration with other licensees wherever possible to minimise redundant breeding.

Our mouse lines are routinely maintained by keeping 2-3 breeding pairs, with around 3-4 litters/year - total 75-100 animals per strain/year. For crosses to enable characterisation of specific phenotypes, in general 5-6 breeding pairs will be kept with 6-8 litters/year - total 350-400 animals per strain/year.

We use local expertise as well as consult with collaborators to optimise the number of animals used.

Whenever possible and when there are no harmful phenotypes we maintain genetically altered mouse lines in appropriate genetic combinations of alleles to reduce the numbers of animals required.

A retrospective assessment of reduction will be due by 23 March 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 mice as a model for mammalian development, as mouse embryos share key features with humans and can be manipulated genetically. Our embryological work is preceded by in vitro and/or in silico studies to test the approach or experimental design before introduction into animals. Failure at this preliminary stage is taken as final and no in

vivo work will take place until this step is successful. In addition, we minimise suffering by maintaining a high health status of the animal population, by attention to feeding regimes and environmental enrichment of cages. We check all stock daily and cull any that show signs of significant illness or deformity. Where surgical or other potentially distressing procedures are required, these are performed under appropriate anaesthesia with analgesia both pre and post operation. Any animals showing signs of distress on recovery from a surgical or other procedure are killed promptly by an approved method.

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

The majority of our work is carried out on embryos prior to 2/3 of gestation and these are not considered sentient. However, to produce these embryos breeding of adult mice is necessary and the generation of new mutant or transgenic lines entails surgical operations followed by recovery of the operated animal. We use mice as a model for mammalian development, as the development of the nervous system and other organ systems of mice shares key features with humans.

For some experiments we use chick embryos. In many cases the similarity in molecular and cellular mechanisms between different vertebrate species allows experiments to be performed on non- mammalian species and the results can be extrapolated to mammals. Nevertheless, in some cases the use of mice is unavoidable.

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

We try to minimise any possible adverse effects. Surgical procedures will be performed aseptically with suitable anaesthesia and animals monitored post-surgery to ensure that they recover well. We will also use suitable analgesia for all surgery.

We choose well-established protocols, known to have minimal harmful effects, whenever possible. Animals produced in this project are not expected (<5%) to exhibit a moderate phenotype. However, it is not possible to fully predict the nature or severity of any potential defect and for all types of mice there will be careful monitoring for possible side effects. Animals exhibiting any unexpected harmful phenotypes will be killed using an approved method, or in the case of individual animals of particular scientific interest, advice will be sought from the Home Office Inspector.

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

We are aware of NC3Rs. We also discuss with colleagues in other research groups and the BRF new improvements that lead to refinement.

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

We will stay up to date via regularly communication with BRF staff, other scientists in the field and regular visits to the following website https://www.nc3rs.org.uk/3rs-resources

A retrospective assessment of refinement will be due by 23 March 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. Mode of action of an anti-inflammatory parasitic worm product

Project duration

3 years 6 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

anti-inflammatory drug development, chronic inflammation, co-morbidities of ageing, parasitic worm

Animal types	Life stages
Mice	adult
Gerbils	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 aim of the project is to increase our understanding of how an anti-inflammatory molecule produced by a parasitic worm works and to investigate how it might be used in the development of new drugs against chronic inflammatory conditions and related health problems associated with ageing.

A retrospective assessment of these aims will be due by 16 September 2025

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?

It is considered that more than 10% of the Western population suffer from one or more of allergic conditions, autoimmune diseases, and the consequences of an unhealthy life style, e.g., cardiovascular disease/type 2 diabetes (T2D), and this number is increasing at an alarming rate globally. Current treatments for these conditions involve the use of drugs that suffer from problems such as unwanted side effects or limited efficacy and indeed there is absolute failure to improve the condition of some patients. Over recent decades, there has been increasing interest in the idea that parasitic worms, through the release of anti-inflammatory molecules, might protect humans against the development of such diseases, each of which is associated with abnormal inflammatory responses. This reflects data emerging from both human epidemiological studies and mouse models of inflammatory diseases. Our work has shown that one particular worm molecule protects mice against a range of distinct inflammatory conditions. If we could fully understand how the molecule is able to do this, then we could use this information in the development of urgently needed new drugs against these conditions.

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

Outputs will include:

1. New information on the mechanism of action of the parasitic worm molecule we are studying

2. New publications incorporating the new information

3. New information relating to the design of anti-inflammatory drugs based on the parasitic worm molecule and the mechanism by which it works

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

The outputs will be of benefit to our efforts to produce anti-inflammatory drugs from the parasitic worm molecule we are studying. To date, we have designed novel synthetic drug-like versions of the molecule, whose success in protecting mice against inflammatory disease development/progression has led to a licensing agreement with a company to progress further development towards clinical trials. However, in spite of having generated a great deal of information, we do not fully understand how the worm-derived molecule works and indeed within the last few years have intriguingly discovered that the molecule is both impacting on the bone marrow and interacting with microorganisms that colonise the gut, when generating its anti-inflammatory effects.

By increasing our understanding of how the worm molecule works, we hope to develop new drugs and treatments that clinicians are able to offer their patients for ailments associated with chronic inflammatory responses as set out above. In addition, our research and publications will likely be of benefit to other immunologists and parasitologists working in this area.

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

We will ensure the wider scientific community is made aware of our work by submitting manuscripts to high quality, open-access journals broadly covering general medicine/ immunology/drug discovery areas, by participating in relevant international scientific conferences and wherever possible, by drawing attention to our website, Facebook and Twitter pages, each of which engages with stakeholders and the public. All of the data generated from the project will also be discussed with the company with which we have a licensing agreement, to enable them to exploit any they wish in their pursuit of new treatments for unwanted inflammatory responses.

Species and numbers of animals expected to be used

- Mice: 1,360
- Gerbils: 80

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 gerbils facilitate development of the parasitic worm that we study. The gerbils constitute a natural host for the worms and suffer no obvious evidence of pathology under the infection rates employed. Mice have easily the best-characterised immune system of all non-human mammals and so represent the optimum model species for studying diseases associated with unwanted inflammatory responses. The appropriate, well-tested, industry-standard models and reagents that we will employ in our models of inflammatory disease are thus based on immunologically mature adult mice. Also, we have previously

shown the parasitic worm molecule that we are studying to have health-inducing effects in the mouse models, e.g., by inhibiting chronic inflammation and expanding male mouse lifespan and thus the mouse can be used to gain understanding of the molecule improves health.

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

Gerbils: Adult male gerbils will typically receive 120 infective larvae of the parasitic worm by subcutaneous injection. These develop into adult worms, the females of which release the microfilaria worm stage which can be detected by examination of blood samples obtained by superficial venepuncture. Once microfilariae are detected, the adult worms may be recovered from the subcutaneous tissues of the gerbil following killing by a humane method. This is usually done within 12 months of infection, but occasionally may stretch to 18 months. Alternatively, gerbils harbouring microfilariae, are employed to infect ticks which act as an intermediate host for the parasitic worm. Here, gerbils are anaesthetised, fur removed from the abdomen and ticks allowed to feed. The ticks detach spontaneously after ~1 hour and the gerbils regain consciousness. It is estimated that ~80 gerbils will be infected during the four years of the project, around 20 of which will be subjected to tick feeding.

Mice - tick feeding: mice will be anaesthetised, fur removed and ticks applied to allow feeding for ~1 hour. The mice will not wake from the anaesthesia. It is estimated that ~200 mice will be required over the four years of the project.

Mice - collagen-induced arthritis model: two injections of type II collagen will be given with a 21-day interval between injections – this stimulates the development of arthritis similar to that seen in humans. The parasitic worm-derived anti-inflammatory molecule that we study or the synthetic derivatives prepared from it will be administered by subcutaneous injection before and/or after collagen injection.

Some mice will receive antibiotics or other substances to alter the bacteria in their gut or improve gut health to assess the impact of gut function on the ability of the parasitic worm molecule or synthetic analogues of the molecule to modify arthritis development and to help understand how the molecules work. On some occasions, mice will receive other substances with defined anti-inflammatory effects for comparative purposes, which will also help us to understand how the parasitic worm molecule works. Some animals will receive cells such as bone marrow or immune system cells or alternatively faecal matter from animals treated with the worm-derived substance or synthetic analogues to assess whether their effects can be transferred to recipient animals. Some mice will undergo blood sampling. The studies will last no longer than 6 weeks from initial collagen injection and all animals will be killed by a humane method with tissue samples retained for a wide variety of analyses.

We estimate about 460 adult mice will be required for these studies.

Mice: obesity accelerated ageing model: Mice will be fed either a Western-style, high calorie diet or a conventional diet for up to 500 days. The parasitic worm-derived anti-inflammatory molecule or synthetic derivatives prepared from it will be given at various time points by subcutaneous injection. No more than 1 injection will be given per week.

Blood samples may be taken monthly to assess markers of inflammation and ageing and the impact of the anti-inflammatory molecules administered. Some mice will receive cells such as bone marrow or immune system cells or alternatively faecal matter from animals treated with the anti-inflammatory molecules to assess whether the effects of the molecules can be transferred by immune system or gut bacteria components. Substances that change gut bacteria composition or improve gut health may also be given to assess the impact of altering bacteria on the ability of the anti-inflammatory molecules to inhibit inflammation and prevent the ill health associated with ageing and also help understand how they work. On some occasions, mice will receive other substances with defined anti-inflammatory effects for comparative purposes, which will also help us to understand how the parasitic worm molecule works.

At the end of each study, all animals will be killed by a humane method. Their ageing parameters (such as obesity [body mass]) will be assessed and tissue samples retained for a wide variety of analyses.

We estimate about 700 mice will be required for these studies.

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

General anaesthesia: no common or likely adverse effects; no more than momentary discomfort/fear; minimised by good technique. Risk of anaesthetic deaths estimated at less than 0.1%.

Subcutaneous injections: no common or likely adverse effects; no more than momentary discomfort; minimised by good technique.

Blood sampling: no common or likely adverse effects; momentary discomfort, minimised by good technique.

Tick feeding: Animals may occasionally have a mild and short-lived adverse inflammatory reaction to tick feeding.

Mouse collagen-induced arthritis procedure:

Animals developing arthritis may show signs of ill health e.g. lethargy, hunching, weight loss. However, we generally use an early time point for experiment termination and previous studies have shown that only around 5% of mice will move into this category. If impaired mobility due to arthritis development impacts upon the ability of the animal to reach food or water, then these will be placed within reach. At the peak of the inflammatory

response during arthritis, animals may show signs of pain e.g. rapid, shallow breathing, grunting on expiration and some loss of limb movement. Analgesia may be given to these animals. Any animal failing to respond to this treatment will be euthanised.

Parasitic worm-derived molecules and their synthetic analogues: these compounds affect the function of the immune system but our studies to date indicate that they have no adverse effects on general health (in fact they improve health being anti-inflammatory, including preventing arthritis). Also, all molecules to be tested undergo pre-analysis of effects on cell viability in vitro.

Intravenous sampling/dosing: no common or likely adverse effects expected; momentary discomfort, minimised by good technique.

Intraperitoneal injections: There is a risk of damage to internal organs during injection but this is minimised by good technique. Thus, no common or likely adverse effects are expected other than momentary discomfort during injection, which is minimised by good technique.

Subcutaneous injections: no common or likely adverse effects expected; no more than momentary discomfort; minimised by good technique.

Freund's Complete Adjuvant: employment of this reagent, which helps boost the immune response, may result in the formation of small ulcers at the injection site in <5% of the animals. However, these should heal and generally do not cause discomfort to the animal. If the ulcers prove to be recurrent or persist (non-healing and moist) for more than 72 hours, then the animals will be euthanised.

Mouse obesity-accelerated ageing procedure:

As with humans, high calorie diet (HCD) can impair health of internal organs such as the gut and liver but there is no indication that animals are in pain or discomfort from the procedure.

Parasitic worm-derived molecules and their synthetic derivatives: these compounds affect the functioning of the immune system but our studies to date indicate that they have no adverse effects on general health (in fact they improve health being anti-inflammatory). Also, all molecules to be tested undergo pre-analysis of effects on cell viability in vitro.

Intravenous sampling/dosing: no common or likely adverse effects expected; momentary discomfort, minimised by good technique.

Intra-peritoneal injections: There is a risk of damage to internal organs during injection but this is minimised by good technique. Thus, no common or likely adverse effects are expected other than momentary discomfort during injection, which is minimised by good technique.

Subcutaneous injections: no common or likely adverse effects expected; no more than momentary discomfort; minimised by good technique.

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)?

Gerbil procedures: 100% mild category

Mouse collagen-induced arthritis (CIA) procedure: Approximately 5% of animals with CIA fall into the severe category, approximately 75% into the moderate category and approximately 20% into the mild category. Animals in the mild category reflect where the experiment is terminated prior to development of joint pathology. Also of note, animals that are administered anti-inflammatory treatments (essentially half the animals) such as the parasitic worm molecule will generally have reduced severity of joint disease such that the proportion in the moderate category may actually be significantly less.

Mouse obesity-accelerated ageing procedure: Ageing and the high calorie diet can impact on the health of organs such as the liver or gut such that the level of severity is considered moderate. However, animals given anti-inflammatory treatments like the parasitic worm product (approximately 50%) will show reduced ill health such that the proportion in this category may actually be significantly less than the expected 100%.

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

Killed

A retrospective assessment of these predicted harms will be due by 16 September 2025

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?

Gerbils: Biologically-active parasitic worm molecule cannot be produced without gerbils. This is because the life cycle of the parasitic worm from which we obtain the product, cannot be maintained in the test tube. The type of gerbil employed is a natural host of the

parasitic worm that we study and infection with the worm at the levels employed does not cause obvious pathology within the host.

Mice: The multifactorial nature of both rheumatoid arthritis and the obesity-accelerated ageing process, dictates that animal use, including cell transfer procedures, is necessary to understand the processes involved in causing disease. Mice constitute the most extensively studied mammalian model to develop well established characteristics of arthritis and ageing and hence have been employed in our studies to date. In addition, our biobanked tissue prepared from previous work, which we plan to employ in the project (thereby saving on new animal use) is derived from mice and therefore our related planned experiments on bone marrow transfer, faecal matter transfer and administration of substances that impact on gut health must logically employ mice for comparative purposes. In addition, there is a wealth of experimental tools for characterising mouse responses, particularly in terms of immunological reagents.

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

Adult worms are required for production of the parasitic worm product that is the focus of the study. The worms must be generated via inoculation of gerbils with infective larva stages as the larvae fail to develop to maturity during in vitro culture. As an alternative approach to production of the worm product, we attempted to modify a free-living (non-parasitic) worm species to produce it. However, in spite of a great deal of effort, we were unable to achieve success.

The stage of the parasitic worm that is infective for gerbils, develops in a species of tick and we employ mice for routine feeding of the ticks. In an attempt to avoid using this procedure, we have tested a technique that involves feeding on sheep blood via an artificial membrane. However, unfortunately we found that this non-animal alternative reduced the ability of the ticks to harbour and transmit the parasitic worm.

Regarding mice, in the past, much of the parasitic worm product's mechanism of action has been elucidated by in vitro experiments but fully understanding disease conditions and attempting to resolve them by molecule/drug-based intervention requires whole animals.

Why were they not suitable?

The alternative procedures we investigated would not allow the maintenance of the parasitic worm life cycle and hence, generation of the anti-inflammatory parasitic worm molecule. Also, in vitro systems cannot provide the required level of understanding of the processes involved in generating unwanted inflammation nor enable investigation of the therapeutic potential of the parasitic worm molecule afforded by mice.

A retrospective assessment of replacement will be due by 16 September 2025

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 to be used is based on 30 years' experience of producing the parasitic worm product and 20 years' experience of testing it in mouse models of diseases associated with unwanted inflammatory responses. The numbers employed are close to the minimum used to generate statistically significant differences between control and experimental groups, as demonstrated in our previous publications.

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

Prior to testing in live animals, derivatives of the worm product are selected by non-animal testing in the laboratory and only those compounds that demonstrate statistically significant evidence of anti- inflammatory activity are selected for testing in mouse models.

Also, in a recently completed project that focused on the obesity-accelerated ageing model, we specifically generated a large biobank incorporating a range of different mouse tissues for employment in the present project. This will significantly reduce the number of mice we require in this new project, likely by up to 200 animals. Moreover, we have built up a considerable stock of the parasitic worm product, which has allowed us to estimate that we have reduced the requirement for gerbils by at least 50% for this new project.

At the same time, we routinely consult the NC3Rs Experimental Design Assistant when planning our experiments, basing our projected animal numbers on widely used statistical techniques associated with our published/pilot data. We also reviewed published literature for any studies of new approaches.

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

The main strategy we have adopted over many years of working on the mouse models of inflammatory diseases is to develop experimental procedures that enable multiple readouts from an individual animal. Thus for example, in our recent obesity-accelerated ageing project, we collected a wide range of tissues and measured over 120 different markers relating to health and well-being. In addition, in experiments where the

effectiveness of procedures has been found to be greater than expected, we have refined our statistical model to further reduce animal numbers

A retrospective assessment of reduction will be due by 16 September 2025

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.

Three models will be employed:

1. **Infection of gerbils with the parasitic worm:** The gerbil is a natural host for the parasitic worm that we study and does not suffer any obvious pain or distress at the levels of infection we employ.

2. **Collagen-induced arthritis mouse model (CIA):** In this model, we induce a condition like human arthritis in mice. The model is a gold-standard method in academia and industry, which reproduces many features of rheumatoid arthritis and has been employed both in investigating the causes of disease and in testing new therapies. In addition, all of our previous related studies and biobanked material are from this model. Overall therefore, it is the most appropriate model for undertaking our investigation. It is likely that animals will suffer pain as a consequence of the inflammatory response arising during the condition but this only reaches the severe category in approximately 5% of animals. In addition, all animals are carefully monitored several times daily for evidence of discomfort, extra bedding and nesting material added for comfort as required, analgesia given if necessary, and animals humanely killed should signs reach the severe threshold. The model is employed for the minimal amount of time required to generate the necessary information (30-42 days) and then mice are humanely killed.

3. **Obesity-accelerated ageing mouse model:** routine administration of a high calorie diet can ultimately (in the long-term) have health consequences for animals but the mice show no obvious signs of suffering or distress at the time-point at which we terminate the experiments.

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

Infection of gerbils with the parasitic worm: Gerbils constitute a natural host for the parasitic worm, which is a member of the filarial nematode group. Due to filarial nematodes as a group of parasites tending to have a restricted range of hosts (e.g., species that parasitise humans and also the gerbil-infecting species that we employ do not undergo normal development in mice) we have been unable to find a less sentient suitable host. In any case the gerbils do not suffer any obvious pain or distress at the levels of infection we employ. A vertebrate species such as a mammal or bird is required as a suitable host for tick feeding. Mice are the most readily available laboratory host and when employed in this process are entirely unconscious during the procedure and when tick feeding is complete, are humanely killed without recovering consciousness from anaesthetic treatment.

Mouse models: Mice constitute the most appropriate mammal to investigate the health conditions associated with ageing and attendant co-morbidities like arthritis and obesity driven by chronic inflammation. There already exists a wealth of experimental tools for use in mouse models of arthritis thereby providing the rationale for our use of them. Mice employed must be 6-8 weeks of age to ensure maturation of the immune system prior to commencing experiments.

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

Animals used in experimental procedures are routinely monitored to ensure well-being and those found to be suffering in any way treated as per conditions in the license. If they reach pre-defined humane end-points, they are humanely killed. We closely liaise with animal care staff and NVS to ensure best care and welfare and we regularly scan the relevant literature for any refinements in our area of research and/or husbandry of the species we use. For example, we use varied environmental enrichment for both mice and gerbils, including social exercise pens for the latter.

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

There are a number of published items providing information on best practice, which will be followed. For example, we have consulted "Ethical Guidelines for the Use of Animals in Research published by the National Committee for Research Ethics in Science and Technology in 2019 and have made use of the "Focus on Severe Suffering" website (https://focusonseveresuffering.co.uk/reports/), particularly with respect to arthritis. We also adhere to the ARRIVE Guidelines for reporting experiments.

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

We routinely consult the NC3Rs website for new advances and via the Experimental Design Assistant explore how to most effectively refine our experiments and reduce the number of animals we use. New information about advances in the 3Rs may also be obtained via information routinely provided by the our Named Information Officer (NIO) and by regular exposure (via publications or presentations) to the research work of colleagues who work in the same area.

A retrospective assessment of refinement will be due by 16 September 2025

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. Preclinical imaging in biomedical research

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

imaging, tool compounds, translational, drugs, models

Animal types	Life stages
Mice	adult, juvenile, aged
Rats	adult, juvenile, aged
Guinea pigs	adult, juvenile

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 project is to provide unique in vivo/ex vivo/in vitro data using imaging and related technologies. Important characteristics of imaging tool compounds (substances required to be administered to enable imaging to be performed) and therapies will be established, in order to determine their suitability and facilitate their use in the clinical

situation. In particular, we aim to understand the effectiveness of the imaging tool compounds and therapies in their distribution to, and engagement with, the target binding sites (proteins within the body that we wish the compounds to bind to). In addition, we aim to use imaging to further our knowledge of normal and disease processes within the body.

A retrospective assessment of these aims will be due by 23 March 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?

Medical imaging techniques may be used in humans to determine the distribution of drugs around the body and their effectiveness in binding to the target of interest. They may also be used to provide information on biological processes within the body in the diseased and normal state. This project is expected to optimise clinical imaging studies in several ways but particularly by the development of tool compounds that are necessary to perform the imaging techniques such as positron emission tomography. In addition, candidate drugs may be evaluated in rodents using imaging techniques prior to human studies, thus facilitating their development, and reducing the potential for failure at a later stage.

There are a number of disease areas in which we will employ imaging techniques including neurodegeneration, neuroinflammation, psychiatric diseases, cancer, inflammation, respiratory diseases, liver disease, irritable bowel disease, obesity and type 2 diabetes. There are currently no cures for these diseases/disorders, or their treatments become ineffective over time. Therefore, there is an unmet need to develop new and better therapies, which we will support with the work under this licence.

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

Many medical imaging approaches require the administration of a substance (tool compound) to the individual being scanned in order to generate the required images. This tool compound may be a novel drug of interest, where its distribution within the body is being measured. However, in many cases, the tool compound is not a drug and can have more than one application. For example, it may be used to understand more about a disease process, such as the concentration of a drug target in a particular tissue or organ, or, by administering it in conjunction with individual drugs of interest, it may be used to demonstrate and quantify the binding of the drugs to the target binding site.

Currently, worldwide, there are a limited number of established imaging tool compounds available and therefore a limited number of disease processes that may be investigated using these highly valuable techniques. The data obtained through this work will support the development of new imaging tool compounds by providing important information on their distribution and binding properties within the body and, therefore, assess whether they possess suitable characteristics to become successful imaging tool compounds. In particular, we will determine whether they reach the target binding site and bind to the site sufficiently, whilst at the same time, exhibiting a low level of binding to other non-target binding sites within the body. Furthermore, we will determine that the target binding site is present at a high enough density within the tissue or organ to be able to measure it using imaging techniques.

In order to assess novel imaging tool compounds and drugs, measurement of their concentration and binding to the target binding site within the tissue will typically be determined using both imaging and post-mortem tissue measurements in rodents. Where a tool compound or drug is being evaluated for later use in a particular clinical disease, an animal model, displaying aspects of the disease, may be needed. This would be the case if the presence or concentration of the target binding site within the body is expected to be much greater in these animals compared to that in naïve animals (animals that have not previously been the subject of a scientific procedure) and the use of naïve animals would not allow accurate measurements to be taken as the target binding site concentration is too low. For example, as in the human condition, the density of certain proteins (amyloid and tau) within the brain is much greater in animal models of Alzheimer's Disease compared to naïve animals. The use of disease models also enables data to be generated in both diseased and normal states within the body providing an understanding of the mechanisms of disease and answering questions on drug targets and processes. It may allow novel target binding sites for the disease to be explored further.

The development of new imaging tool compounds will support the successful application of imaging techniques to human studies and may subsequently be used in both preclinical and clinical imaging studies. They may be used to evaluate multiple novel drugs for conditions including neurodegeneration, neuroinflammation, psychiatric diseases, cancer, inflammation, respiratory diseases, liver disease, irritable bowel disease, obesity, and type 2 diabetes. Where an imaging tool compound for a particular target binding site is already established and available, this may be used under this licence to further understand the disease process or to evaluate the binding of drugs to that binding site within the body.

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

There are a number of benefits of conducting the work under this licence. In the short-term, the work is intending to answer specific questions about novel imaging tool compounds and drugs of interest, and/or target binding sites within the body using rodent models. Where the studies are investigating the distribution of a drug within the body and/or the binding of the drug to the target binding site, this may allow assessment of the suitability of potential drugs at an early stage in development.

Where previous data have been collected indicating a therapeutic effect of a potential drug when administered to rodents at a particular dose level, we will be able to provide data under this licence to quantify the level of binding to the target binding site required to illicit this effect. These data will be beneficial to future studies in humans.

Often, the development of a successful imaging tool compound will lead to its further use to explore the nature of a disease and/or the binding characteristic of novel drugs for a particular disease. Therefore, a medium-term benefit of this work will be the availability of this imaging tool compound for such measurements. This, as well as the advancement in basic knowledge of healthy and diseased states that will be achieved through our studies, will be of interest to researchers worldwide. Where results are not commercially sensitive, data will be submitted for publication in peer-reviewed scientific journals or for presentation at international conferences available to such researchers.

More longer-term, the work under this project licence will support the use of imaging technologies in humans by the development of new imaging tool compounds and by the optimisation of methodologies.

It will allow assessment of new drugs in humans early within the development process and directly within the living tissue of interest, reducing development timelines by at least several months and reducing very costly late-stage failure of drugs.

The ultimate impact from the work will be to contribute to the successful development of new therapies for patients. The new imaging tool compounds, together with the new therapies that are being investigated, are being developed for a wide range of therapeutic areas, including neurodegeneration, neuroinflammation, psychiatric disease, ageing, systemic inflammation, cancer, irritable bowel disease, metabolic and respiratory diseases and as such have potential to benefit a significant patient population. These disease areas are of particular interest as they have an unmet need for new and better therapies.

The disease models to be used under this licence are all well established and internationally accepted models that mimic pathological aspects of human disease (please see below):

Neurodegeneration, Neuroinflammation and Psychiatric Diseases:

Models of neurodegeneration, neuroinflammation and psychiatric disease may be generated by the administration of substances that cause an effect in the brain such as the loss of brain cells, an inflammatory reaction or, changes in brain chemical levels. In many cases, the substance will need to be administered directly to the brain as otherwise it would not pass the blood brain barrier and get into the brain to illicit it's effect. Alternatively, a particular group of brain cells may be cut with a specially designed knife allowing investigation into the effect of brain cell integrity on the development of a disease. These animal models exhibit certain aspects of disease without any corresponding effects on their welfare and may be used to explore disease process, develop novel imaging tool compounds or, evaluate novel drugs for diseases such as Parkinson's Disease,

Alzheimer's Disease and Schizophrenia. Over the years, these types of models have been used under our previous licences to answer a range of experimental questions and have provided the necessary information to support the conduct of subsequent human studies.

Subcutaneous and Intra-cranial Tumours:

Tumour models may be generated by the administration of tumour cells either under the skin (typically on the shoulder or side of the body) or into the brain of rodents. The former route of administration may be used to investigate a range of tumour types and has little impact on the welfare of animal. The administration of tumour cells directly into the brain will be carried out only where the blood brain barrier would otherwise prevent the tumour cells from entering the brain. Tumours, in situ within the brain, may be necessary when, for example, we are investigating whether a novel drug can reach the target binding site. The effect of the blood brain barrier on the delivery of the drug to the tumour cannot be accurately reproduced otherwise. Animals with tumours within the brain are not expected to experience a significant impact on their wellbeing.

Respiratory Diseases:

Models of asthma, chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis (formation of fibrous connective tissue within the lung) may be generated by the administration of substances that cause inflammation in the lungs. These models are well established and used extensively worldwide in the field of lung disease research, as they have been shown to exhibit many pathological characteristics of the human conditions. The substances to be administered are generally well tolerated by the animals and have no significant impact on their wellbeing. As these conditions are multifaceted, there isn't a single model that captures all the characteristics of the clinical situation. Therefore, the choice of disease model to be used under this licence will be highly dependent on the experimental question being asked.

Non-Alcoholic Fatty Liver Disease (NAFLD):

Models of NAFLD may be generated by an altered diet or by the administration of a substance that is toxic to the liver. This results in fat build-up within the liver that can progress to the formation of fibrosis (fibrous connective tissue). The body weight of the animals may increase significantly or in some cases decrease. The manner in which the clinical disease develops is complex and therefore the choice of disease model is highly dependent upon the experimental question being addressed. The models to be used under this licence will develop NAFLD via different routes in order to accommodate the complex nature of the disease.

Ageing:

The developmental stage of a rodent can have a profound effect on the functioning of biological systems in the body. The use of an inappropriate age of rodent could result in

variable or irreproducible data being collected. Therefore, aged animals (>15 months old) may be used to study the ageing process and/or ageing related diseases such as Alzheimer's Disease.

Irritable Bowel Disease (IBD):

An acute (short-lasting) and a chronic (long-lasting) mouse model of IBD may be used. Both are generated by the administration of the same substance, dextran sodium sulphate (DSS), by modifying the concentration of DSS and the frequency of administrations. The only way to truly judge the effectiveness of new drugs for IBD is in living animals and the DSS mouse model is a well-established model used in the field of IBD research throughout the world. It has been shown in the literature to be a relevant model for the human disease. Due to the inflammatory nature of the IBD disease model, animal discomfort cannot be avoided. DSS causes damage to the cells lining the intestine resulting in an immune response. The subsequent inflammation can cause bloating, inappetence and general abdominal discomfort. Animals will typically exhibit diarrhoea and weight loss. Where signs of discomfort are observed in the animals, supportive treatment such as warming and/or soft food will be provided and the animals will be closely monitored for any signs of deterioration. It is not expected that pain will reach levels that will impact normal behaviour, but we will monitor animals daily for any signs of pain or discomfort. Any such clinical signs will be carefully managed to minimise their effect on the welfare of the animal. The dose of DSS to be used and the duration of the study will both be kept to a minimum commensurate with the aims of the study.

Systemic Inflammation:

Induction of inflammation in rodents by the administration of specific pro-inflammatory (inflammation- inducing) substances allows precise control of (i) the inflammatory mechanisms activated, (ii) the degree of inflammation induced, (iii) the site and spread of inflammation induced and (iv) the nature of the inflammatory response elicited. Therefore, using this approach under this licence will allow a detailed understanding of the ways in which imaging tool compounds and drugs interact with specific inflammatory pathways within the body. Different pro-inflammatory substances elicit their effects via different pathways. We may be required to activate or trigger more than one inflammatory pathway during the lifetime of the licence. Where possible, mild pro- inflammatory substances will be used, but it may also be necessary to use strong pro-inflammatory substances. This includes lipopolysaccharide, which is commonly used to generate systemic inflammation in rodents and is well described in the scientific literature as it is very effective at eliciting an immune response. As the administration of LPS and other strong pro-inflammatory substances has the potential to result in serious signs of ill-health such as pain or discomfort in rodents, including death, low doses will be used to generate systemic inflammation and the duration of the studies will be the minimum required to answer the

experimental question. Using this approach, we do not expect animals to experience adverse effects that are more than of moderate severity.

Pulmonary Arterial Hypertension (PAH):

One of the most commonly used animal models of PAH involves the administration of Monocrotaline (MCT) in rats, and this model will be used under this licence. MCT administration leads to arterial damage, alterations in the blood vessels of the lung and eventually hypertension (high blood pressure). At around 4 weeks after the administration of MCT, the changes seen within the lungs of the rats appear to mimic those changes seen in the lungs of PAH patients who are experiencing limitations in physical activity.

Therefore, this model will be used to support the development of novel imaging agents and therapies for PAH, and also to provide a greater understanding of the disease process.

The rats tolerate MCT well for the first few weeks after administration but then start to exhibit changes in their breathing pattern and some changes in body weight. Animals exhibiting breathing difficulties, together with other signs of ill-health, such as a hunched posture, will be humanely killed.

Obesity and Type 2 Diabetes:

Genetically modified and/or diet induced models of obesity and diabetes may be used that are well established and internationally accepted models. These models gain weight but, otherwise, tolerate the diets well. Any other signs of ill-health that may occur will be carefully managed.

In summary, the animal work conducted under this licence will determine if novel imaging tool compounds and drugs have the appropriate characteristics to be administered to humans and in many cases will also support the optimisation of the study design in human studies. Animal models of disease may be used where the experimental question cannot be answered using naïve animals only. The ultimate impact from the work will be to contribute to the successful development of new therapies for patients.

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

The assessment of novel imaging tool compounds and the advancement in basic knowledge of healthy and diseased states that can be achieved through our imaging studies will be of interest to researchers worldwide. Where results are not commercially sensitive, data will be submitted for publication in peer-reviewed scientific journals.

Species and numbers of animals expected to be used

- Mice: 5700

- Rats: 5400
- Guinea pigs: 350

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.

Under this project we will use mice, rats and Guinea pigs as they are the species with the lowest sentience that can be used to answer our experimental questions. Mice will be used as they are the species with the lowest sentience (capacity to experience feelings and sensations) that shows a relatedness to humans and the disease models that we will use, particularly genetically altered animals, are well established in mice. Rats are immediately after mice in the evolutionary tree and will also be used. Again, many of the disease models, including some genetically altered models, are well established in rats. Guinea pigs will be used where the use of mice or rats would not allow the experimental question to be answered due to species differences.

The imaging equipment to be used under this licence is especially designed for imaging rodents. There is a considerable amount of historical data using these species in both imaging studies and drug development. In addition, a large proportion of the substances to be administered will have previously been administered to rats and mice, and therefore information will typically be available on their toxicology in these species. Due to the spatial resolution (characteristic of a scanner that determines how accurately the images are measured) limitations of preclinical imaging, rats and Guinea pigs, as larger species, provide a better opportunity to quantify biological processes within regions of organs. In addition, the blood volume of these species allows serial blood samples to be taken during the scans to allow full quantification of the imaging data.

Typically, promising imaging tool compounds and therapies identified under this licence, will subsequently be administered to adult humans in clinical studies. The developmental stage of a rodent can have a profound effect on the functioning of biological systems in the body. The use of an inappropriate age of rodent could result in variable or irreproducible data being collected. Therefore, young adult, adult and aged animals may each be used under this licence depending upon the disease model and study objectives. Young adults or adults will be used in most cases. Aged animals will only be used to study the ageing process and/or ageing related diseases such as Alzheimer's Disease.

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

Typically, an animal (genetically altered or wildtype rodent) will be placed under general anaesthesia and will undergo the administration of a substance(s) by intravenous injection, followed by multiple blood samples being taken. This will typically occur on 1-2 occasions and, each time, the animal may be imaged on a scanner specially designed for scanning rodents. Imaging sessions will typically last for up to 5 hours. Other procedures may be carried out on occasion such as fasting of the animals overnight prior to the scan, the surgical implantation of devices to measure parameters such as body temperature or, devices for dosing and/or sampling to/from the animal, and/or behavioural assessments. Animals will be killed at the end of the series of procedures.

Some animals may undergo a procedure to generate a model of disease (please see below). These animals would then typically undergo the procedures above:

Administration of a substance directly to the brain under general anaesthesia to generate models of neurodegeneration, neuroinflammation or psychiatric disease.

Cutting of brain cell pathways using a specially designed knife under general anaesthesia to generate a model of brain cell loss.

Generation of a tumour under the skin, typically of the shoulder or side of an animal or, within the brain by the injection of tumour cells or tissue under general anaesthesia.

Administration of a substance that causes inflammation in the lungs to generate a model of asthma, chronic obstructive pulmonary disease or idiopathic pulmonary fibrosis.

Alteration of the diet for up to 7 months and/or administration of a substance to generate a model of non-alcoholic fatty liver disease.

Alteration of the diet for up to 6 months to generate a model of obesity and/or type 2 diabetes.

Injection of a substance to generate a model of systemic inflammation or pulmonary arterial hypertension.

Administration of a substance in the drinking water to generate a model of irritable bowel disease.

The total experimental duration will be dependent upon the time necessary to generate the disease model, and this could be up to several months in some cases, e.g. diet induced models of obesity**.**

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

Under this licence, a significant number of the animals will undergo imaging procedures whilst under general anaesthesia and will remain under the anaesthesia until they are killed. However, some animals will undergo procedures without anaesthesia, such as the injection of substances and behavioural assessments. In most animals, these procedures are expected to result in no more than mild transient distress and no lasting harm to the animals. Following a surgical procedure under general anaesthesia, e.g., the implantation

of a device for continuous administration of a substance or the administration of substances directly to the brain, it is possible for an animal to experience some transient pain and discomfort, however, this will typically be alleviated with painkillers. Some animals will be singly housed, typically for up to 5 days but in some cases maybe longer. The duration of single housing will be kept to a minimum as it may be stressful to the animal.

The development of signs of ill-health such as pain and/or discomfort is expected in some animal models of disease as it is not possible to separate such clinical signs from the biological changes required for the model induction and progression of disease. One of the most likely adverse effects under this licence is body weight loss and in many cases, this is a good indicator of the severity of disease. We have a humane endpoint of 20% weight loss specified for most models, at which point the animals will be humanely killed. However, on the rare occasion that an experiment is to be undertaken where bodyweight loss may be expected to exceed 15%, this will be discussed with the named animal care and welfare officer, named veterinary surgeon and other relevant persons as necessary during pre- study planning and advice taken on amelioration and appropriate humane end points to apply. This will provide assurance to the Animal Welfare Ethical Review Body and Animals in Science Regulation Unit that the 20% limit will only be applied after careful consideration and discussion with the named persons regarding the scientific need to exceed a 15% limit.

Animal models of irritable bowel disease will typically exhibit signs of abdominal discomfort, bloating and inappetence, together with changes in stool consistency, diarrhoea, and blood in faeces/rectal bleeding as the disease progresses. If other signs of ill-health were to be exhibited by these animals, that does not improve following withdrawal of the induction substance, then the animals would be humanely killed.

Where the experimental induction of a disease model or, a genetic alteration, affects the brain (including the introduction of a brain tumour), movement and behaviour may be affected in some animals.

Subcutaneous tumours are not expected to impact on the wellbeing of the animals as they will typically be in the shoulder or side of the body, however, if a tumour was to grow too large then it might impact on the movement of the animal. This will be avoided by closely monitoring the size of the tumours and humanely killing an animal if a tumour grew too large.

Models of respiratory inflammation and fibrosis may show signs of respiratory distress such as laboured breathing, but this typically resolves itself within a few hours. The generation of a model of pulmonary arterial hypertension in rats will lead to inflammation and changes within the blood vessels of the lung. Rats will usually tolerate this with little evidence of illness for the first few weeks (typically 3 – 4), but then develop progressive breathing difficulties and weight changes compared to control animals. If breathing

difficulties were to be present in combination with other signs of ill-health, then the animal would be humanely killed.

Models of obesity, type 2 diabetes and liver disease often exhibit excessive weight gain, which typically leads to reduced mobility, increased drinking and urination, and fatigue. Animals dosed with an inflammatory substance may appear to show transient signs of being unwell, including altered posture and inappetence but these symptoms will be completely resolved within 72 hours. Any irritation seen at the site of administration will typically resolve within 24 hours.

Most animals used under this licence will be young adults or adults. However, on occasion, it will be necessary to use aged animals, where we are studying the effects or aging or age-related diseases such as Alzheimer's disease. As animals age beyond 15 months, they will start to develop reduced mobility and a general reduction in organ function, which can lead to cataracts, greying fur, alopecia, dermatitis, overgrowth of incisor teeth and other age-related symptoms.

All animals under this licence will be closely monitored at least daily for adverse signs. Where adverse signs are seen, the animals will be monitored more frequently, including weighing, and provided with refinement measures such as extra bedding, fluids, warming or soft food as needed. If unexpected adverse effects occur, advice will be sought from the vet. All animals exhibiting signs of pain and/or discomfort, over and above those expected for the particular disease model, will be humanely killed.

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)?

Species	Severity	Percentage (%)
Mice	Non-recovery	25
	Mild	28
	Moderate	42
	Severe	0
Rat	Non-recovery	33
	Mild	27
	Moderate	39
	Severe	0

Guinea pig	Non-recovery	31
	Mild	20
	Moderate	49
	Severe	0

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

- Killed

A retrospective assessment of these predicted harms will be due by 23 March 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?

Compounds will be evaluated as candidate drugs and imaging tool compounds. Studies using blood/tissues/cells or computer simulation will typically be used to investigate the binding characteristics of novel compounds and/or their targets. However, as these compounds are being developed for administration in humans, it is important to understand their effects on the body as a whole and also the effect of the body on the compounds themselves. Therefore, they need to be investigated in live animals. Furthermore, some biological processes, such as the metabolism and clearance of a compound from the body, may only be investigated in live animals.

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

Non-animal approaches are routinely used by our team to provide important data that contribute to the evaluation of new imaging tool compounds and drugs. By using tissue, blood/plasma and/or cell preparations; or computer simulations, many data describing the characteristics of the tool compounds and drugs may be obtained.

There are a number of criteria that typically need to be fulfilled to optimise the development of imaging tool compounds. These include adequate availability of the binding site of interest within the body; appropriate distribution of the compound throughout the body following administration; effective engagement of the compound at the binding site; and the time-course of the compound within the body. Furthermore, novel

drugs also need to show sufficient occupancy at the relevant binding site in order to illicit a therapeutic effect. In vitro assays (conducted outside a living animal) are able to provide information on the density of a binding site within a particular tissue or organ, and specificity of the binding of a compound to that binding site.

Computer simulations (in silico) may also be used to screen multiple compounds and determine the most likely to be successful based on given criteria. However, the distribution of a compound once it is administered, together with its time-course within the body may only be measured using live animals.

Under this project, in vitro assays, and in some cases in silico assays, will be used in the initial evaluation of many new imaging tool compounds and drugs. Where appropriate in vitro/in silico data for the compound or related compound warrants further investigations, experimentation in live animals will be considered.

Why were they not suitable?

There are currently no effective in vitro or in silico assays that are able to replicate the complexities of the live animal, due to the diverse nature of differentiated tissues. Without the use of animals, we would be unable to fully predict the suitability of novel compounds prior to human use.

A retrospective assessment of replacement will be due by 23 March 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 to be used for each protocol has been estimated based upon previous experience of typical study designs and the number of studies expected. Many of our studies will be conducted using normal animals, however, rodent models of disease will be used on occasion, where the experimental question cannot otherwise be answered. The number of animals used for each type of study (e.g. occupancy, biodistribution, dosimetry) will be determined by a combination of previous experience developed under a former licence authorising work for the same purpose; adaptive experimental design

facilitated by ongoing mathematical modelling of the data; and where applicable, predicted disease model variability.

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

We are a multidisciplinary team of biologists, mathematicians, chemists and physicists, that has many years of experience of designing and conducting the types of studies to be carried out under this project, both internally and together with external collaborators. Many of our projects have an initial in vitro screening component, whereby important binding characteristics of the compounds and the tissues of interest are determined. Only where the data from these assays indicate that the compound and/or tissue has the appropriate characteristics will the compound be administered to live animals.

Over the years we have developed standard study designs in live animals to answer specific experimental questions that are routinely conducted and we always use the minimum number of animals for our studies, whilst ensuring that robust scientific data are collected. Where possible, data have been published in peer reviewed scientific journals. Furthermore, we often employ an adaptive approach to our studies, meaning that interim analysis is conducted by our mathematicians whilst the study is ongoing. This avoids the use of further animals unnecessarily.

Where standard study designs are not appropriate to answer the experimental question, previous experience will be used to help establish animal numbers.

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 imaging approaches for many of our studies. This allows data to be collected longitudinally from one of more organs of the body over time, thus negating the use of multiple animals killed at various timepoints to provide the same information. As animals may be imaged on more than one occasion, they can act as their own control, again keeping the number of animals used to a minimum.

Furthermore, at the end of the final imaging session, animals will be killed, and typically multiple tissues taken for further analysis, including additional in vitro assays.

When using a new animal model of disease, a pilot study in a small number of animals will typically be conducted initially to optimise the study design and to validate the model within our laboratory.

A retrospective assessment of reduction will be due by 23 March 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.

Disease Models

We will use several rodent disease models to answer key experimental questions for a range of different therapeutic areas (please see below). These models are typically those that are widely accepted and used by international experts in the relevant field.

Neurodegeneration, Neuroinflammation and Psychiatric Diseases

We will use disease models of neurodegeneration, neuroinflammation and psychiatric diseases that are established by (i) the administration of substances such as toxins and inflammatory agents directly to the brain or to the periphery of rats and mice and/or (ii) genetic alterations in the pathways related to these disease areas in rats and mice. Any administration of substances to the brain will be carried out under general anaesthesia. Administration via other routes is not expected to cause more than transient mild discomfort. The models themselves are not expected to suffer any significant adverse effects that result in abnormal behaviour, discomfort, or distress. However, if adverse effects did occur, such as body weight loss, these would typically be transient and would resolve within 72 hours. If an animal were to show signs of pain and/or distress, it would be closely monitored, and further refinements provided such as moist diet. Where it would not interfere with the outcome of the study, analgesia would be provided. In the unlikely event that an animal did not improve, the animal would be humanely killed.

Fimbria-Fornix Transection

Naïve and/or genetically altered rats and mice may be used for this model of brain cell loss/injury. This model will be generated under general anaesthesia by cutting brain cell pathways within the brain using a specially designed knife. Animals are expected to make an unremarkable recovery from the surgical procedure. If an animal were to show signs of deviation from normal health, supportive treatment would be provided, and the animal humanely killed if it did not improve.

Oncology

Mouse and rat tumour models will be used under this licence. An alternative model, the Drosophila fruit fly, which is lower on the evolutionary tree, is not suitable to use due the aims of our work and methods

that we are using. We are aiming to develop novel imaging tool compounds for use in clinical studies using techniques such as positron emission tomography and single photon emission computed tomography. Due to the spatial resolution limitations and practicalities of these techniques, it would not be possible to image fruit flies due to their small size.

The tumour models that we will use will typically be chosen based upon previous experience and will have known characteristics that are deemed appropriate to answer the experimental question. The use of tumour models which are known to have a high incidence of tumour ulceration (breakdown of the surface cells) will usually be avoided.

Tumour cells or tissues will typically be implanted under the skin (subcutaneous) or into the brain of immunocompromised (low immune system defenses) mice or rats to generate tumour models. The subcutaneous administration of tumour cells or tissues will typically be into the shoulder or side of the animal, areas that are considered to cause the least stress.

The animals may show some weight loss, which is typically short-lived, but are not expected to suffer any pain or distress. If tumours were allowed to grow too big, they could impact on the normal behaviours of the animal. For instance, a very large subcutaneous tumour could prevent the animal from reaching food and water. A large brain tumour could cause neurological effects including reduced mobility. These adverse effects will be avoided by carefully monitoring the size of the tumours and humanely killing the animals before the tumours become too big.

Respiratory Inflammation and Fibrosis

We will use rodent disease models of respiratory inflammation and fibrosis (formation of fibrous connective tissue) that are established by (i) the administration of inflammatory agents or pro-fibrotic (cause fibrosis to develop) agents to the lungs and/or (ii) genetic alterations in the pathways related to these disease areas.

As described above for Oncology, the Drosophila fruit fly may be considered as an alternative model for research into aspects of asthma, particularly when studying susceptible genes. It has also been used as a model for chronic obstructive pulmonary disease, including the generation of a cigarette smoke induced model. However, as previously described, fruit flies are not an appropriate model for our research due to the aims of the work and the methods employed.

Inflammatory or pro-fibrotic agents may be administered by injection which may cause brief discomfort or otherwise will be administered under general anaesthesia. The choice of agent will be based on the experimental question. Rats or mice will be used but guinea pigs may be used where the use of rats or mice is not suitable to answer the experimental question. The administration of inflammatory or pro-fibrotic agents may occasionally cause

short-lived respiratory distress but this typically resolves within a few hours after the administration. Some animals may also lose a small amount of body weight. Any animal that showed signs of respiratory distress, significant weight loss, or further signs of pain or distress which did not resolve quickly, would be humanely killed.

Non-Alcoholic Fatty Liver Disease (NAFLD)

An animal model of NAFLD may be generated in rats or mice by the administration of an altered diet (e.g. high fat) and/or the administration of a hepatotoxic compound (causes toxic damage to the liver).

Genetically altered models of obesity and type 2 diabetes may be used in addition to immunocompromised animals and naïve animals for the generation of these models. Animals on a high fat diet will typically gain weight but few other side effects are expected. The animals tolerate the diet well. Other altered diets and/or the administration of hepatotoxins may result in body weight loss, decreased appetite and fatigue. Models of obesity and type 2 diabetes may show signs of diabetes such as increased drinking and urination and where they do, water bottles and bedding will be changed more regularly. The animals would be humanely killed if they exhibited other signs of ill-health that were not resolved quickly.

Alternative animal models of NAFLD have been described in the literature. These models are generated by genetic alteration or chemical/diet induction in zebrafish or medaka fish, animals which are considered to be less sentient than mammals. However, there are limitations to imaging these smaller species, due to the spatial resolution of the scanners that we are using, and the methods required to enable quantification of the data being generated.

Aged Rodents

Some rats and mice, both naïve and genetically altered, will be aged up to a maximum of 25 months. Ageing is associated with a range of potential adverse effects, such as weight changes and reduced activity, as a result of the ageing process. If symptoms of ageing were causing distress to the animal which could not be ameliorated under advice from the vet, or the animal was showing signs of pain, then the animal would be humanely killed.

Irritable Bowel Disease (IBD)

IBD will be induced in mice by the administration of dextran sodium sulphate (DSS) in their drinking water, a stress-free route of administration as opposed to other chemically induced models of IBD, where the substances are administered by injection or directly into the rectum.

Due to the inflammatory nature of the disease, the mice may experience abdominal discomfort, bloating and inappetence as a result of colitis induction. Following DSS

exposure, animals will typically exhibit weight loss, change in stool consistency, diarrhoea and blood in faeces/rectal bleeding as the disease progresses.

Currently there are no adequate methods that can simulate generating the disease as an alternative to using living animals. It is not expected that pain will reach levels that will impact normal behaviour, but we will monitor animals daily for signs of ill-health such as discomfort. Where signs of discomfort are observed in the animals, supportive treatment such as warming will be provided and the animals will be closely monitored for any signs of deterioration. The duration of the studies will be kept to a minimum. Any animal that loses significant body weight or exhibits other signs of ill health in addition to the IBD symptoms will be closely monitored and if these additional symptoms are not quickly resolved, then the animal will be humanely killed.

Systemic Inflammation

Inflammation can be associated with substantial welfare harm associated with impairment of body health and tissue damage. There are many ways of inducing local/systemic inflammation however many are generally imprecise (e.g. surgically-induced peritonitis or administration of live pathogens)

and/or produce relatively long-lived welfare harms (e.g. arthritis models). We are using a highly targeted approach by the administration of specific inflammatory agents to rats and mice that allows precise control of (i) the inflammatory mechanisms activated, (ii) the degree of inflammation induced, (iii) the site and spread of inflammation induced and (iv) the nature of the inflammatory response elicited. This allows the mechanistic pathways of interest to be activated directly and specifically and means (i) there is less opportunity of welfare harm associated with uncontrolled activation of unrelated inflammatory pathways or pathogen growth, (ii) in many cases milder, targeted agents can be used (with associated reduced severity) and (iii) the inflammatory response induced is rapid and predictable allowing a reduced number of animals and shorter experimental duration.

Where possible, mild inflammatory substances will be used, but it may also be necessary to use strong inflammatory substances. Mild inflammatory agents are not expected to cause any adverse effects beyond mild brief injection discomfort. However, the administration of strong inflammatory substances has the potential to result in an impaired health status of the animals, with associated weight loss and reduced movement. The dose of all inflammatory agents will be kept to a minimum and this should reduce the potential for and severity of any adverse effects. Any adverse effects observed will be short-lived and should be resolved within 72 hours. If the adverse effects were not resolved within 72 hours, then the animals would be humanely killed.

Model of Pulmonary Arterial Hypertension (PAH)

PAH will be induced in rats by the administration of Monocrotaline which leads to inflammation around the blood vessels of the lung, resulting in a gradual onset of high blood pressure in the lungs over time. Rats are to be used rather than mice as they have a

more consistent and predictable response to Monocrotaline. A single injection of Monocrotaline will be administered with no more than short-lived discomfort. There may be some irritation at the site of injection but this will typically resolve within 24 hours. Rats will tolerate the administration with little evidence of illness for the first few weeks (typically 3-4) but then will start to develop progressive laboured breathing and increased respiratory rate. At the doses of Monocrotaline to be used, non-lung blood vessel damage should be

minimal. However, if additional signs of ill-health were to be exhibited, that didn't resolve quickly, an animal would be humanely killed.

Obesity and Type 2 Diabetes

Genetically altered and/or diet induced rat and mouse models of obesity and diabetes will be used. These models will typically exhibit excessive weight gain, reduced movement, decreased body temperature, increased drinking and urination and other adverse effects associated with these conditions. Due to the genetic alterations necessary in these models, adverse effects cannot be avoided. These models will be closely monitored for adverse effects and the duration of studies will be kept to a minimum. Where increased drinking and urination is observed, water bottles and bedding will be changed more regularly. Where an animal shows signs of an altered health status that does not resolve quickly, the animal will be humanely killed.

Zebrafish are considered as a good alternative, less sentient, model for the study of obesity and type 2 diabetes. They have the key organs important for the regulation of energy homeostasis and metabolism and also possess key functions such as insulin regulation. However, as previously described for other therapeutic areas, the aims of our work, together with the imaging techniques that we are using, make the zebrafish an inappropriate model for use under this licence.

Methods

Our team consists of highly trained researchers, who will closely monitor the welfare of the animals under study, together with support from experienced named animal care and welfare officers. As project licence holder, I review protocols and assess the skills of the team members to ensure that high standards continue to be met, there is adherence to regulatory requirements and appropriate refinement methods are used. Under previous licences, we have improved and refined methods to minimise the suffering and distress of animals, particularly those associated with dosing and blood sampling. The duration of all studies will be kept to a minimum provided that it is consistent with study and/or scientific objectives. As much information as possible will be gained from each animal undergoing a procedure. For example, multiple post-mortem tissues will typically be analysed at the end of a final imaging session.

One of the main techniques to be used under this licence is imaging, which is minimally invasive and carried out under general anaesthesia. Imaging allows the same animal to be used as its own control and/or to be investigated longitudinally, instead of requiring a

group of animals per time point studied, thus minimising the number of animals used. The earliest possible endpoints will always be applied that provide adequate scientific data, particularly when working with a model of disease. Where any new disease model is being implemented within our lab, we will discuss the characteristics of the model with researchers and/or a vet with experience of the model and undergo any necessary training in the model induction. A pilot study will then be conducted in our lab using a small number of animals. Monitoring systems will be tailored to each model and strict humane endpoints will be applied to minimise suffering.

Substances may need to be administered to the animals for a number of reasons, including the generation of disease models as detailed above or to determine their suitability as drugs and/or imaging tool compounds. Doses will be kept as low as possible within the constraints of the study's purpose and all available information will be used to minimise the risk of adverse events. Where there is limited information on the substance to be administered, a small number of animals will be dosed in the first instance. The routes, volumes and frequencies of administration themselves should result in no more than brief discomfort and no lasting harm. The dose and frequency of dosing will typically be determined by previous data from in vitro approaches or computer simulations; previous studies; and/or the literature. The routes used will be typical for the species under study. Serial blood samples may be taken from some animals but no more than 15% of the blood volume will be taken during a 28-day period from conscious animals or animals under recovery anaesthesia.

Animals under general anaesthesia will have their body temperature and respiration rate measured. All surgical procedures carried out under recovery anaesthesia, including the placement of telemetry devices and dose delivery/sampling devices, will be conducted under aseptic (sterile) conditions. Local anaesthetic, analgesia and/or antibiotic will typically be given unless it would interfere with the outcome of the study. Following surgery, an animal may be singly housed to protect the wound. Single housing may also be conducted for other reasons such as when fasting a single animal before scanning. The duration of single housing will be kept to a minimum and additional refinement will be provided whenever possible.

Opportunities for further refinement of methods will be sought throughout the duration of the licence.

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

Many of our imaging studies will be conducted in naïve animals under terminal general anaesthesia. However, terminally anaesthetised animals cannot be used to replicate the pathology of human disease. In order to answer some experimental questions, we will use rodent models of disease. The generation of these models typically takes several days to several months to be completed. Hence, it will not always be possible to use young animals.

Rodent models will be used. We cannot use non-mammalian species to evaluate compounds for future administration to humans due to a lack of anatomical and physiological similarity between species. In mice, we are using the least sentient species that allows our scientific objectives to be met. Mice are the species with the lowest sentience that shows a relatedness to humans. Rats are immediately after mice in the evolutionary tree and will also be used. Guinea pigs will be used where mice or rats are not suitable to answer the experimental question.

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

We will continually aim to refine procedures throughout the lifetime of the licence, by reviewing available literature and by using our previous experience, together with that of our colleagues.

All animals will be provided with environmental enrichment (modifications to the environment to enrich the welfare of the animals) and will be housed in social groups whenever possible. Intravenous dose administration and arterial blood sampling in conscious animals or animals under recovery anaesthesia will typically be carried out via thin tubes temporarily inserted into suitable blood vessels. Volumes and frequencies of doses and samples will be kept to a minimum. Staff will be trained in any new surgical procedures by others that are experienced in the technique and will be deemed as competent in the procedures by a vet or authorised training officer prior to conducting the procedure themselves. For some of the behavioural assessments to be conducted, including rotarod and novel object recognition, animals will typically be trained.

Animals will be monitored daily. Progress sheets will typically be completed for animals that have undergone procedures to carefully monitor their welfare. Warming and/or soft food will be given to animals displaying adverse effects. During surgical procedures in animals under general anaesthesia, gel will be administered to the eyes to prevent dryness. Fluid therapy will be given to animals undergoing prolonged anaesthesia. Post-surgery, animals will be placed in a warming box whilst they recover from anaesthesia and, unless it would interfere with the outcome of the study, analgesia will be provided. In addition, moist food will be placed on the floor of the cage for easy access.

Furthermore, each animal model of disease will be closely monitored, and further refinements provided as described below:

Neurodegeneration, Neuroinflammation and Psychiatric Diseases:

Animals will be weighed daily for the first 7 days post initiation of the model, then at least weekly thereafter.

Subcutaneous and Intra-cranial Tumours:

Animals will be weighed regularly, at least 2x per week. A body condition scoring regimen will be implemented at least 3x per week. Subcutaneous tumours will be measured by callipers or imaging at least 2x weekly. Brain tumours will be assessed by body weight or imaging. If animals with brain tumours reach ≥8% body weight loss, monitoring and weighing will be increased to daily.

Respiratory Inflammation and Fibrosis:

Animals will be weighed regularly, at least 2x per week, and where weight loss is expected, animals will be weighed daily. In addition to warming/soft food, extra bedding may be given to animals displaying adverse effects and those animals will be assessed more regularly.

Non-Alcoholic Fatty Liver Disease and Obesity/Type 2 Diabetes:

Animals will be weighed regularly, at least 2x per week, and monitoring frequency will be increased if there are concerns about an animal's wellbeing. Chew sticks and/or cardboard items will be provided to ameliorate overgrown teeth and the teeth will be checked regularly. If there are signs of diabetes such as increased drinking and increased urination, bedding will be changed more frequently.

Aged Animals:

Enhanced welfare monitoring will be conducted at least every 2 weeks from the age of 15 months, increasing to weekly from 18 months of age. Monitoring frequency will be increased should signs of ageing become apparent. Mice can develop idiopathic ulcerative dermatitis (skin condition leading to hair loss, itching and eventually to the formation of ulcers), and where they do so, they will have their hind toenails trimmed to reduce scratching trauma. Any adverse signs seen on the skin will be treated under the advice of the vet.

Irritable Bowel Disease:

A Haemoccult test will be carried out daily in acute studies to detect any non-visible blood in the faeces and the animals will also be weighed. In chronic studies, a Haemoccult test will be performed weekly, and the animals will be weighed every three days. Where signs of ill-health occur, the administration of the disease inducing substance, dextran sodium sulphate, may be ceased.

Systemic Inflammation:

Animals will be weighed regularly, at least 2x per week. In addition to warming/soft food, extra bedding may be given to animals displaying adverse effects and those animals will be assessed more regularly. During the peak of adverse effects, the frequency of monitoring will be increased to at least every 4 hours.

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

We will follow LASA, PREPARE and ARRIVE guidelines and when using cancer models, we will follow the NCRI guidelines. Although there is no specific guidance published covering the other models that we use, we will always search, and review published information to find the best ways to perform our experiments.

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

We will always aim to follow the best possible practice in performing any kind of experiment including those on animals by searching and reviewing up to date published literature. We are advised of advances in the 3Rs via regular correspondence (email) from the National Centre for the Replacement, Refinement and Reduction (NC3Rs) and follow best practice guidelines from PREPARE, ARRIVE and the National Cancer Research Institute, in order to design, perform and report experiments to the highest standards. Staff from the establishment attend workshops and symposia organised by NC3Rs.

A retrospective assessment of refinement will be due by 23 March 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. Development of novel therapies for heart failure and investigations on myocardial repair and regeneration

Aim and duration

What's the aim of this project?

The primary aim of this translational/preclinical project is to develop innovative treatments for heart failure, including cell therapy, gene therapy, and new pharmacological therapy. We also aim to obtain novel scientific knowledge, including cellular and molecular mechanisms underlying development of and/or recovery from heart failure.

Why is it important to undertake this work?

Heart failure remains a major cause of human disability and death. Millions of patients are suffering this disease globally. Treatment of these patients cost billion pounds, with huge labours by hospital staff, at the NHS every year. This issue will become more and more critical all over the world due to the increase in the aged population. Therefore, development of efficient cost-effective strategies is a high priority. The results obtained in this translational project will suggest promising clinical protocols for cell therapy, gene therapy and new pharmacological treatment that will provide an adequate therapeutic benefit with the least risk of complications. In the near future, we believe our new treatment can be applied to patients and will hopefully help improve mortality and quality of many heart failure patients with reducing the cost and labour for the treatment/management of these patients.

In addition, the scientific results obtained in this project will provide a range of important new knowledge in basic biomedical science, which will be of great value in understanding cellular and molecular mechanisms by which heart failure develops and recovers. This will contribute to the advance of medical sincere and biology.

Project licence duration

5 Years 0 Months

Benefits

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

Outputs can include new information, publications, or products.

This project will develop preclinical evidence to validate new treatments for different types of heart failure, enabling further development of the innovative approaches forward to clinical application. In addition, this project will provide important new biomedical and scientific knowledge to understand the mechanism for heart failure development/recovery and shed light on new biological insights, which will pave the way to future medical and

scientific research. These pieces of new information will be presented at international conferences and published in academic journals.

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

The impact of these outputs may be seen in the short-term, or they may not be fully realised until you've completed the project. Consider all timescales in your answer.

Various people and sections will receive benefits from the outputs of this project.

Researchers (immediate): This basic and translational research project will provide a range of novel biological information regarding a mechanism by which heart failure is developed and/or recovered. This new scientific information will provide impact and benefits to researchers in a wide range of areas of cardiovascular research. Also, through this research we will be able to provide high-standard training for the research staff and students involved in this cutting-edge project.

Patients, Clinicians and National Health Service (future): Heart failure is the leading cause of death and disability. This project will propose novel approaches for the treatment of heart failure, which will be further developed by subsequent clinical studies. The new treatment proposed in this project is expected to effectively treat different types of heart failure, improving the survival and quality of life of the patients. This will in turn reduce labour and cost to maintain these patients. Thus, successful development of such a treatment will offer great benefits on patients, clinicians and the National Health Service in the future.

Business and Society (immediate and future): Scientific success of this project will attract large numbers of younger generations to enter scientific disciplines as a career opportunity. This will also improve opportunities for advanced life science research training in cutting-edge technologies in the UK and attract national/international postgraduate and undergraduate students to our Institute as well as to the UK. Successful preclinical information obtained from this project will attract interest from commercial companies in progressing development of this innovative treatment towards clinical application, as such a therapy possibly has a huge market. Through the effective dissemination of our results, the general public will be more aware of the benefits of developing and using an innovative therapy, including cell therapy and gene therapy, to treat human diseases. This will allow the full potential of such advanced therapies to be recognized and support the UK in maintaining its reputation in medical research.

Will this work be offered as a service to others?

Yes

What are the benefits of offering this work as a service?

This work may be offered as contract research with a company, which will bring an earning to our university and laboratory.

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

For example, collaboration, dissemination of new knowledge, or publication of unsuccessful approaches.

To achieve the maximum impact from this project, we will fulfil all aspects of communications, in cooperation with the departments for Research & Development, Intellectual Property and Public Engagement, according to our dissemination and engagement strategies as follows.

1. Publication and presentation: As soon as sufficient data is obtained in the proposed project, it will be published in high-quality peer-reviewed academic journals subject to consideration of protection of their potential for intellectual property and commercial exploitation. Our publication strategy will be taken in accordance with the institutional policy on "open access" publishing. The obtained results will also be presented and discussed at scientific meetings/seminars (institutional, national and international). This will help to disseminate the obtained results to academics, clinicians and companies in the relevant field. In addition, to raise awareness of the potential of our new therapeutic approach in the hospital sides, we will introduce our project to clinicians, nurses, and other hospital staff at clinical seminars/lectures and via hospital newsletters.

2. Data sharing: Data produced in the proposed project will be preserved within our Institute for sharing among the researchers according to our institutional policy on data sharing and preservation. Raw data will be retained and available for possible review/audit, in digital format and as original materials. We will encourage collaborations (internal, external and international) via our website with a link to this project and through communications during the conferences and publications.

3. Exploitation: Obtained results may include patentable intellectual property. Intellectual Property Department in our institute will help handle intellectual property issues, licensing, and commercialization of the exploitable results produced in this project.

4. Public engagement: Our institute has active public engagement programmes. Results produced in this project are open to the public on their homepages and will be published in the Bulletin/Newsletter. Our publications contain special lay sections to make data accessible to public. If appropriate, the results of this project will be disseminated by media broadcast and newspapers. These engagements will collectively enhance public awareness of the importance of this project. In addition, our institute, particularly the Public Engagement Department, runs a number of outreach projects, including annual science festival, designed to reach local school pupils to raise their career aspirations through activities that include science experiments, work experience placements, visits to the institution and talks from international leaders in medical research. Our project will be introduced during such public activities.

5. Business: This project will preclinically develop an innovative therapy for a number of types of heart failure. Thus, there is a good opportunity for business development, potentially attracting the interest of biomedical ventures or mega-pharm. Through our dissemination strategy above, we will maximize such an opportunity. Filing intellectual properties is important to this end. I will be responsible for identifying new intellectual properties and establishing appropriate protection of the data from this project before public dissemination.

6. Clinical medicine: Although the knowledge obtained in this project cannot be directly applied to the patients, this project is one of the major steps to reach the ultimate goal of the clinical establishment of a novel therapy for heart failure. To raise awareness of the potential of our new therapeutic approach in hospitals, we will disseminate the progress of the project among doctors and other hospital staff through clinical seminars/lectures/conferences and hospital newsletters.

Anticipated harms

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

The proposed project requires evaluation of heart failure in clinically relevant settings. To this end, we will use models in adult rats and mice, which are the most suitable for this basic/pre-clinical investigation. Basic pathophysiology on heart failure is sufficiently compatible between human and rodents. Lower species have significantly distinct anatomy and physiology of the heart and are not suitable for our purpose. Furthermore, there are different types of human heart failure, all of which can be adequately represented in established rodent models. In addition, in the rodent models, we will be able to use genetically altered animals. The use of these sophisticated animal models enables in-depth investigation of the molecular and cellular mechanism for heart failure development and recovery.

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

For example, injections and surgical procedures. Include any relevant information about the duration of experiments and the number of procedures.

One of the different types of heart failure will be induced in mice or rats. Genetically altered animals will be used in some experiments.

Then, the animals will be treated with one of the proposed innovative treatments, including administration/implantation of cells, genes, chemicals, biomaterials, or their combination, which will have been proven or suggested safe and effective by our prior studies or published literatures. The most suitable administration route of these substances will be utilised or chosen through comparisons. The effect of a combination of ventricular unloading to improve the outcome of these treatments will also be investigated (Protocol 4).

At chosen time (between 1 day and 6 months, typically within 4 weeks, following the treatment), its safety and efficacy will be assessed by measuring cardiac function and structure. At the end of this Protocol, animals will be humanely killed, and their tissues and organs will be collected for further investigations.

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

Examples can include pain, weight loss, tumours, or abnormal behaviour. State the estimated duration of these effects on an animal.

Unfortunately, in order to mimic clinical heart failure, which is fatal and the major cause of human death, these models needs to have a substantial severity, causing about 10% death in animals. Surgical procedures required to induce heart failure or deliver a treatment will cause pain and distress, which is usually temporary and of mild-moderate severity, to animals. However, we have developed the least invasive models to minimise death and suffering of animals (see the 3R section). At the end of the Protocol, animals will be humanly killed, and tissues and organs will be collected for further examinations, including investigations on the mechanisms by which heart failure recovers.

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

The expected severities are severe (Protocol 1 and 2), moderate (Protocol 3, 4 and 7) and mild (Protocol 5, and 6). The majority of animals in each Protocol will suffer adverse effect of these severities. Thus, collectively, 39%, 14% and 46% of mice will experience the severe, moderate and mild severities, respectively. In rats, 61%, 17% and 22% of animals will experience the severe, moderate and mild severities, respectively. Suffering will be prevented or minimised with appropriate monitoring and care, along with the specific definition of humane endpoints.

Fate of animals

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

- Killed
- Used in other projects

Replacement

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

Our translational/pre-clinical research requires evaluations of safety and efficacy of new treatments in a clinically relevant models of heart failure, particularly needing assessment of changes in global function and structure of the heart and complications such as

arrhythmia occurrence in a quite long term post-treatment. The biological process for recovery from heart failure is extremely complicated, involving multiple cell types as well as a wide range of molecules and signalling. Although we have made all possible efforts, it is impossible to generate a meaningful in vitro or ex vivo replica of such complex clinical processes. Computer-based systems, physico-chemical techniques, the use of lower organisms and embryo stages and cells, tissues, and organ cultures are not adequate for our proposed projects (the web site: www.frame.org.uk was checked). This can only be undertaken in whole beating hearts, thus requiring the use of living animals.

As complementary models, we used and will use in vitro and ex vivo models using cell cultures (primary cells or cell lines of cardiomyocytes, fibroblast and endothelial cell lines, or these cardiac cells derived from stem cells), vibratome-cut heart slices, and isolated perfused hearts. These models are useful as a preliminary data to suggest promising treatments for heart failure. However, these in vitro and ex vivo methods do not allow themselves to “replace” the use of living animals as discussed above.

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

We considered the use of in silico, in vitro and ex vivo models using computer-based systems, lower organisms and embryo stages of animals, 2-dimensional and 3-dimentional cultured cells (both stem cell-derived and primary cells), organoids, heart slices, and perfused hearts. Whenever suitable, these methods will be utilised in this project as preliminary or supplementary studies. Some of these materials are available without killing animals, while others will be obtained in this Project or from other animals killed by the humane procedure.

Why were they not suitable?

These non-animal models are useful to suggest promising treatments for heart failure. However, these do not allow themselves to “replace” the use of living animals as discussed above. This is because any non-animal alternatives cannot appropriately represent complex in vivo processes associated with heart failure development/recovery, which include multiple cell types, factors and signalling. These cells/factors work together closely, affecting each other, toward heart failure development or recovery as a whole. Only living mammals can be meaningful models that mimic the clinical scenario of heart failure treatment.

Reduction

Enter the estimated number of animals of each type used in this project.

- Mice: 2800
- Rats: 1800

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

Do not mention POWER calculations here. If relevant, there will be an opportunity to provide these details elsewhere.

Out of aforementioned estimated numbers, 46% (1,300 of 2,800) of mice and 22% (400 out of 1,800) rats will be used to maintain and produce genetically-altered animals. These numbers are estimated based on those in our previous and ongoing similar types of projects. The proposed methods, experimental designs, and methods for analysis of the quantitative results have been discussed with the Statistical Service Unit in our Institute. The design of individual experiments generally involves factorial designs, which maximizes the information obtained from the minimum resource. The numbers of animals required will vary according to the particular experimental model/treatment and the estimate of the coefficient of variation, etc. For most of the quantitative experiments, sample sizes are set using power analysis as described in each Protocol. However, such estimation may be often difficult. In this case, we will conduct an in vitro and in vivo pilot study to estimate the degree and variation of the effect, using which the accurate group size in each study will be determined. For the qualitative experiments, the amount of material required will be the minimum necessary to provide an adequate description

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

You may want to reference online tools (such as the NC3R's Experimental Design Assistant) or any relevant regulatory requirements.

Experiments were in principal designed by referencing to the NC3Rs Experimental Design Assistant. It is known that the degree of heart failure is affected by induction procedures. Good Laboratory Practice should prevent, or at least minimise the introduction of bias into the experiments. This will, in turn, help significant reduction of the animal numbers used in this project.

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

This may include efficient breeding, pilot studies, computer modelling, or sharing of tissue.

In principal, we will refer to the NC3Rs Experimental Design Assistant. Animals will be bred efficiently using a Standard Operational Protocol in our Biological Sciences Unit. In vitro pilot studies will be carried out to exclude less-promising approaches to treat heart failure. Our in vitro and ex vivo studies will provide important information to reduce the numbers of animals required in the in vivo investigation by limiting the number of treatment protocols to be tested Tissues/organs collected will be used to assess as many

biochemical parameters as possible, which will also help to minimize the number of animals required.

Refinement

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.

Choice of species: We have chosen to use rats and mice. Although there are some differences in cardiac biology between humans and rodents, the basic pathophysiology of heart failure is acceptably compatible. Lower species have significant difference in heart morphology and physiology and are not suitable to our study. Large-animal species are phylogenetically closer to humans, but unsuitable for large-scale studies like our proposed research. Small rodents are thus the most suitable for our projects. In addition, in the rodent models, we will be able to use a range of genetically altered animals. This is a great advantage in the purpose of dissection of the mechanism of the therapeutic effects, identification of donor cells, and obviation of immunorejection by donor cells (immunodeficient animals). Genetically altered animals (Protocol 5-6) and bone marrow chimera animals (Protocol 7) are useful to investigate the mechanisms underpinning the effects of treatments by focusing on one particular gene or cell types.

Choice of heart failure models: We will use six types of acute and chronic heart failure models in rat and mouse: left coronary artery ligation-induced myocardial infarction and ischaemic heart failure (Protocol 1), dilated cardiomyopathy induced by doxorubicin, myocarditis and genetic abnormality (Protocol 2) and transverse aortic constriction-induced hypertrophic heart failure (Protocol 3). Each of these represents a major type of clinical heart failure and has been well validated in rat and mouse, and widely used in relevant research. Because these heart failure types are different in the pathogenesis, clinical features, and response to a treatment, the optimal treatment must be considered separately. All heart failure models result in discomfort for the animals; however, we have optimised the procedures to minimise the mortality and discomfort.

Treatment models: Effects of administration of cells, genes, drugs, biomaterials or their combination will be compared among different types of promising administration methods (Protocols 1-3). Only cells/genes/drugs and delivery methods, which are suggested to be promising by prior research or pilot studies, will be tested. Combined therapy with left ventricular unloading will be achieved by heterotopic transplantation of the heart (Protocol 4). This is available in rodents.

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

For example, animals at a more immature life stage, species that are less sentient, or animals that have been terminally anaesthetised?

There is no established model of heart failure in less sentient animals due to technical difficulty. In addition, the basic anatomy and pathophysiology of the heart is likely to be unacceptably different between human and less sentiment animals. Thus, less sentient animals are not suitable in this project.

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

I will stay informed about the latest advances in the 3Rs through information/newsletters from the Home office and NC3Rs, attendance at relevant academic conferences, regular literature search and communications with peers in the same research field. Identified new methods/protocols will be discussed with our Biological Sciences Unit staff and Home Office Inspector for the implementation.

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

Potential refinements include increased monitoring, post-operative care, pain management, and training of animals.

We have used the same/similar procedures to induce heart failure in rats and mice for the last 20 years, which have now been well established and significantly refined. However, we will continue our effort to further refine the procedures to minimize the animal suffering. This may be achieved by:

- Animals will be given the most appropriate analgesic to reduce post-surgical pain. We will use newly introduced, more effective analgesic regimen and/or antibiotics when available.

- To prevent infection, surgery will be conducted using aseptic technique which meets the standards set out in the HO Minimum Standards for Aseptic Surgery in a specifically regulated recovery surgery room. Appropriate antibiotics will be given following the latest knowledge.

- Less-invasive surgery, including minimizing the incision and reducing the surgical time will be attempted. During surgery, body temperature is maintained by heating pad. Heart rate and oxygen saturation may be monitored and controlled.

- After surgery, animals will be intensively observed in an appropriate recovery cage until complete recovery. They will be closely and continuously monitored using the score sheet system to detect their suffering at an earliest opportunity.

- The limits of collected blood volume are set to prevent hypovolemia and anaemia.

- We set clear humane endpoints in each Protocol.

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

I will regularly refer to the Home Office homepage and documents, NC3R homepage and documents, and ARRIVE or PREPARE guidelines. This ensures full transparency in producing and reporting all the in vivo experimental data.

26. Mechanistic studies in pre-clinical stroke, cognitive impairment and small vessel 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

Stroke, Vascular dementia, Hypertension, Cerebrovascular disease, Therapy

Animal types	Life stages
Mice	adult, pregnant, embryo, neonate, juvenile
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 better understand what happens in the brain in a model of stroke or vascular dementia with a view to identifying novel pathways, processes and targets for development of new drugs to treat these conditions.

A retrospective assessment of these aims will be due by 06 April 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?

Stroke is a risk factor for dementia and dementia predisposes to stroke. Around 1/3 people will develop stroke, dementia or both and the incidence of both increases with age. Stroke and dementia are inter- related; they share similar risk factors and each risk increases the risk of the other. Because a stroke doubles the chance of developing dementia and stroke is more common than dementia, more than a third of dementias could be prevented by preventing stroke. Stroke and dementias account for almost 90% of all deaths originating from the brain. Only 2 treatments exist for those who have stroke and there are currently no drugs that prevent vascular dementia. Hence, the unmet clinical need for both of these linked conditions is without question.

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

- Publications in scientific journals of research findings.
- Presentation of research findings at local, national and international conferences.
- In advancing knowledge and understanding in the area of stroke, vascular dementia and small vessel disease our work will have significant impact on the scientific community, both within this specialised research area and further to other diseases which affect the brain negatively or those where blockade of a blood vessel is the cause of disease.
- The data generated will be used to support future grant applications.

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

The expected benefits arising from this project are multifold, and will be in relation to both the advancement of scientific knowledge (short-medium term) and further to potential clinical translation of novel therapies to people with stroke and/or dementia (longer term).

In advancing knowledge and understanding in the area of stroke, vascular dementia and small vessel disease our work will have significant impact on the scientific community, both within this specialised research area but also further to other diseases which affect the brain negatively or those where blockade of a blood vessel is the cause of disease. There is a huge unmet clinical need to develop new treatments for these conditions. Dementia is the only condition in the top 10 causes of death in the UK without a treatment to prevent, cure or slow its progression.

Ultimately, any positive findings which lead to changes in clinical practice will clearly have substantial impact on stroke sufferers and those with vascular dementia and the families

who care for them should they be left with long-term disability. Through enhanced quality of life as a result of reduced deficit following stroke or reducing the risk of vascular dementia it is these patients, and those closest to them, who will benefit most.

A collateral benefit will be that staff and students working on this project will benefit from learning relevant skills such as project management, independent and critical thought, appreciation and understanding of the use of animals and implementation of the 3Rs. In training a highly skilled researcher in specialised surgical techniques (a skill recognised by funding bodies and pharmaceutical companies as being in decline) there will be wider impact to the research community as a whole as this person can then go on to pass on their expertise to others as they progress through their research career.

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

As a small research group, we are always looking to establish new collaborative links to maximise outputs. I encourage researchers in my group to attend conferences to allow them to present their work and provide them with networking opportunities with leading scientists in the field. This benefits the researcher by equipping them with presentation skills but also the wider research community as it allows dissemination of data generated at an early stage that may lead to new collaborations through shared material to strengthen or consolidate a body of work. I also encourage interaction with scientists in other disciplines to maximise potential research avenues and within our institute, regular internal seminars facilitate this. We regularly publish completed studies in peer reviewed scientific journals.

And journals such as J Cereb Blood Flow & Metab have the facility to publish neutral or negative studies, something that is vital to allow the field to advance.

Species and numbers of animals expected to be used

- Mice: 1000
- Rats: 2000

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 rat and mouse models we use are carefully selected based on their unique genetic profiles. We will be studying adult animals. Some of the models we use will have established hypertension (high blood pressure), which occurs spontaneously (naturally) in adulthood. This is known to be a major risk factor for both stroke and dementia. This animal is also considered the best available spontaneous model of cerebral small vessel disease (SVD). By including an animal model which displays some of the key risk factors

associated with the development of stroke and/or dementia and/or SVD (eg high blood pressure), we hope to improve translation from pre-clinical to clinical studies. This is also a key recommendation of some of the guidelines related to pre-clinical stroke studies. Some of our mouse models have been genetically modified to inhibit (knock out) or add in (knock-in) the function of specific genes, which allows us to understand the role these genes play in the development of cardio- and cerebrovascular disease. The most appropriate models will be chosen based on our ongoing studies or from the published scientific literature.

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

During this project we will maintain colonies of rat and mouse models of cardio- and cerebrovascular disease, and we will use these models to examine the underlying disease mechanisms. We will conduct our investigations in control animals and in animals which have been genetically modified to alter specific genes or proteins that may play a role in the disease process.

We will carry out a range of methods for assessing the characteristics of the cardio- and cerebrovascular system in our rodent models. These methods typically include repeated behavioural testing (to assess memory and brain function), blood pressure monitoring and echocardiography to examine changes in heart function and structure as these have been found to be altered after brain injury. Echocardiography is a noninvasive method used to image the heart and how it is functioning requiring brief anaesthesia for imaging purposes. Blood pressure monitoring is typically carried out on a weekly basis by non-invasive means. However, when scientifically relevant, blood pressure can also be monitored continuously and in greater detail using a probe that is surgically implanted into the animal. These studies may also involve collection of blood and urine samples at regular intervals.

Typically, animals will remain on procedure for up to 8 weeks.

Some of our studies will also involve modifying the function of the cardio- and cerebrovascular system in our rodent models by administering drugs or altering the function of genes which may be involved in the disease progression or of master switches which control the expression of these genes in order to modify the disease processes. These genetic studies will use either viruses, similar to those used in vaccines, or “extracellular vesicles”, which are small particles that circulate in the body carrying messages between different parts/organs as an engineered delivery driver. These intervention studies will also involve monitoring of blood pressure and non-invasive imaging repeated at regular intervals, as well as the collection of blood and urine samples. Typically animals will remain on procedure for up to 12 weeks (with a maximum limit of 16 weeks).

Typically, two thirds of rats or mice would undergo a surgical procedure to reduce blood flow to the brain - either abruptly, to model a stroke or more chronically/slowly to lead to progressive decline similar to that seen in vascular dementia. The stroke surgery involves

blockade of the main blood vessel in the brain that gets blocked in people who have a stroke. For the dementia model, this involves narrowing the 2 main arteries in the front of the neck that supply blood to the brain using either little coils or cuffs that squeeze/narrow these blood vessels or by tying a thread around them. The animals would be closely monitored daily for the 1st three days after either surgery with regular checks on body weight.

Typical behavioural assessments would be repeated between 3 days and 6 weeks post-surgery, following recovery from the procedure. During the entire 2 month period, there may be an MRI scan. The animals will typically be humanely killed by perfusion fixation or organ retrieval under terminal anaesthesia at the end of the study and tissue harvested for investigations. A typical animal would undergo one surgical procedure, blood pressure monitoring (bi-weekly), echocardiography (bi-weekly), blood sampling (bi-weekly) and behavioural testing (weekly), and would be humanely killed after 2 months.

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

Some of the rat models used in this project contain harmful mutations in their genes which result in cardio- and cerebrovascular disease characteristics of moderate severity (i.e. hypertension, cerebral small vessel disease and stroke). We observe approximately 6% incidence of stroke in male rats. The majority of these strokes are sustained after the age of 4 months, which is the time of established high blood pressure (hypertension). Spontaneous strokes are almost never encountered in female hypertensive rats (<1%).

The mouse models detailed do not show any changes to their behaviour or health as a result of any change to their genes.

Pain as a result of surgical procedures: all animals undergoing surgery may experience some pain. Analgesics will be given in consultation with the NVS and for as long as necessary.

Anaesthetic death (1%)

Weight loss from repeated anaesthesia (1%).

Weight loss following experimental stroke or vascular dementia may approach 20% due to reduced eating and faecal output in particular in the early 1-3 days after surgery, any further loss then the animal will be immediately humanely killed.

A rapid negative reaction/response may be seen to the maximum molecular switch (viruses, some of which are the same as those used in vaccines) in our in-house colony of rats (5%). Sensitivity may be increased in rats from external sources. This rapid reaction/response is not anticipated in mice. Any animal displaying signs of acute toxicity (e.g., pale colour, collapse, rapid breathing) will be immediately humanely killed.

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)?

Approximately 70-80% of the rats and mice used in this project will contain mutations in certain genes that result in cardio- and cerebrovascular disease of moderate severity.

The expected severities of the methods used for characterisation and monitoring range from mild to moderate. All animals will experience one or more of these monitoring methods.

60-70% of the rats and mice will undergo acute induction of cerebral ischaemia to model stroke or chronic induction of cerebral ischaemia to model vascular dementia (maximum severe severity). This may also include drug intervention and/or the use of viruses and/or extracellular vesicles or combinations of these as novel therapeutics.

20% of the rats and mice will undergo gene expression modification using virus or extracellular vesicle- mediated delivery or drug intervention (maximum moderate severity).

The cumulative effect of those animals not undergoing stroke or dementia modelling (30-40%) but who will experience repeated monitoring measures, drug, virus or extracellular vesicle-mediated delivery, is not expected to exceed moderate severity as the least harmful route and lowest volume of administration will be chosen for delivery of substances, the nature of the monitoring methods are broadly non-invasive and animals will be acclimatised or familiarised with these within the study design. In our previous experience of similar studies involving multiple assessment/intervention steps in combination with genetic modifications, we have identified no lasting harm as a result of cumulative adverse effects. Animals on these studies maintain weight, show normal behaviour and do not exceed scoring sheet limits of moderate severity.

A small number of animals may show sensitivity to dietary or pharmacological intervention resulting in body weight loss. These animals are carefully monitored using welfare scoring sheets and are removed from study or killed before exceeding moderate severity limits.

Some animals will experience surgical procedures that are classed as moderate. All animals undergoing these procedures are given analgesia and are allowed time to fully recover from the effects of surgery before undergoing further procedures.

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

- Killed

A retrospective assessment of these predicted harms will be due by 06 April 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 nature of stroke and dementia and complexity of these diseases makes finding alternatives to animal models extremely difficult. A search using the suggested guidance provided on the FRAME website offered no specific alternatives at present that could replace the use of animals. Although cell- based models are available to examine normal and disease mechanisms in cells from blood vessels and the brain there are limitations associated with interpretation of results from these models. These cells are mainly from juvenile or neonatal animals or tumour cell lines and may not be predictive of what happens in an intact adult animal. Usually a single cell type will be grown in isolation while in the body different cell types develop together and function as units.

More recently, cell models have been described where several cells within the brain are grown together, an "organ on a chip". However, a major limitation of all cell models is that they have no blood supply. In the body the circulation and sensory nerves that link organs in the body to the brain/spine (central nervous system), contribute to stroke and vascular dementia by providing a pathway for immune, inflammatory and reflex signals and influences between the brain and the rest of the body.

Hence, disruption in the blood supply is a major driver to the injury seen in stroke or vascular dementia. Modelling of the flow of blood (or other fluids in the case of cell models) is challenging and requires highly specialised equipment and it is still limited to quite smooth surfaces of cells when these are not reflective of blood vessels in those who go on to have a stroke or develop dementia. Therefore, when positive data are generated in cell systems, the next step is to progress to animal models, normally first in rodents and then in primates, before final translation to clinical trials in humans.

Advances have been made to the bench to bedside translation of research findings through guidelines devised by groups of industrial and academic researchers (eg the STAIR guidelines) and through guidelines relating to animal welfare (IMPROVE guidelines) and reporting of animal studies (ARRIVE guidelines). All of these combined, have improved the quality and reproducibility of animal studies in stroke and dementia, in particular. And the recent ESCAPE-NA1 clinical trial, showing a new drug to protect brain cells (neurons) in people who have had a stroke and improve outcome in these people,

supports this. The drug used was 1st tested in rodent and non-human primates and found to be effective in these models.

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

Where possible we use non-sentient alternatives to live animals. Research on the disease processes contributing to stroke and on the efficacy of new therapies can start with studies of cells or brain slices grown in culture. And we will continue to use these. Within my research group we have established models of low oxygen (hypoxia) in cell culture which mimics what happens in the brain after a stroke. Furthermore, cell culture is used to develop and optimize delivery systems in some of our studies before animal testing is performed or to investigate the functional analysis of master switches of gene expression and pathways of interest. We study many cells within the brain – cells that line the blood vessels (endothelial cells), the main "wiring" cell in the brain (neurons) and the brain's resident immune cells (microglia).

Why were they not suitable?

For some of the early studies, the cell culture models are suitable. But there reaches a point where there are limitations associated with interpretation of results in these models; cells are mainly derived from neonatal animals or tumour cell lines and therefore results may not be predictive of the cellular response in the adult brain. Usually a single cell type will be grown in isolation while in the brain different cell types develop together and function as units. Also cell systems have no blood supply, while in the body the circulation and sensory neurones that link organs in the body and the central nervous system (brain and spine).

Therefore, when positive data are generated in cell systems, the next step is to progress to animal models, normally first in rodents and then in primates, before final translation to clinical trials in humans. Although cell-based models can provide important detailed information on mechanisms occurring within the cell, and will be used alongside our rat and mouse studies, they cannot fully replace the use of live animals for investigation of cardio- and cerebrovascular disease progression where an intact cardio- and cerebrovascular system is necessary.

A retrospective assessment of replacement will be due by 06 April 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?

These numbers are for the full 5 year duration of a project licence and are based on ongoing projects within the group and projected plans for future studies. Initial pilot studies have been conducted (under authority of a previous licence) to establish the lowest appropriate group sizes for investigation of cerebro- and cardiovascular parameters in our models. Estimated numbers of rats and mice have been based on these group sizes for the appropriate comparison of treatment and control groups using a randomised block design.

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

Reduction has been achieved by using power analysis calculations on existing data sets (or if unavailable from the literature) to determine the minimum number of animals required per group for a given study. Studies may also include specialised MRI to allow imaging to be repeated over the course of a study to determine the evolution of damage non-invasively in a single animal. This removes the need for multiple groups of animals to be humanely killed at each time point. This also increases the statistical power of the studies whereby the effect of an intervention can be assessed in the same animal over time. Also, since data for each time point come from the same group of animals, there is reduced variability in the data sets and consequently smaller group sizes are required when power calculations are carried out for study design.

In the design of this project we have adopted the quality standards required for clinical trials (eg randomisation, blinding, sample size calculations) and considered the IMPROVE guidelines [doi: 10.1177/0271678X17709185] relating to preclinical stroke studies. All findings will be reported according to the ARRIVE guidelines [https://doi.org/10.1371/journal.pbio.3000410].

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

Pilot studies to determine efficacy/dose will be performed where appropriate. Animals undergoing procedure, we routinely harvest organs which may not be the primary focus of the research project as these may be relevant or interesting to other groups in the wider scientific community. Sharing of material and collaborations to allow this occurs already both from and back to my group. Hence, if we are able to obtain material without the need for further animals to be used - we always look to do so.

Furthermore, we will use efficient breeding strategies striking a balance between the numbers of animals required to assure continued genetic integrity and reduction, replacing

breeders before reproductive performance declines, replacing non-productive breeders as soon as possible, using experienced males.

A retrospective assessment of reduction will be due by 06 April 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 model(s): The rat and mouse models we use are carefully selected based on their unique genetic profiles. Some of the models we use will have established hypertension (high blood pressure), which occurs spontaneously (naturally) in adulthood. This is known to be a major risk factor for both stroke and dementia. This animal is also considered the best available spontaneous model of cerebral small vessel disease (SVD). By including an animal model which displays some of the key risk factors associated with the development of stroke and/or dementia and/or SVD (eg high blood pressure), we hope to improve translation from pre-clinical to clinical studies. This is also a key recommendation of some of the guidelines related to pre-clinical stroke studies. Some of our mouse models have been genetically modified to inhibit (knock out) or add in (knock-in) the function of specific genes, which allows us to understand the role these genes play in the development of cardio- and cerebrovascular disease. The most appropriate models will be chosen based on our ongoing studies or from the published scientific literature.

Disease model(s): In order to study stroke in an animal model, a blood vessel in the brain is blocked either permanently or for a shorter defined time period, and the consequence is irreversible damage to a specific brain region and a functional deficit which reflects where in the brain the injury occured (as occurs in the clinic in humans). In order to study new therapeutic strategies, you must have a reproducible amount of damage which is large enough to detect therapy-induced reductions in the area of the brain that is damaged and dies, and in a location within the brain which will produce a functional (sensorimotor) deficit. Therefore, all stroke protocols come under the severe severity band. However, most rodents recover very quickly (usually within 1-3 days as brain swelling subsides) and develop compensatory behaviour to overcome some of the deficits caused by the brain

injury. In any case, we have developed clear humane end-points in order to limit potential suffering and these will be implemented as necessary.

To model vascular dementia, the two main blood vessels in the neck that supply blood to the brain will have small coils, cuffs or a thread wrapped around them to decrease, but do not eliminate, blood flow to the brain. This in turn leads to inflammation, the blood brain barrier breaks down and the animals show signs of progressive cognitive impairment as is seen in people with dementia. Hence this type of brain injury falls within the severe severity band.

Our protocols have been chosen to provide the maximum detailed information necessary for understanding all the factors that contribute to these diseases, whilst at the same time ensuring that the animals under investigation experience the least pain, suffering, distress or lasting harm.

Administration of substances will be carried out by the least severe/painful method available; for example administering drugs in food or drinking water rather than by injection. The substances we propose to use for our gene transfer studies are well characterised, and the maximal dose for our rat and mice colonies has already been established. We will ensure that this maximum dose is never exceeded. Again, the least invasive/painful route of delivery will be chosen.

Animals will always be housed in pairs or groups unless individual housing is required due to welfare reasons or for scientific purposes.

For all studies involving animals we follow ARRIVE guidelines to ensure good practice and transparent scientific reporting (e.g. we randomise our animals when including them in our experimental studies and our researchers remain ‘blinded’ to the treatment groups until after the study is completed. This reduces unconscious bias in experiments. We will also consider the IMPROVE guidelines for preclinical stroke studies.

The ultimate benefits arising from the research programme will be improved diagnosis and treatment of those with stroke or vascular dementia or both.

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

The ultimate aim of any new treatment for stroke or dementia is to improve quality of life. Clinically, this is measured with scales to assess the degree of disability or dependence in the daily life or through tests of executive function and memory. To reflect this in preclinical models to allow the impact of experimental stroke or vascular dementia to become evident, animals need to recover from surgery and measures of cognitive decline or neurological deficit given time to develop. Hence, terminal anaesthesia procedures are not compatible with this key outcome. Similarly, stroke and vascular dementia are diseases of the elderly and therefore, to improve translation, animals at more immature life stages may not be reflective of this as the brain may still be developing and so be more likely to rewire than would occur in an adult animal. This would confound result interpretation. Mice are

the least sentient vertebrate group in which the process of hypertension development and its associated target organ damage in the intact animal can be interrogated. However, the rat is the more tractable model of the human cardio- and cerebrovascular system, displaying greater similarity with humans in terms of disease pathophysiology.

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

For animal research, the stroke model used (e.g. vessel blocked permanently or vessel blocked temporarily) is dependent on the scientific question being addressed or the drug under test. The mildest insult (e.g. how long the vessel is blocked for) which produces a reproducible amount of damage which can be measured using tests to see the effect on functional outcome will be used, to minimise animal suffering and post-operative care (regular monitoring, subcutaneous fluids, maintained body temperature, softened diet, etc.) provided. For stroke related therapies, it is important to establish efficacy in a range of models. If a drug shows potential to reduce brain damage and improve functional outcome in one model, further studies will test its efficacy in a range of models (incorporating co-morbidity/risk factors, ageing, sex etc.) Animals under procedure will be checked regularly for any deterioration in their condition or adverse effects of therapy. Should this occur, prompt veterinary advice will be sought. If the severity limits of the licence are approached, the animal will be promptly and humanely killed.

All of our animals are monitored on a daily basis. Those animals undergoing surgery or other interventions to modify the cardiovascular system will be scored according to a numerical scoring sheet for pain/discomfort/distress in mice & rats, and if necessary will be humanely killed.

Regular non-invasive monitoring by blood pressure measurement or how the heart functions (echocardiography) will typically identify harmful changes in the cardiovascular system prior to the development of overt clinical signs.

To reduce stress, animals are regularly handled, and will be acclimatised for short periods in metabolic cages, trained for behavioural measures, functional outcome and in tests of memory and familiarised with the blood pressure recording procedure prior to the actual measurement phase of the study.

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

Percie du Sert et al - The IMPROVE Guidelines (Ischaemia Models: Procedural Refinements Of in Vivo Experiments) (2017) - J Cereb Blood Flow Metab 37 3488-3517.

Percie du Sert et al - The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research (2020). PLOS Biology https://doi.org/10.1371/journal.pbio.3000410

Lapchak et al - RIGOR Guidelines: Escalating STAIR and STEPS for Effective Translational Research (2013). Transl Stroke Res 4 279–285.

Fisher et al - Update of the Stroke Therapy Academic Industry Roundtable Preclinical Recommendations (2009). Stroke 40 2244–2250.

Macleod et al - Good Laboratory Practice: Preventing Introduction of Bias at the Bench (2008). Stroke

40 e50-e52.

Local guidelines.

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

The Project Licence holder and members of the research team will ensure continued professional development in the 3Rs area through regular attendance at meetings/workshops such the Animals in Science Regulation Unit (ASRU) annual meeting, 3Rs workshops/symposiums held at local or national Research Institutes, and also attendance at local training workshops organised by the NTCO.

The NC3Rs webpage (https://www.nc3rs.org.uk/) is a site we regularly visit. I regularly discuss best practice with colleagues who have direct experience and expertise in related models - animal or disease (e.g. stroke or dementia) - to those we use.

A retrospective assessment of refinement will be due by 06 April 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. Safety evaluation of substances administered to man

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

Toxicity, Safety, Toxicokinetics

Animal types	Life stages
Mice	juvenile, adult, pregnant
Rats	juvenile, adult, pregnant
Rabbits	adult
Minipigs	adult
Beagles	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

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 licence is to assess the safety of substances that may be administered to humans, including new pharmaceutical drug products, food and drink additives and supplements and novel foods.

A retrospective assessment of these aims will be due by 05 April 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 vital to ensure new pharmaceutical drugs, and food and drink products, are safe for humans to use. It is generally accepted that the way in which a material is metabolised within a living body has a significant effect on how it works and its potential toxicity. Therefore, the work conducted under this licence requires the conduct of studies in living animals in order to assess for toxicity of tissue, organs and systems following single or repeated administration (e.g. the cardiovascular, respiratory and reproductive systems). Without these studies, new medicines could not be developed and there are risks that unsafe food and drink related products may be used.

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

Data derived from this project will be used to identify possible effects on the body/organ systems caused by a substances such as pharmaceuticals and food stuffs (additives, supplements, ingredients and novel foods). This data will then be used to inform scientists of possible adverse effects that may occur and to allow regulatory authorities to make judgements on whether to permit clinical studies to proceed, to licence a drug or to grant a marketing application.

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

Safety assessments detailed in this licence will ensure the safe use and management of products for human consumption/administration. In the short-term, this is achieved by providing high quality data to guide decisions regarding the suitability and the safety of the product for further development.

Ultimately, these outputs will ensure that humans are not exposed to hazardous products in foods or drinks, and in the case of pharmaceuticals and therapies, the data generated

will support the development of safe new medicines designed to improve the health and quality of life of patients.

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

While much of the substance specific data generated is covered by confidentiality agreements, work on novel biomarkers, refinements in methodologies, protocols and techniques that permit a reduction in the number of animals required for specific study designs, or to achieve specific end points, are freely shared and discussed at Scientific conferences and other forums (e.g. attendance at regular NC3R meetings).

Although most studies will require the use of concurrent Controls to provide contemporaneous data for direct comparisons (to represent animals undergoing the same regulated procedures, administered the same vehicle etc), data generated from Control animals is held in reference databases to provide information relating to normal biological variation, thereby enhancing interpretation of study findings.

Species and numbers of animals expected to be used

- Mice: 35,000
- Rats: 35,000
- Rabbits: 2500
- Minipigs: 1000
- Beagles: 2000

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.

It is generally accepted that the way in which a material is metabolised and distributed within a living body has a significant effect on how it works and its potential toxicity. Unfortunately, at this time, effects on complex interacting biological systems cannot yet be replicated using in-vitro or ex-vivo methods (non-animal models). Consequently, the use of animals is still an essential part of safety testing.

All studies performed under this licence will use the least sentient species practicable for achieving the study objectives. The majority of cases (circa 90% of studies) will involve the use of rats and mice. On occasions where rodents are not regarded as a suitable species i.e. they are not able to provide the safety data necessary, or regulatory guidelines requires that studies should be performed in a rodent and a non-rodent species, then the rabbit or pig may be used. When no other species will generate the safety data required to meet regulatory requirements, then the beagle dog will be considered.

In many cases, regulatory guidelines dictate the life stage of the animal that will be used, and this will be commensurate with that age in which humans will be exposed to the material being tested. To this aim, the majority of animals will be classed as adult. There will, however, be occasions where the test substance is intended for paediatric use, i.e. will be administered to children. On such occasions safety testing will be performed using juvenile rats or mice.

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

The majority of studies will follow a similar paradigm; Animals will be dosed with the test substance (for example a pharmaceutical product) using a route that will be used clinically e.g. oral administration, injection into a vein, or dermal application. The duration of administration will be dependent on the objectives of the study, but will range from a single dose on one occasion only, to daily administrations for up to 104 weeks (usually in rats and mice) when the pharmaceutical is intended for long-term use, i.e. the treatment of a chronic disease and the objective of the study is to assess for carcinogenic potential.

During the course of the study the animals will be well cared for and will be closely monitored for reactions to treatment. Blood and/or urine samples may be collected in order to assess for clinical condition and treatment related effects. Similarly, an ECG will be taken at intervals during the study (usually in dogs or minipigs) to establish if there are any effects in the activity of the heart. Other end points will also be included as required to address specific concerns (e.g. functional observation tests, toxicokinetic evaluation, blood pressure assessment).

At the end of the study the animals will typically be euthanised and a necropsy undertaken. This is essential because it is important to establish if the internal organs have been affected in any way and this can only be achieved by pathological examinations by a qualified Pathologist.

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

It is anticipated that some animals will lose weight, or at least fail to gain weight at a rate consistent with normal weight gain. This may be attributed to a reduction in food consumption (also a potential adverse effect), but may be present even with normal food consumption.

Animals will be closely monitored for signs of discomfort and particularly signs of pain. Any animal showing such signs will be closely monitored and will be inspected by a Vet if considered necessary. No animal will be allowed to endure pain and remedial action will always be taken.

As animals get older, particularly those on long-term studies, they may develop tumours. This may be sporadic and due to 'old' age, but may be attributed to the administration of the test substance. Animals will be assessed for development of internal and external

tumours by visual assessment and gentle palpation. Any animal with a tumour will be closely monitored and steps taken to ensure that they are not in undue discomfort, that their mobility is not impaired and that they are still able to eat and drink. If the size and location of the tumour is considered to significantly impair the health and welfare of the animal then it will be humanely killed.

Ageing

Alterations in clinical condition may be encountered during long-term studies (typically greater than 26 weeks in duration) due to the age of the animals as the studies progress. Examples of this include reduced body weight (loss and/or reduced weight gain) and development of masses; these will be monitored in accordance with the humane end points outlined in other sections of this licence relating to body weight and tumours.

Additional examples associated with ageing include abscess formation on the preputial glands in rodents. Such abscess(es) will typically resolve with time, allowing the animal to return to a normal state. Provided there is no other impact on the animals' clinical condition, and the animal is not demonstrating signs of distress, animals with preputial gland abscesses will be monitored daily for up to 10 days following identification of the abscess to allow recovery. If there are no signs of improvement within this period, the animal will be euthanised by an appropriate method.

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)?

It is expected that the majority of animals (circa 75%) will experience no more than Mild discomfort e.g. a small effect on clinical signs, body weight and/or food intake. Other effects are possible, but will be transient and fall into the category of Mild severity.

It is feasible that a further 20% of animals will experience effects considered to be of Moderate severity

e.g. a more significant effect on weight loss and/or food consumption, as well as other effects such as lethargy and ptosis (half closed eyes).

In rare cases, certainly in less than 5% of all animals used, a more severe reaction to treatment may be experienced. This is typically due to unexpected toxicity in particularly susceptible animals. Animals reaching severe severity will be euthanised without delay in order to prevent further suffering.

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

Killed
Kept alive

Rehomed
Used in other projects

A retrospective assessment of these predicted harms will be due by 05 April 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 generally accepted that the way in which a material is metabolised within a living body has a significant effect on how it works and its potential toxicity. Also the potential direct effects of a test substance on living tissue are often different to that predicted by ex-vivo or in-vitro methods e.g. due to in-vivo protective mechanisms. Consequently, for the majority of substances forming part of the work it is imperative they are tested on living animals in order to assess for toxicity to tissues, organs and systems (e.g. the cardiovascular, respiratory and reproductive systems) following single or repeated administration.

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

Sponsors will typically utilise non-animal alternative assays as part of the development programme for a substance (e.g. in silico modelling and in vitro metabolism studies). Such assessments would not be conducted under this project, however, where available, these data would be used guide the selection of the most appropriate species and to confirm the in vivo study design (e.g. to confirm the species which show similar patterns of metabolism to those expected in man).

Whilst information from these alternative assays will contribute to the overall safety assessment, they cannot replace the need for in vivo studies at this time.

Why were they not suitable?

The use of alternative methods, including the use of dead animals cannot, at this time generate relevant data which supports the submission of safety data to international regulators. The fundamental aspects of the safety data we require involves assessing physiological, behavioural and biochemical effects following the administration of a test substance and this can only be achieved by using live animals.

Alternative methods such as in-vitro techniques will be used as much as practicable to supplement the work involving protected animals.

A retrospective assessment of replacement will be due by 05 April 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 establishment maintains detailed records pertaining to the numbers of animals used on projects each year, as well as the number of study types undertaken. By analysing annual trends and having knowledge of industry requirements, it is possible to project the number of study types we will undertake during the life of this licence thus enabling the estimation of the number of animals required.

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 will be used, recognising the fact that reduction is not achieved by using too few animals to achieve the objectives of the study. For Regulatory studies, guidelines require the number of groups and animals per group to be adequate to clearly demonstrate the presence or absence of an effect of the test substance; core study designs are based on international guidelines where these exist. Otherwise reference is made to internal guidance on study designs to provide the optimum number, balancing the need to achieve study objectives while avoiding excessive animal use. Project specific variations are used as required. The core study designs have been used extensively under the previous project licence and in other facilities and we have a track record of successful submissions and ability to eliminate unsuitable compounds. They are generally in line with those used throughout the pharmaceutical industry.

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

Statistical input is sought, where appropriate, to strengthen the overall scientific quality and relevance of the studies to be performed, with power-sample size calculations performed for specific studies if necessary to determine group size. Where group sizes are sufficient

(rodent studies), data from definitive toxicity studies are analysed statistically. Group sizes in dog and minipig studies are usually smaller than in rodent studies and consequently are of low statistical power. However, these smaller group sizes are feasible due to the multifactorial nature of toxic changes, assessment of toxicity in these species is made by examination of data from each animal and by correlation of in-life and post mortem findings within an individual, rather than simply assessing group mean values and statistical parameters.

A retrospective assessment of reduction will be due by 05 April 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 most cases the studies will be performed using internationally recognised guidelines (e.g. ICH and OECD) which define the most appropriate methods and scientific models to use.

The majority of animals used throughout this project will be rats and mice. On occasions however, and when scientifically justified, or specifically required by the regulator, the rabbit, beagle dog or minipig may be used. The selection of an appropriate species will be a combination of ethical, scientific and practical consideration.

The species chosen will be the lowest neurophysiological sensitivity to achieve the objectives of the study and one which responds to the primary pharmacodynamic effects of the test substance; In most cases, this will be the rat or mouse.

Most pharmaceuticals need to be tested on a second species, with the second species being a non- rodent (CPMP/ICH286/95) modification). The selection of a suitable non-rodent species is of paramount importance as it will maximise human safety, clinical benefit and animal welfare. At this time, the dog is the primary non-rodent species used because of historical/data experience, practicalities and legislative requirements; and as such, will be the principal non-rodent species used in this licence.

The methods (procedures) used will be validated, well established and commonly used within the research community. The administration of test substance, removal of blood, collection of urine for example will cause no more than transient discomfort or distress. Any signs of distress will be carefully monitored including the onset and severity of treatment related effects. Appropriate and swift action will be taken to avoid any undue pain or distress.

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

The majority of studies performed require changes in physiological, pharmacological or behavioural activity to be monitored. Performing procedures under terminal anaesthesia or using immature life stages, for example, would not permit these important findings to be observed, thereby preventing a detailed safety profile of the test substance from being produced.

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

The procedures required will be undertaken by competent staff, each having undergone extensive training and competency assessment. These procedures also undergo continual refinement to ensure the methods remain the most appropriate for minimising pain, suffering and distress to the animals.

Animals will be monitored immediately after undergoing a procedure for any signs of adverse effects and will continue to be monitored at appropriate intervals until it is deemed that further observations are not required.

Animals will be habituated to procedures whenever considered necessary i.e. when it is deemed that by habituating animals to a procedure distress will be reduced. Similarly, where appropriate animals may undergo training to perform certain tasks thereby, minimising distress by removing the need to restrain an animal or involve direct contact during its performance.

In addition to this, the company is an industry leader in the application of microsampling techniques for obtaining blood samples in toxicology studies, which not only reduce the volumes required but also reduce the severity of the sampling procedures.

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

The volumes administered to the animals and the volumes of blood taken will be in compliance with industry accepted guidelines. The primary guideline used is:

"A Good Practice Guide to the Administration of Substances and Removal of Blood, Including Routes and Volumes"; Karl-Heinz Diehl, Journal of Applied Toxicology J.Appl. Toxicol. 21 15-23 (2001).

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

Staff maintain a proactive attitude towards the 3R's. Several members of staff are already participants in Industry Forums which discuss the 3R's in some detail and report any advancements to relevant persons. These advancements will be discussed further and implemented into our standard practices, where appropriate. The company is an established leader in the development and application of the 3Rs in toxicology studies.

A retrospective assessment of refinement will be due by 05 April 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. The penumbra as a therapeutic target in cerebrovascular 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

ischaemic stroke, brain tissue recovery, comorbidities, stroke outcome, metabolic support

Animal types	Life stages
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?

Cerebrovascular disease, such as ischemic stroke, are a devastating family of conditions that interfere with the blood supply to the brain. Since the brain does not contain any significant oxygen or glucose reserves to support cellular metabolism during a cerebrovascular event, brain tissue swiftly and irretrievably dies during stroke. The only effective treatment is a swift reopening of the blocked artery, called recanalization, to restore the flow of oxygen- and glucose-containing blood. The penumbra is a tissue at risk that still receives a residual blood flow that, however, is insufficient to keep it alive over

time. In case of timely recanalization, the penumbra can be salvaged but otherwise turns into infarcted tissue within a few hours. This also means that only patients with a penumbra benefit from recanalization.

Recanalization is only possible in dedicated major healthcare centres. Often, the penumbra is already lost by the time patients get there. Thus, only very few patients (much below 10%) currently benefit from recanalization.

Pharmacological support of the penumbra until as such time as recanalization procedures can be attempted may thus be of benefit in the brain affected by an acute cerebrovascular event. Such support may be provided through antagonism of downstream damaging and energy-sapping events, such as the activation of glutamate receptors, or the neutralization of reactive oxidative species, and the stimulation of vascular growth through stems cell and neurotrophin-based approaches, but equally, targeting upstream events, such as depletion of cellular adenosine triphosphate (ATP) may prove useful. This is because ATP is the universal fuel that supports the majority of cellular activity. Thus, ATP-restoring or ATP-sparing therapeutic interventions may be of value in cerebrovascular disease.

As an example of an ATP-restoring approach, we have shown in a rodent model that it is possible to pharmacologically restore ATP levels in damaged brain tissue after stroke with the use of compounds that are already in use in man for different purposes. Further validation of such metabolism-based approaches to salvage the penumbra may lead to new and faster point-of-care therapy for patients suffering cerebrovascular events. We hypothesise that the compounds and reagents to be investigated during the tenure of this Project Licence could support brain energy metabolism particularly in the penumbra and therefore significantly enhance the chances of a good outcome after stroke. Thus, the overall aim is to preserve the penumbra by providing energy support. Preserving the penumbra even for a few hours will allow many more patients to benefit from recanalization approaches, and to a much larger extent.

A retrospective assessment of these aims will be due by 29 April 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?

After a cerebrovascular event, such as stroke, it is crucial to return cerebral blood flow to affected areas as fast as possible to restore the cerebral metabolic state. However, the

current thrombolysis/thrombectomy approaches face limitations: a narrow time window; the lack of facilities at smaller centres; the potential for insufficient reperfusion despite recanalization, and –importantly– not addressing the loss into the blood stream of ATP metabolites necessary for ATP resynthesis. The therapeutic time window could be widened and improve outcome if the brain tissue could be metabolically supported before and after recanalization.

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

If successful this project may lead to the development of simple yet effective strategies to reduce acute and long-term effects of cerebrovascular disease, which can be used alongside current clinical practice.

Some of the compounds we want to further investigate are already in clinical use for other purposes - we thus expect that the route of translation from preclinical research to patient treatment with these repurposed compounds will progress much quicker than for entirely novel compounds or more complex procedures.

We will gain new insights to help to improve stroke treatment as our study aims to investigate the influence of the purine salvage pathway on the possible recovery and/or rescue of brain tissue affected and damaged by, for example, ischemic stroke.

Further, we aim to share our obtained discoveries with the scientific community in openly accessible peer-reviewed scientific journals. We hope that our investigations will

significantly contribute to the understanding of metabolic pathways in pathological conditions and enable the rapid development of efficient point-of-care treatments.

At the end of the project we hope to have established:

- whether metabolic and related support can preserve the penumbra in relevant stroke models

- whether this improves overall outcome in animal models of recanalization, and is compatible with existing recanalization strategies

- whether animals with comorbidities mimicking the situation of most human patients also benefit from the approach

- how long and to what extent the benefit can last

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

We hope to deliver significant benefits for the outcome of stroke patients, in particular ischemic stroke patients who suffer the highest incidence of common cerebrovascular events. Some of the strategies we propose are based on compounds already in use in the

clinic for different reasons, while other strategies utilise known medicinal chemistry approaches to deliver false nucleotides in the treatment of cancer and viral infections.

The major benefit we predict of these ATP-restoring approaches are the limited (if any) side effects, and the ease of delivery – for example intravenously at the primary point of care. As such, it could be already delivered in a pre-hospital setting through paramedics, which would preserve the affected but salvageable penumbra brain tissue for longer until recanalization attempts or other interventions in a specialised facility can be performed. This would ultimately lead to reduced mortality and morbidity in patients and facilitate a successful rehabilitation. The results generated by the proposed project could serve as an important prerequisite for the next steps to be taken for successful translation to the clinic and to the general benefit of patients, including for those suffering traumatic brain injury, where similar ATP deletion occurs.

In the long run, we hope that the number of patients benefiting from successful intervention can be significantly increased, and that individual patients benefit more. This would reduce individual burden as well as burden of caretakers and the NHS. The intervention is relatively simple so many patients could benefit worldwide.

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

Our study design ensures that we will be able to understand the overall effectivity of the approach including metabolic and neurobiological basis. We will gain information on the time window for intervention and, importantly, whether comorbid subjects (representing a significant part of human stroke patients), can equally benefit.

Moreover, we plan a series of regular meetings of stroke survivors, their carers and families to share with them developments in this project and elsewhere. This will be an opportunity to meet informally with scientists, doctors and other health-care professionals involved in stroke. In addition, we will maintain a website with project updates and readily digestible descriptions of stroke research that people affected by stroke and other cerebrovascular disease may find interesting and informative. The outcomes from this study will be reported to local stroke support groups, and at Stroke Association meetings, should the opportunity arise. Outcomes will be reported to the scientific and clinical community through publication in Open Access form in peer-reviewed journals and written in accordance with ARRIVE guidelines. Additional routes for informing people will occur via social media platforms and through our study website.

Species and numbers of animals expected to be used

- Rats: 800

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.

Rodents, including the rats we propose to use, are commonly used in preclinical stroke research. This is because, while it is recognised that there are differences between the rodent and human brain, the pathological event that we are targeting, ATP depletion, is common to, and highly similar, in both rodent and man. Thus, the model is appropriate to study the outcome of manipulating this most fundamental of cerebrovascular disease-induced pathologies. Rodents have been invaluable in understanding the basic pathophysiology of stroke, in appreciating the concept of tissue-at-risk after cerebral ischaemia, in revealing ischaemia-related cortical spreading depolarisations and in demonstrating the protective effects of hypothermia. We will use adult normotensive rodents (e.g. Sprague Dawley, Wistar) and comorbid (e.g. spontaneously hypertensive rats; SHR), together with their appropriate control (e.g.

Wistar Kyoto, WKY). Comorbid rats exhibit a cardiometabolic risk profile typical for stroke patients including hypertension, hypercholesterinaemia, insulin resistance, and others, and their use is a necessary step on the clinical translation pathway in stroke research.

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

The procedure will use rats and involve the induction of cerebral ischaemia under general anaesthesia and the delivery of compounds typically via an intravenous cannula to test whether they improve outcome. Cerebral ischaemia will be induced by the insertion of a filament to occlude the middle cerebral artery (middle cerebral artery occlusion, MCAO), which is one of the three major arteries that supply blood to the brain, for a defined period of time to mimic successful recanalization of a human cerebral artery. Following the induction of cerebral ischemia under anaesthesia, the animal will either i) be recovered to allow sensorimotor, behavioural or neurological assessment for a period not exceeding 28 days, or ii) will remain under general anaesthesia for up to 8 hrs for the direct assessment of such parameters as cerebral blood flow and oxygenation, cortical spreading depolarisation, seizure activity and purine and other neurometabolite release, after which time they will be euthanised.

The evaluation of sensorimotor function will be primarily performed through several neurofunctional tests (please refer to Table 1. These tests all utilise natural rodent behaviours such as exploration, locomotion and grooming and are not considered stressful (sub-threshold). Some of the more complex require mild stressors as outlined in Table 1.

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

It is not anticipated that the animals will suffer pain as a result of the stroke or the treatment given. Careful post-surgical monitoring for pain (grimace scale, weight loss, coat condition, abnormal behaviour or unusual behaviour) or infection (redness/inflammation, discharge) will ensure animal welfare is maintained as a top priority. Any signs of ill health (e.g. reduced ability to feed/ thrive/ interact) will be treated promptly in consultation with

the animal facility's Named Animal Care and Welfare Officers (NACWOs) and, if necessary, the Named Veterinary Surgeon (NVS). If it is not possible to mitigate the ill health the animal will be euthanised via an appropriate method, with tissue salvaged where possible to conduct ex vivo investigations, for example into the cause of the ill health. If no post-anaesthesia recovery (ability to feed, groom, urinate/defecate, move freely) occurs within a reasonable time (~24 hrs) the animal will be euthanised and according to pre-defined humane endpoints.

Unexpected mortality will be assessed by a post-mortem examination. It is of highest interest that the animals receive immediate resolving treatment, e.g. analgesic treatment in

presence of pain. If these measures fail to improve the state of the animals, they will be euthanised. We estimate the overall severity of the procedures for the animals as moderate.

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 estimate the overall severity of the procedures for the animals as moderate. The animals will receive a sufficient acclimatisation period of 7-10 days as recommended by and in compliance with the ASPA Guidance Notes and the Code of Practice, and the LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery. For neurofunctional assessment, which involves behavioural testing, the animals will be trained prior to any procedures to habituate them to being handled and familiarise them with the different test environments and conditions. The overall burden for the naïve animals occurring from behavioural testing can therefore be estimated as mild. All surgeries planned in this project will be in compliance with ASPA and the Code of Practice and therefore performed with the highest scientific standards to ensure the highest possible animal welfare. Although the surgeries are highly invasive and the animals will perform the previous trained behavioural tests post-surgery, we expect the overall severity to be moderate as we will ensure to reduce the impact on the animals' welfare as much as possible. The animals will be closely monitored, preventive pain medication and housing enrichments (e.g. nesting materials, cardboard tube, and shelter) will be conducted to ensure that no animal is experiencing unnecessary and extensive pain and discomfort.

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

- Killed

A retrospective assessment of these predicted harms will be due by 29 April 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 seek to study the effects of novel treatments on the outcome after cerebral ischaemia in animal models that are clinically relevant to human stroke, including animal models that have comorbidities often seen in human stroke patients. One of the common comorbidities seen in stroke patients is hypertension, which is why, for example, the SHR is a highly suitable model for our endeavour, and could not otherwise be modelled in silico or even in vitro. Important outcome measures will involve analysis of brain lesion volume and sensorimotor and cognitive function (e.g., motor function and tactile function, spatial working and reference memory), which is why the study cannot be performed using reduced cell culture-based in vitro systems or brain slices. Furthermore, the treatment regimens to be investigated are, therefore it would be unethical to use these compounds in potential human clinical investigations without statistically robust evidence of their efficacy and safety in a suitable animal model.

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

This study requires an animal model in which the outcome of stroke is comparable to that in man. To evaluate the outcome of stroke it is necessary that tests can be conducted which evaluate the neurological function in a manner that is comparable to methods used in stroke patients. It is therefore necessary to perform the study in rats, as there are no species of lower sentience that meets the required criteria, and the manipulations and outcomes cannot be modelled in silico.

Why were they not suitable?

There is currently no in vitro or in silico model that can simulate the complex pathophysiology of stroke and cerebral blood supply, particularly in the context of comorbidities, to an extent that comes close to the human situation. To investigate whether stroke outcome can be improved it is essential to examine the potential effects in an animal species with comparable physiology to humans. The outcome not only depends on neuronal mechanisms, which could be mimicked to a certain extent in cell culture-based approaches but most importantly on physiological mechanisms that involve motor and cognitive function, cerebral blood supply, blood pressure and others. It is therefore not possible to replace the use of animals with in vitro methods or in silico systems. Further, this study is based on the results of several comprehensive experiments in reduced in vitro

systems using brain slices in which the effect of the active agents was thoroughly investigated. The convincing results of these previous studies led to the design of this study as the obtained data suggest a potential significant benefit in the treatment of stroke patients and therefore provided a strong body of evidence to justify animal experiments.

A retrospective assessment of replacement will be due by 29 April 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 is based upon a) the need to replicate the observations to the point of statistical robustness given the variability seen in experimental models of stroke, b) the range of recovery and non-recovery experimental studies we plan to conduct in both normotensive and comorbid animals, and c) the inevitable mortality associated with the induction of experimental stroke. Data obtained in the underlying pilot study of this project were used to estimate the number of animals needed to obtain convincing data to evaluate whether novel treatment approaches are worth translating to a clinical trial with human stroke patients. We are confident that we have specified the minimum number of animals necessary to achieve statistically robust and clinically relevant results based on the findings of our previous studies, and considering a realistic inter-subject variability and mortality (~10%, most commonly under anaesthesia).

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

We have based our study on best practice for preclinical animal studies in stroke as described in the PREPARE, STAIR and IMPROVE recommendations in compliance with the ASPA Guidance Notes and the Code of Practice. In addition, we will follow the recommendations of the Stroke Recovery and Rehabilitation Roundtable (SRRR) international taskforce as they relate to the monitoring and the recovery of experimental rodents after stroke. These guidelines are designed to facilitate the design, performance, reporting, interpretation, reproducibility and clinical relevance of preclinical stroke studies while equally considering animal welfare and the 3Rs. These guidelines include reference to the randomisation of experimental animals to treatment groups and the blinding of

researchers and analysis to experimental treatments, and will be followed diligently during the course of this project.

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

We have used the pilot data from our previous stroke study to arrive at estimates of animal numbers. While we anticipate a certain level of mortality (~10%, most commonly under anaesthesia; potentially higher for comorbid animals - 15-20%), we have taken this into account in our calculations. We will also employ a continuous data analysis paradigm. A researcher not enrolled in animal experimentation and data collection will continuously analyse all data. This researcher with a strong background in statistics will not communicate to the experimental team to keep blinding and randomisation unaffected. The research will be stopped in case a statistically significant intergroup difference was reached before the requested number of animals was used, or in case it becomes clear that statistically significant results cannot be obtained anymore with the remaining number of subjects. This avoids using animals without generating further knowledge.

A retrospective assessment of reduction will be due by 29 April 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 rodent model of middle cerebral artery (MCA) occlusion is the state-of-the-art model to simulate an ischaemic stroke caused by cerebral artery occlusion through thrombotic events as occurring in humans. It is more reproducible than clot models, therefore requiring fewer animals per group, while the pathophysiology is highly comparable. In this model, animals will be kept in deep anaesthesia while the respective blood vessel, which supplies blood to a particular brain region (the MCA in our case), will be occluded through the insertion of a (typically) silicon coated filament. The filament can be removed after a pre-defined time period to restore blood flow, or kept in place to mimic the unfortunate

human condition of unsuccessful recanalization. The animals receive pain medication during and/or immediately after the surgery is completed and the surgical wound is treated and before the animals gain consciousness. Refinements to post-surgical behavioural testing include using sensorimotor tests recording spontaneous normal animal behaviour (exploring, locomotion, reaching, rearing, grooming). These tests will be used from early post-stroke phases (from day 1) as they are not too challenging and or associated with stressors.

The water maze introduces a mild motivational stressor (cool water, minimally 19°C), and the need for active swimming for its completion. These stressors are offset by the abbreviated duration of testing (up to 12 days), which includes a number of different phases, some of which could be removed (e.g. reversal learning) to shorten the duration of testing to minimise the impact on the animal. An alternative form of training is the Barnes maze, which, though typically longer (up to 21 days if all phases are included) does not require swimming, only walking over a circular platform to locate an escape hole.

We anticipate using these tests when post-stroke recovery of gross motor function is advanced (typically 7 days) and thus is not expected to interfere with recovery.

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

This study requires an animal model in which the outcome of stroke is comparable to that in man. To evaluate the outcome of stroke it is necessary that tests can be conducted which evaluate the neurological function in a manner that is comparable to methods used in stroke patients. It is therefore necessary to perform the study in rodents such as rats, as there is no species of lower sentience or alternative model that meets the required criteria.

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

The surgery model is the current state-of-the-art procedure to reliably simulate ischaemic stroke as seen in humans. The method is well described and a comprehensive database is available to identify crucial steps that contribute to the minimisation of unnecessary harms caused to the animals. The greatest possible welfare of the animals is fundamental for the conduct of this study not only in the context of best practice in animal studies but also to ensure that the data obtained possess the best possible qualities and that crucial parameters such as genuine motor functions are not compromised by behavioural alterations solely dependent on possible discomfort of the animals. The animals will be extensively monitored by specifically trained scientific staff and an appropriate and well established pain or antibiotic medication will be administered as the need arises. The behavioural tests and when they occur and how many are administered per day are refined to be minimally stressful.

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

We will adopt refinements in preclinical stroke research as described in the IMPROVE (Ischaemia Models: Procedural Refinements Of in Vivo Experiments) guidelines.

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

We will adopt refinements in preclinical stroke research as described in the IMPROVE (Ischaemia Models: Procedural Refinements Of in Vivo Experiments) guidelines, which includes reference to surgical procedures such as anaesthesia, analgesia, and post-surgical considerations of bedding, housing, feeding, nutrition and monitoring. These guidelines are regularly updated by experts to ensure the best possible and reproducible conduct of our experiments; we frequently check regarding latest updates. The principal applicant is embedded in the UK and international stroke community, and indeed contributes to pre-clinical stroke research guidelines. We will thus be kept well informed as to refinements in the relevant areas. We note that such refinements are expected to be implemented by reputable journals, a further driver to adopt and to adhere to best practice.

A retrospective assessment of refinement will be due by 29 April 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. Animal models of autoimmune 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

autoimmunity, therapy, T cells, B cells, inflammation

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 overall purpose of this project is to discover novel therapeutics which will improve quality of life for patients suffering from autoimmune diseases. A particular aim is to move beyond the current situation of achieving simple control of symptoms and develop treatments which allow patients a long-term remission in the absence of continuous medication. In order to achieve this purpose, this project has two interconnected aims 1) to

optimise animal models of autoimmune disease in order to ensure their relevance to human disease and 2) to determine the in vivo efficacy of novel therapeutics.

A retrospective assessment of these aims will be due by 12 April 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 significant unmet clinical need still exists in the treatment of autoimmune diseases, currently available therapeutics do not provide effective symptom control in all patients. Additionally, even in patients where effective symptom control is achieved continued use of the treatment is often limited by the side effect profile. Research into novel disease pathways may result in the discovery of new drug-able targets, leading to more clinically effective treatments with improved safety profiles.

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

Short term outcomes from this project may include an increased understanding of immune response mechanisms in disease models and the identification of novel drug targets.

Where possible novel findings will be published and shared with the wider scientific community

The long-term aim of the project is the discovery of novel therapeutics for the treatment of autoimmune disease.

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

Short term outputs of this project will contribute to a broader scientific understanding of immune pathways involved in autoimmune disease and the mechanisms of action of novel targets and therapeutics.

The ultimate aim of this project is the discovery and development of novel therapeutics however this is a process with a long life cycle. Typically, this requires a time span of approximately 10 years from the initial idea through basic research and clinical development before a therapeutic will be available on the market to benefit patients with autoimmune disease.

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

Where possible key learnings', successful or unsuccessful will be published at conferences or in the scientific literature.

Species and numbers of animals expected to be used

- Mice: 5200
- Rats: 700

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.

Rodents (mice and rats) will be used in this project. The immune system of rodents shares many similarities with the human immune system. Hence rodents can be used to research novel pathways and the mode of action of novel therapies and the data can be translated to human.

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

Animals may undergo procedures involving injections. In most cases animals will be dosed with therapeutic agents via a needle or oral gavage, this is not expected to cause more than a transient discomfort. It is not anticipated that animals will receive more than 40 needle stick injuries during their lifetime. In the case of skin transplantation animals will undergo minor surgery and be bandaged around the abdomen for 5-7 days post-surgery, this may give rise to moderate discomfort. For all procedures' anaesthesia/analgesia will be used where appropriate and animals will be carefully monitored to ensure that discomfort to the animal is limited. Gaseous anaesthesia will be used and typically this will not be more than 3 times in any one day (each separated by at least 1 hour), 7 times in one week or 31 times in a month. The duration of experiments on this project are typically 1 month or longer although animals are not expected to experience adverse events for the entire duration of the protocol; in models of graft versus host disease (GvHD) and systemic lupus erythematosus (SLE) for example these changes occur gradually. All procedures have been ethically reviewed and all animalsundergoing procedures will be looked after by trained staff that work closely with a veterinary surgeon. At the end of each experiment all animals will be humanely killed.

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

Reproducing aspects of autoimmune diseases such as Multiple Sclerosis (MS) and SLE or the pathological mechanisms associated with GvHD and transplant rejection will lead to

animals displaying some of the signs and symptoms of the human disease. These are chronic debilitating conditions and are anticipated to give rise to moderate discomfort in most cases. However, in the case of experimental autoimmune encephalomyelitis (EAE) and GvHD, it is anticipated that this may give rise to severe discomfort. This is required to ensure that the animal models effectively represent specific elements of human disease pathology. Expected symptoms for all diseases would include a gradual decline in bodyweight, changes in appearance and behaviour. In the case of SLE and some GvHD models, protein will be detected in the urine this is a consequence of the kidney damage which occurs in these diseases. In the case of EAE models a gradual paralysis of hind limbs will be seen. The duration of clinical symptoms differs for each model but effects will typically develop gradually over a period of a few 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)?

Based on the numbers presented for use in each protocol and the proportion of each severity within the protocols, across the project it is anticipated approx. 20% of mice will experience the severe category, 55% moderate and 25% the mild category.

For rats the expected proportions are: 50% severe, 25% moderate and 25% mild.

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

- Killed

A retrospective assessment of these predicted harms will be due by 12 April 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?

Our current understanding of autoimmune diseases and transplant rejection shows that these are highly complex processes requiring multiple cell types and mediators that are necessary for a chronic inflammatory response. Consequently, it is difficult to model the disease process in its entirety using in vitro cell assays and/or computer models.

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

Wherever possible, in vitro cell based assays will be used to study functional responses of immune cells in isolation or combination before the use of an in vivo model. An up-to-date knowledge of the literature and latest technology will help replace in vivo experimentation wherever possible.

This includes the following databases for alternative methods:

- The John Hopkins Centre for Alternatives to Animal Testing (http://altweb.jhsph.edu)
- Animal Welfare Information Centre (http://awic.nal.usda.gov/)
- European Centre for the Validation of Alternative Methods (http://ecvam.jrc.it/index.htm)
- Fund for the Replacement of Animals in Medical Experiments, FRAME (http://www.frame.org.uk/)

Why were they not suitable?

At present no in vitro assays or computer models fully replicate the complex interplay of cell - cell interactions and soluble mediators which occurs in the in vivo micro-environment.

A retrospective assessment of replacement will be due by 12 April 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?

These numbers are based upon the typical n numbers required in an individual experiment and the number of experiments likely to be performed over a 5 year period.

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

Optimum group sizes have been established, based on previous experience with these protocols taking into account experimental variability and level of disease incidence to ensure that scientifically meaningful data is generated.

Experimental data will be analysed using appropriate statistical tests. The institution has its own statistics group and statistic courses are run in house specifically for scientists to help with experimental design. With help from our statisticians we are also currently looking into the use of Bayesian analysis where appropriate, this allows the use of historical control data (positive and negative) in order to reduce the number of animals used within an experiment. Where possible the effect of a therapeutic will be determined on multiple readouts from a single experiment, thereby reducing the number of animals used overall. The advances in technologies such as flow cytometry, CYTOF, multiplex cytokine analysis - allows more parameters to be investigated within a sample allowing more information to be obtained from a single animal than was previously possible.

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

A significant proportion of drug discovery is carried out using cells and cell lines (in vitro), with many thousands of potential drugs being screened to identify the most promising compounds/antibodies. However, in order to study complex inflammatory responses, further testing is required in animals in which disease relevant pathways have been induced. To ensure the fewest number of animals are used, only the most effective compounds/antibodies that have been pre-screened for activity in vitro will be examined in animals. Only a limited number of these compounds/antibodies will be evaluated in this project and those molecules will have been previously evaluated in simpler in vivo mechanistic models.

An initial pilot study consisting of a single high dose group plus control may be conducted initially to determine efficacy prior to larger multi dose group studies.

A retrospective assessment of reduction will be due by 12 April 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.

This project includes a number of animal models namely EAE, SLE, GvHD and skin transplantation.

Multiple models of solid organ transplantation are described in the literature. Skin transplantation is proposed to be used in this project as the surgery required for this is minor, compared to that required for models such as heart transplantation, as it does not require the body cavity to be opened up.

Refinements to this model have also been established in house such as changes to the type of bandaging used to minimise discomfort and the use of a heated rack with different temperature zones for recovery post-surgery.

The models of EAE are described in the literature and each reproduces different elements found in the human disease. The acute model (protocol 1) only replicates the initial inflammatory phase of human disease. Where relevant, therapeutics will first be assessed for efficacy in this model before using protocols 2 and 3. Refinements to these protocols have been introduced in house, including adaptations to housing and bedding materials as animals develop disease. Additionally, provision of wet food and drinking water at floor level in the cage.

The development of adverse events typically occurs gradually in models of GvHD and SLE. The humane endpoints used in these protocols will be kept under review.

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

Rodents (rats, mice) are the most appropriate mammalian species on which these chronic diseases can be modelled. These models require the symptoms of disease to develop over an extended period of time hence cannot be carried out under anaesthesia.

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

We aim to limit the animal suffering through use of distress scoring sheets. Humane endpoints are employed to limit suffering and disease burden and are constantly under review.

Throughout the disease phase additional food and water are supplied at various points around the cage floors in the EAE protocols, thus reducing the distance the animals need to travel in order to feed/drink. Changes are also made to the bedding material of these animals in order to avoid products which may become entangled around their limbs. Cages may also be placed on a special rack which has heated and unheated zones.

During the disease phase of EAE animals will typically be monitored twice rather than once daily

A number of refinements to the skin transplantation protocol have been made, specifically reducing the anaesthesia time, reduction of sores due to ‘sharp’ bandaging. Female mice are also used whenever possible. Due to behavioural differences (reduced fighting) with male mice it is easier to house female mice in groups over the relatively long timeframe of these types of experiment. Analgesia regimes will be kept under review and modified according to best practice in consultation with the NVS.

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

Guidance will be taken from organisations such as the NC3Rs and LASA.

LASA have previously published a paper on refinements in EAE models (Wolfensohn et al, 2013; J Pharmacol and Tox Methods, 67 (3)) and if this guidance is further updated their advice will be taken into consideration.

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

The nc3rs.org website is a useful source of information regarding advances in the 3Rs which can be consulted for updated advice. Advise will also be sought from the named veterinary surgeon (NVS) who provides continuous professional development (CPD) seminars on a range of topics. We also have the animal welfare and ethical review board (AWERB) and another inhouse meeting specifically for in vivo scientists which are useful ways of cascading information gained from external congresses or webinars.

A retrospective assessment of refinement will be due by 12 April 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. Platelets in thrombosis, haemostasis and myocardial infarction

Project duration

5 years 0 months

Project purpose

- Basic research

Key words

Platelets, Thrombosis, Heart attack

Animal types	Life stages
Mice	adult, juvenile

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 advance understanding of the mechanisms regulating platelets, a critical ‘gatekeeper’ cell in the bloodstream that serves multiple roles. The objectives of this project address key clinical needs relevant to patients with bleeding disorders or recovering from heart attack.

A retrospective assessment of these aims will be due by 12 April 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?

It is estimated that approximately 150,000 people die annually in the UK as a result of cardiovascular disease. Platelets play a dual role in cardiovascular disease in that they are responsible for triggering the blockage of blood vessels whilst also playing a role in promoting the regeneration of heart muscle following a heart attack. This project aims to advance understanding of the mechanisms regulating platelets and to investigate whether these can be manipulated to further enhance the regeneration of cardiac tissue following a heart attack.

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

It is to be expected that the studies will identify new molecules and pathways involved in the regulation of platelet numbers and function. In so doing the work is expected to contribute to the development of new diagnostic tests and treatments for cardiovascular disease, including following a heart attack or stroke. A key output will be research publications, in standard journals in the field, for dissemination of the findings.

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

In the short term, the beneficiaries of these studies will be researchers working in the field of heart disease and stroke. In the medium term, the findings of the research will benefit biotechnology company's working to develop new and specific diagnostic tests and treatments for cardiovascular disease. In the long term, the work is expected to benefit clinicians treating and patients suffering from cardiovascular disease through the introduction of better diagnostic test and treatment options into the clinical setting.

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

The work will be conducted in collaboration with other groups nationally, including biotechnology companies interested in the development of cell based therapies. These collaborations will facilitate the rapid translation of relevant findings into the clinical setting in the form of improved diagnostic and treatment options for cardiovascular disease. The findings of the study will be published in peer reviewed journals, including online open access platforms such as Biorxiv and dissemination at national and international conferences in the broad field (including the International Society for Thrombosis and Haemostasis and the American Society for Hematology annual meetings), to ensure the rapid dissemination of the new knowledge gained.

Species and numbers of animals expected to be used

- Mice: 9900

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 of choice for the outlined studies because their microcirculatory patterns and platelet-endothelial interactions are well characterised and are similar to humans. Very little work has been done on platelet function in non-mammalian species, and at present such work is limited to fish. Even in fish, the available information is very poor compared to mice. Mice are the only mammalian model to which forward and reverse genetics can be applied to assess gene function, which is essential for determining the molecular mechanisms of platelet function during blood clot formation and the repair of tissues following vascular injury. In addition, the use of genetically altered mice with cell-specific reporters is essential for tracking cell fate and behaviour in the context of platelet production and release. The work will be undertaken in mice from 6 weeks of age, when the majority of growth has occurred in the species and the vessels and organs of the body, including the heart, can be considered of adult size and function. This is important to allow assessment of genes in platelet function in thrombosis and repair in formed organs, without assessment involving any potential developmental role for these genes.

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

The majority of animals used in these studies will be bred with genetic alterations relevant to platelet function however, these alterations are not expected to impact on the animal's wellbeing. Approximately 70% of the animals bred will be killed humanely, without undergoing any further procedures, in order to provide tissues for use in laboratory-based studies. Around 10% will be used in procedures that cause no more than transient pain or suffering, such as blood sampling or being given drugs by injection. The remaining 20% of animals will be used in studies involving a surgical procedure, performed under general anaesthesia, to induce models of either veinous thrombosis (moderate severity) or the induction of an injury that replicated the situation when a blood vessel becomes blocked, as occurs in heart attacks. The myocardial infarction model and limb ischaemia model are of high severity in which mice are allowed to recover from the anaesthetic after surgery, will be given pain relieving drugs and monitored closely post-surgery. In the immediate post-surgical period, animals will be monitored with continuous presence of an observer until recovery from anaesthesia, followed by checking twice in the ensuing 24 hour period, and thereafter once daily until the end of the study or an end point is reached. Recovered mice, which would be approximately 85% after surgery, are expected normally to resume normal behaviour within 24 hours of the procedure and thereafter to continue to thrive until the end of the study, at which point they will be humanely killed. The end point of the study is time-limited for all protocols (normally 48 hours but up to 1 week for venous thrombosis; up to 2 months for MI; normally 2 weeks but up to 1 month for limb ischemia), but a set of suffering end points are also used, beyond which the animals would be killed. Animals would therefore not be in a state that deviates from normal for more than 24-48 hours (see details in Protocols).

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

The vast majority (>90%) of the animals used in these studies are expected to experience no more than mild transient pain or suffering, incurred as a result of tissue sampling, for genotyping, blood sampling or the injection of a drug.

A small proportion (<10%) of animals will undergo a surgical procedure under general anaesthesia. Upon recovery, all animals will be given pain control nevertheless, all animals

are expected to experience some post-operative pain during the first few days following surgery. Animals that have undergone surgery on the hind limb are expected to show reduced limb function and may develop necrosis of the toes but otherwise continue to behave normally. Animals that have undergone cardiac surgery show reduced activity levels for the first day or two post-surgery but resume normal activity levels thereafter. Animals that have undergone these procedures will be closely monitored and promptly killed if suffering exceeds that expected for these 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)?

Mild: about 70% of mice wild be expected at this category

Moderate: 20% of mice are expected within this category

Severe: 10% of mice are expected within this category

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 April 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 outlined studies form part of a multidisciplinary investigation to advance understanding of the events that underlie platelet production and function. Much of this work will be conducted in studies performed using platelets derived from human donors. However, some of the primary functions performed by platelets, such as triggering blood clotting or promoting tissue repair, can only be studies using living animal. In addition, platelets lack a nucleus and therefore can’t be grown or genetically manipulated in culture to study platelet function. Consequently, it is only possible to address the stated objectives by altering the genes of interest in living animals.

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

Laboratory based studies utilising platelets isolated from donated human blood will be used to support the outlined studies. During the course of the study programme, attempts

will be made to develop a non-animal based cell culture system for generating platelets based on stimulating stem cells (human induced pluripotent stem cells) to differentiate into megakaryocytes, the precursor cell responsible for platelet production. When successful, this would
eventually replace much of the work that currently required mice for tissue generation (many of the 5000 mice bred in Protocol 1), leaving the in vivo analyses. In other words we would estimate, eventually, that the work would approximately halve the number of mice required for platelet experiments.

Why were they not suitable?

Laboratory based study on platelet release and blood clot formation are of limited value as they do not accurately reflect these processes as they occur in life. Consequently there is no viable alternative to the use of animals for these studies. Tissue obtained from animal material is essential for many of the laboratory based experiments, in particular the use of heart tissue as there are no mammalian heart preparations available from non-sentient sources and there are no cell lines that reproduce fully differentiated adult cardiomyocytes. This is true not only regarding the expression of ion channel and transporter genes that regulate electrophysiological properties of adult cardiomyocytes, but also of the cell structure and localisation of membrane receptors. Furthermore, while laboratory based studies simulations ischaemia-reperfusion injury will provide useful clues towards regulatory roles for platelet- derived proteins, these findings will always need to be tested in animals models as the injury/adaptation that occurs is also influenced by other circulating cells that contribute to inflammation and therefore cannot be modelled in cell culture.

A retrospective assessment of replacement will be due by 12 April 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 estimated the numbers of animals needed for our research project based on:

Pilot experimental data which give us the information for statistical estimation of minimal number required.

Previous experimental data and information from the published literature.

3) Prior experience running similar projects.

Approximately 70% of the animals in the project will provide the breeding lines and tissue for isolation (principally blood and bone marrow) from genetically altered animals, to allow detailed understanding of the role of platelet genes in their function and generation (Protocols 1-3). The remaining 30% of animals will support Protocols 4-7, where in vivo analysis of complex multicellular and multitissue function and interactions will be analysed, to understand platelet role in thrombosis, haemostasis and repair of tissue from ischemic damage (the myocardium in particular). All this is supported by the current and planned funding (understanding how platelets are made, and development of in vitro platelet generation systems understanding how platelets function in tissue repair processes). The numbers of animals for each protocol have been estimated from an understanding of effect sizes to be measured (from our experience or from the literature), and application of power calculations (where appropriate) to determine optimal group numbers. Examples of power calculations are provided with the protocols.

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

In order to minimise animal usage and maximise the data generated, laboratory-based experiments will be coordinated to enable tissues harvested from a single animal to be used in multiple assays. This will enable multiple data sets to be generated from one animal, such as functional assays on platelet aggregation, secretion, signalling, and calcium responses, all from a single blood sample.

For all animal experiments, we will use the NC3R's Experimental Design Assistant (EDA) tool to help our experimental design and , where appropriate, perform power calculations to determine the smallest group size needed to attain statistical significance. Effect sizes will be estimated from current literature on related subjects, or from past experience, particularly in the field of platelet function where we have over 30 years of experience. Where we have previous data, we will use this with EDA when calculating group size. We also emphasise training and competency and encourage our researchers to update and improve their skills and techniques in animal experiments so as to minimize errors and avoid unnecessary repeats.

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

For novel studies, group size will initially be based on estimates for effect size but will be re-evaluated once pilot data are obtained. A statistical significance of 5% will be used and a power of 80%. Before performing tests of significance, data will be assessed for normality and equal variance to determine whether parametric or non-parametric tests should be employed. Unpaired and paired Students t-test signed rank and Kruskall-Wallis one way ANOVA are examples of tests that will be performed as appropriate. Statistical advice will be sought from our helpdesk and other professional contacts which will allow us to maximise the potential benefit from any data and to minimise animal usage.

In relation to animal breeding, in Protocols 1 & 2, we breed 2 types of animals: (i) ‘standard’ whole animal constitutive gene knockouts and (ii) conditional knockouts where the gene is deleted in specific tissues. Over the past few years we have increasingly moved to the latter approach, because it provides a refined way to address gene function specifically in platelets, which is particularly useful for the analyses in Protocols 4-7 where multiple tissues and organs are involved and measured. The breeding associated with these two approaches is different. For standard knockout generation we have used

breeding of heterozygote pairs to produce littermate-matched offspring, which provide very well- matched controls for the experiments and consequent reduction in numbers of animals required.

However, there is wastage associated with generation of ‘spare’ heterozygote animals. Conditional knockouts generate only homozygote knockouts and controls, with no heterozygote generation, and in this way there is no generation of ‘spare’ mice. This approach is therefore more refined experimentally (because of the tissue-specificity of the approach) and reduces animal usage.

A retrospective assessment of reduction will be due by 12 April 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 the species of choice for the outlined studies because their microcirculatory patterns and platelet-endothelial interactions are well characterised and are similar to humans. Furthermore mice are the only mammalian model to which forward and reverse genetics can be applied to assess gene function, which is essential for determining the molecular mechanisms of platelet function during blood clot formation and the repair of tissues following vascular injury. In addition, the use of genetically altered mice with cell-specific reporters is essential for tracking cell fate and behaviour in the context of platelet production and release. The genetic alterations used in these studies are not expected to have any impact on the wellbeing of the animals. Approximately 70% of the animals bred will be killed humanely by a schedule 1 approach, without undergoing any further procedures, in order to provide tissues for use in laboratory-based studies.

Around 10% will be used in procedures that cause no more than mild transient pain of suffering, such as blood sampling or being given drugs by injection.

The remaining 20% of animals will be used in studies involving a surgical procedure, performed under general anaesthesia, to induce models of either vein thrombosis or coronary heart disease. Mice that are allowed to recover from the anaesthetic will be given pain relieving drugs and monitored closely until fully recovered. Recovered mice are expected to resume normal behaviour within 24 hours of the procedure and thereafter to continue to thrive until the end of the study, at which point they will be humanely killed. In all cases the earliest timepoint consistent with obtaining the required data will be used.

The methods chosen have been selected to generate the greatest amount of data for the fewest animals, whilst also minimising pain, suffering, distress, or lasting harm to the animals. Where possible, we will isolate different tissues from the same animal, thereby derive multiple data sets from one animal, for example conducting multiple platelet functional assays (aggregation, secretion, signalling, and calcium responses) all from a single blood sample to maximise the use of the animal. To this end we will use modern approaches, such as FACS analysis which can be performed using very small sample sizes. Intravital microscopy is also now the gold standard approach to analysis of thrombopoiesis and thrombosis, because it allows multiple measurements of platelet release, function and interaction with blood vessels to be done simultaneously, generating large amounts of data from a single animal. For protocols that involve surgery under general anaesthesia with recovery (Protocols 5- 7), these are the most refined with respect to pain, suffering, distress, or lasting harm to the animals. We have reviewed alternative approaches and have proposed the most refined procedures currently possible, to minimise severity. t will be induced by a surgical procedure performed under general anaesthesia by an experienced surgeon using full aseptic precautions. All animals will be given pain control during the post-operative period (initiated intraoperatively) which will be maintained until animals exhibit no overt signs of suffering. All animals will be monitored for adverse signs and will be killed at the earliest timepoint consistent with obtaining the data needed to meet the objective of the study.

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

It is essential for the study that the species used has microcirculatory patterns and platelet-endothelial interactions that have been characterised and shown to be similar to humans. It is also essential that the species is amenable to genetic alteration. Mice are the least sentient of the species that meet these criteria. The work needs to be undertaken in animals at a fully-formed adult life stage, in order to assess functionality of genes in conditions that best parallel disease situations in humans, where venous and arterial thromboses and myocardial infarction events are principally in adults. Use of adults in the study will thereby also exclude confounding interpretations of genetic function in development, which would otherwise cause problems of interpretation of gene function assignment.

Wherever possible procedures will be conducted under terminal non-recovery anaesthesia however, the prolonged time course of studies investigating the role of platelets in promoting tissue healing and thrombus formation means they cannot be performed under terminal anaesthesia.

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

We actively seek advice from experts in the field and undertake training to improve competency. We also regularly review our procedures to ensure they are the most refined including post-operative care, pain management and the appropriateness of the pain relief agents and methods being used. Model refinement is constantly ongoing, and for example we have recently refined the myocardial infarction model (Protocol 7) to incorporate damage to the vessel using chemical approaches as well as ligation. This allows a more realistic mimicking of arterial damage in conronary artery disease, allow for better modelling and more meaningful datasets. It also allows for a better understanding of the pathology of myocardial infarction and the assessment of appropriate novel therapeutic approaches.

We now incorporate a comprehensive assessment table for humane endpoints, detailed in Protocols 5, 6 & 7. This is very helpful to structure our approach to humane management of the animals under our care, particularly in these protocols post-operatively. This structures our assessment of behavioural and clinical signs of distress, discomfort and pain. We always work on the basis of giving pain control if we suspect the animal is in pain. We therefore give pain control at the end of surgery and reassess the behaviour of the animal for signs of pain at 6 to 8 hours. Any animal showing any signs indicative of pain or deviating from normal behaviour will then be given further pain control and reassessed again at 6 to 8 hours. This process ensures that we minimise post-operative pain and maximise post-operative care and attention. Additionally, surgical procedures, performed under general anaesthesia, are done by an experienced surgeon using full aseptic precautions. All animals will be monitored for adverse signs and will be killed at the earliest timepoint consistent with obtaining the data needed to meet the objective of the study.

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

We routinely consult The National Centre for the Replacement, Refinement & Reduction of Animals in Research (NC3Rs) website (https://nc3rs.org.uk/) for guidance on performing procedures on animals, particularly noting developments in analgesia, monitoring and endpoint setting, for refined assessment of disease progress and recovery from surgical procedures. We have regular weekly seminar meetings within our group to share and disseminate novel ideas and best practice so as to refine and raise the standards of the procedures conducted on animal. All surgical procedures will be performed in compliance with LASAguidelines on aseptic surgery.

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

Members of the research group will attend events and meetings organised by our NC3R's representative to share and disseminate good practice and novel methodologies in addition to participating in national and international meeting relevant to our research field.

A retrospective assessment of refinement will be due by 12 April 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. Rodent toxicity, tumorigenicity and safety studies with medicinal products

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

Toxicity, Regulatory, Safety, Rodent, Tumorigenicity

Animal types	Life stages
Mice	adult, aged
Rats	adult, aged
Hamsters	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?

This licence authorises the conduct of studies in small laboratory animal species (rats, mice and hamsters) with the aim of evaluating the toxicity and tumorigenicity (ability to cause cancer) of medicinal products (human or veterinary/animal health), including

medical devices. This is to aid in the development of new medicines and devices, and to provide mandatory information to regulatory authorities to allow human/veterinary trials or marketing approval.

A retrospective assessment of these aims will be due by 20 April 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?

Governments require (and the public expects) that substances we are exposed to are safe or that their potential hazards are well understood and documented.

The data generated from the studies performed under this project will be used to inform decision- making processes on substances under development and, where appropriate, to satisfy governmental regulatory requirements necessary to gain clinical trial approval, marketing authorisation or product registration.

This safety assessment is of immense importance along with other non-rodent and non-animal studies in demonstrating to governments and the public the safety of these substances.

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

The overall benefit of this project is that it supports the development of safe, new medicinal products to improve the health and quality of life of human and veterinary patients by generating high quality data that is acceptable to regulatory authorities and enables internal decision making within our clients' organisations.

Achievement of the objectives of this licence will enable safe drug development candidates to progress and will also help to remove unsuitable candidates from the development pipeline at an early stage, thus saving animals and resources.

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

Manufacturers of drugs will benefit, as the data generated will allow them to progress their medicinal products under development and, where appropriate, to satisfy governmental regulatory requirements necessary to gain clinical trial approval or marketing authorisation.

Patients and animals will benefit from these studies as this work will contribute to the development of new drugs that help alleviate human conditions. These new drugs may work better in the clinic, relieve or cure diseases and have better side effect profiles. We may, by our work, also contribute to better knowledge and understanding of these types of drugs, and that knowledge may be used to develop further new drugs. Similarly for medical devices, they may be better than existing devices or a new device which can improve patient outcomes (examples of medical devices include artificial hip replacements and dressings for wounds).

The toxicity information obtained is important when planning future trials in humans and animals, to make sure any starting dose in a clinical trial is safe for the participants taking it.

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

The work will be shared with drug manufacturers who will use it to determine their future strategy, or for submission in documents required by regulatory authorities. Whilst we have no direct control over what happens to the data after we have shared it, we trust from information given to us that it is used for regulatory purposes or to support regulatory purposes (e.g. to support drugs/devices progressing to clinical trials). Previously however, we have collaborated with customers and shared data we have produced in the form of scientific publications that are in the public domain.

Species and numbers of animals expected to be used

- Mice: 33400
- Rats: 55000
- Hamsters: 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.

Most of our experiments will be carried out on conventional adult mice and rats as these are the smallest relevant species that we can use that have body systems that are comparable to humans. In some specialist cases we may use the hamster because what we are trying to find out is better performed in that particular species rather than in the rat or mouse.

The only other time we would use a species other than a mouse or rat is to continue work that has been previously done in that species. For instance if previous work, and results gained, had been carried out in a hamster, it would make no scientific sense to start the next stage of a programme of work in a rat or a mouse.

In some protocols we may use genetically altered animals. These animals may be more sensitive to developing tumours (which means fewer animals and a shorter study can be conducted), they may be immunosuppressed so that they do not reject the test substance (for example, biological test substances such as human stem cells) or these alterations mean the animals produce specific medical conditions that we need to assess toxicity against. The use of genetically modified animals is low compared to conventional animals.

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

Typically on this project, animals are dosed over a period of time with test substances, and usually sampled (e.g. blood or urine) before having tissues taken after they have been humanely killed for extensive toxicology analysis. Studies would range from a single dose, to those which last a matter of days (much less than a month) although some can last for 1, 3 or 6 months, and sometimes up to 2 years (to specifically examine whether a test substance can induce cancer). Study durations are dependent on the specific regulatory test being performed. Some animals are left dose free for a few weeks after dosing is complete to see if any effects of the test substances can be reversed.

Dosing of animals is commonly done orally using a flexible tube, or by injection using a syringe and needle, maybe directly into a vein, into a muscle in the leg, or under the skin. Other common routes are used such as inhalation (when animals are dosed in specially designed tubes) or dermal (via the skin).

Blood samples are usually taken from easily accessible veins in the neck or the tail of rats or mice. We are limited to how much blood we can take at once or, cumulatively, over a month. If we need a large blood sample, we would do this when the animal is anaesthetised and we would not let them recover consciousness.

Where possible, we try and take as many of the tissues and samples we need after the animals have been humanely killed after all dosing had been completed.

Some animals we use are genetically altered, so they better represent disease more applicable in humans, and make toxicity testing more relevant (and often shorter).

In some protocols we also have to surgically prepare animals for testing, when a normal animal would not be suitable. This maybe, for example, to implant a cannula into a vein for prolonged intravenous dosing, or for intravenous dosing over a period of hours. Surgery may also be required as part of the safety evaluation of a medical device that will be surgically implanted into a body cavity, or other therapeutic agent that requires injection into an internal tissue. Surgery is only performed when there is no other way forward.

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

When dosing an animal by injection or taking blood, the amount of pain an animal feels is similar to what a patient would feel having an injection done by a doctor. If we have to

repeatedly inject animals using a needle and syringe, we would choose different sites to do this where possible. If we can take blood samples when an animal is deeply unconscious then we do. If we need to take repeated blood samples or need to dose repeatedly then we try and use different sites. Of course everyone who performs these procedures are trained to a high standard.

Animals undergoing surgery receive the same sort of care as a patient would in hospital. We discuss their pain relief and use of antibiotics with a vet before we start and administer drugs as necessary..

The genetically modified animals we use are usually immunocompromised or modelling human disease.

Routinely we need to take a urine sample for analysis, so we would then put an animal into a special cage which is smaller than their normal cage. The animal can still move around. Virtually every animal will get used to their new cage within about 15 minutes and are fine.

Dosing with drugs and chemicals may cause adverse effects in some studies. Experience shows that the majority (~65%) of animals are not expected to show any clinical signs of suffering (either no clinical signs or normal background signs expected of the rodent strain). A small percentage (~15%) may show transient subtle to mild clinical signs. Moderate signs of adverse effects may be seen in some animals (~20%), usually in the higher dose groups. Lethality and/or severe effects are not study objectives in any of the protocols within this licence, but for preliminary studies that may be the first animal studies with limited data available, a very small percentage of animals may inadvertently show severe findings before they are immediately and humanely killed.

We do observe our animals at least twice a day, and the people who do this know the signs when an animal is ill. If an animal is ill, we would check it more frequently, and get more senior staff involved in its care for advice, including vets.

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)?

On the last project, about 85% of animals displayed mild severity, and around 10% of animals were classified as having displayed moderate severity. This is because these studies can last between a few days and weeks to up to a year, and although the individual procedures are usually mild in nature on their own, the cumulative effects make them moderate overall.

It's impossible to predict the proportion of severities expected on a service licence like this, as this will be dependent on what study types we are asked to perform.

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 20 April 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?

There is currently no regulatory and scientifically acceptable alternative to the use of rodents in these studies. These studies are run to satisfy the regulatory requirements of governments around the world to ensure pharmaceuticals and medical devices are safe for humans and animals. These tests are very specific as to what they require in terms of testing in animals to ensure this.

We maintain a constant awareness of regulatory guidance and ensure that where non-invasive methods exist which fulfil the regulatory requirement, they are used in preference to animal studies.

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

There are no other non-animal alternatives for the work being undertaken on this project. The regulations we are following will not allow safety decisions to be made on non-animal systems alone.

In vitro and in silico methods (test tube or computer work not using animals) are used in combination with animal studies to inform study designs and assist in understanding of potential toxicity but cannot yet replace in vivo (animal) studies.

Why were they not suitable?

Although there are test tube tests that can model some parts of how drugs and chemicals get into our bodies, and how our body deals with them, and can identify undesirable effects, for example, there is no series of test tube tests that brings all these complex happenings together, like we see in animals and humans.

That is why we need to test new drugs and medical devices in animals, as they have similar physiology and processes as humans, and that testing gives us a good idea what may happen if they were ever tested in, or exposed to humans.

A retrospective assessment of replacement will be due by 20 April 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 we have used are based on figures of previous usage from previous projects, or a projection thereof (based on estimated incidence) based on requests received from drug manufacturers in the past. It is, however, impossible to accurately predict the number of studies that may be performed, in the circumstances.

The regulatory guidelines we follow for each study usually indicate the number of animals in a study; otherwise, the number used is the minimum to achieve the aims of the study.

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

Studies are designed to provide maximal data and statistical power (where appropriate) from the minimum number of animals considering that it is better to increase the number of animals used to achieve the objective than to use too few animals and risk having to repeat the study. For regulatory studies, guidelines require the number of groups and animals per group to be adequate to clearly demonstrate the presence or absence of an effect of the test substance; core study designs are based on international guidelines where these exist. Otherwise reference is made to standard study designs with input from the Department of Statistics, where appropriate, to identify the optimum number balancing the need to achieve study objectives while avoiding excessive animal use. These internal designs are reviewed and updated in line with changing external guidelines and internal refinements that either minimise numbers or reduce severity.

Whenever possible, common species of animals are used such that a large amount of control background data is available. This reduces the need for large control groups.

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 try and get as many outputs as we can from a single animal where possible, without adversely affecting its welfare. So if we need to get several different samples, for example, we will often do that in the same animal, rather than use separate ones, when possible.

Before our main studies, we use smaller groups of animals to get an idea of the doses we need to use for the main studies. These studies are important as it gives us confidence that the doses we are using are correct prior to testing them in bigger groups of animals required by global regulators.

A retrospective assessment of reduction will be due by 20 April 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.

Most of our models involve dosing animals with test substances or devices, and sampling them, with many outputs taken after the animals have been humanely killed. This is generally the least invasive set of procedures that can be done to give meaningful outputs to make scientific decisions about further tests, or to determine the safety of a test substance/device.

Throughout our studies, our animals are checked at least twice a day. This allows us to see over a period of time, whether dosing each individual animal is causing any adverse clinical signs. if this is the case, we can take action: get veterinary advice, add food supplements and extra bedding if needing, and even reduce dose levels or stop dosing completely.

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

Rodents (rats, mice and hamster) will be used in all of the studies conducted under this licence. Rodents are considered to be of the lowest neurophysiological sensitivity (their

brain function and physiology) that will allow us to achieve the study aims and are considered suitable for predicting what's likely to happen in humans (or animals).

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

Many of the procedures performed on our rodents like blood and urine sampling, cause only transient distress to the animals. Blood sampling procedures are similar to and about as painful as having a blood sample taken by a doctor or a nurse. Blood volumes are kept to a minimum within rigid volume guidelines. Confining animals in special cages to allow us to take urine samples is similarly of little distress to the animals.

For inhalation dosing, where animals are restrained in tubes, training of the animals occurs for increasing periods prior to treatment commencing to accustom the animals. Dosing and sampling procedures 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 and will be the minimum consistent with the scientific objectives of our studies. In addition, suffering will be further minimised by implementing clearly defined humane endpoints.

Animal welfare is of utmost importance and Good Surgical Practice will be observed for any animal undergoing surgical procedures. Surgery will be conducted using aseptic techniques (to prevent infection) which meet at least the standards set out in the Home Office Minimum Standards for Aseptic Surgery. Before we start surgery, we agree with a vet what pain killers or antibiotics the animals need both before and after the surgery. When recovering from surgery, we give the animals extra heat and monitor them closely until they start behaving normally again. We then check them at least twice daily before they go on study.

In addition, care is taken to provide as much environmental enrichment as possible. This includes plastic shelters in their cages, wood blocks and balls to gnaw on and push around; mice are given swings, mice and hamsters are given extra bedding for warmth and food supplements are given as appropriate.

In some tests we use animals that are genetically altered, to mimic conditions seen in humans or more commonly, transgenic mice because of their susceptibility to tumours. These animals are specially bred and don't display any harmful clinical signs due to their conditions.

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

For blood sampling and dosing then the following guidelines/literature will be followed:

Diehl et al. A Good Practice Guide to the Administration of Substances and Removal of Blood, Including Routes and Volumes, Journal of Applied Toxicology: 21, 15-23 (2001).

Gad et al. Tolerable levels of nonclinical vehicles and formulations used in studies by multiple routes in multiple species with notes on methods to improve utility. International Journal of Toxicology: 1-84 (2016).

LASA/NC3Rs: Guidance on dose selection for regulatory general toxicology studies for pharmaceuticals.

LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery

Guidance on the Operation of the Animals (Scientific Procedures) Act 1986. UK Home Office 2014

Regulatory guidelines.

This is not an exhaustive list and principally focuses on UK and EU documents:

European Parliament and Council Directive 2001/83/EC of 6 November 2001 of the Community Code Relating to Medicinal Products for Human Use, OJ L311/67-128, 28 November 2001 as amended Commission Directive 2003/63/EC, OJ L159, 27 June 2003.

Guidance on Repeated Dose Toxicity. Committee for Human Medicinal Products (CHMP), European Medicines Agency (EMA). 2010. CPMP/SWP/1042/99 Rev 1

Note for Note for Guidance on carcinogenic potential (CPMP/SWP/2877/00) of the European Agency For the Evaluation of Medicinal Products.

Guideline on the evaluation of control samples in non-clinical safety studies: checking for contamination with a test substance. Committee for Medicinal Products for Human Use (CHMP), 2005. CHMP/SWP/1094/04

CHMP SWP conclusions and recommendations on the use of genetically modified animal models for carcinogenicity assessment. CPCHMP SWP conclusions and recommendations on the use of genetically modified animal models for carcinogenicity assessment. CPMP, 204. CP4. CPP/2592/ev 1

ICH Safety Guidelines:

- Guideline on the need for carcinogenicity studies of pharmaceuticals S1A
- Testing for Carcinogenicity of Pharmaceuticals S1B
- Dose Selection for Carcinogenicity Studies of Pharmaceuticals S1C(R2)
- Note for Guidance on Toxicokinetics: The Assessment of Systemic Exposure in Toxicity Studies S3A
- Pharmacokinetics: Guidance for Repeated Dose Tissue Distribution Studies ((S3BB)
- Duration of Chronic Toxicity Testing in Animals (Rodent and Non Rodent Toxicity Testing) S4
- Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals S6(R1)
- Immunotoxicity Studies for Human Pharmaceuticals S8

- Nonclinical Evaluation for Anticancer Pharmaceuticals S9
- Guidance on Non-clinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorisation for Pharmaceuticals M3(R2)

ISO 10993 Biological evaluation of medical devices – Tests for genotoxicity, carcinogenicity and reproductive toxicity (Part 3); Tests for local effects after implantation (Part 6); Tests for irritation and delayed-type hypersensitivity (Part 10) and Tests for systemic toxicity (Part 11).

Notification Number 24 of the Pharmaceutical Affairs Bureau, Japanese Ministry of Health and Welfare, dated 11 September 1989, as amended notification number 88 dated 10 August 1993, notification

number 655 dated 5 April 1999.

Notification Number 1607 of the Pharmaceutical Affairs Bureau, Japanese Ministry of Health and Welfare, dated 1 November November 199

EMEA Committee for Medicinal Product for Human use (CHMP) EMEA/CHMP/410869/2006: Guideline on human cell-based medicinal products.

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

This will be achieved by regular discussions with our Named Information Officer, colleagues in Animals Technology, and by attending appropriate training courses and conferences, or getting feedback from such events.

A retrospective assessment of refinement will be due by 20 April 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. Imaging studies of cancer

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

cancer, imaging, therapy

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?

The aim of this project is to utilise cancers grown in mice to develop and apply imaging methods for visualising and quantifying tumour features in situ, and establish their use for the assessment of cancer therapies.

A retrospective assessment of these aims will be due by 21 April 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?

There is a continuing need to develop new technologies for faster and more accurate cancer detection, diagnosis and monitoring, thereby enabling personalised adaptation of treatment for improved patient outcomes. Advances in non-invasive imaging techniques provide a means of defining quantitative metrics to map and monitor tissue structure and function in vivo, which can enable accurate tumour detection, an understanding of the local anatomical environment in which the tumour is growing, and inform on tumour drug delivery and treatment response.

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

As results emerge, they will be presented at appropriate national and international conferences such as the National Cancer Research Institute (NCRI), American Association for Cancer Research (AACR), World Molecular Imaging Congress (WMIC) and International Society for Magnetic Resonance in Medicine (ISMRM). Ultimately, manuscripts will be drafted and submitted to high quality, high impact, openly-accessible journals with an appropriate target audience (e.g. Cancer Research, JNCI, Radiology).

Ultimately the research will facilitate the adoption of imaging methodologies into aligned clinical imaging-embedded investigations/trials which, working with our radiology and oncology colleagues and the wider UK cancer imaging network, we are strongly positioned to deliver.

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

Short-term impacts:

The work will be highly relevant to both pre-clinical and clinical imaging scientists interested in the development and application of new/innovative methods for investigating tumour pathophysiology and treatment response in vivo.

This project will establish best practice for the acquisition of multiple types of imaging data obtained in a single scanning session. This will enable image co-registration and data fusion, a better understanding of the spatial relationships of the quantitative features afforded by each imaging technique, and potentially provide additional mechanistic information.

This research will impact on pre-clinical drug developers. The use of rodent tumour models coupled with non-invasive imaging represents a powerful research strategy with which to more accurately evaluate new therapeutics, either accelerating the clinical development of an effective agent, or forcing early closure of a project unlikely to produce a useful drug, saving both considerable time and money. Imaging investigations can also reveal any treatment-induced effects on normal tissues, informing on drug safety and toxicity.

These studies will also highlight improved pre-clinical imaging-embedded trial designs which enable the evaluation of any synergistic or additive effects of combination therapy, and better guide any subsequent clinical trial.

These studies will also impact on the refinement and reduction of animals in biomedical research, specifically the use of refined animal models of cancer, coupled with the use of non-invasive imaging. In this way, each animal acts as its own control, enabling the use of paired statistical tests and hence reducing the size of animal groups required to still generate meaningful data.

Longer-term outputs

The research outputs will impact on patients suffering with cancer, and their carers, whose disease management will be improved through the clinical adoption of specific imaging methodologies identified in these studies, and be treated with new smarter, kinder treatments in clinical development.

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

Our institute is committed to the widest dissemination of its research results, both in the scientific literature and to the general public, and is a signatory on the Concordat on Openness on Animal Research in the UK.

Research presentations have been previously made at meetings organised by NC3R's, LASA and the IAT. Outreach is supported by researchers who act as ambassadors for "Understanding Animal Research" (UAR).

The UK cancer research community is the only collective to monitor and generate guidelines on best practice for the welfare and use of animals in cancer research, and which includes pre-clinical imaging strategies. Refinements identified through this project will be incorporated and disseminated through the latest revision of these guidelines that is currently being collated.

The pre-clinical and clinical imaging teams meet frequently to discuss ongoing and planned pre-clinical and clinical imaging investigations, playing a major role in many national and international cancer imaging collaborations, underpinned by integrated research activities with leading non-imaging cancer research programmes within our institution and their networks.

Species and numbers of animals expected to be used

- Mice: 4100

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 cancer research, the majority of animal tumour models use adult mice. They are the simplest species in which tumours are relatively straightforward to grow. The use of mice can produce reproducible tumours in practical experimental group sizes, and whose welfare is easy to monitor, manage and care for.

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

Cancers will first be established in mice typically using an injection of tumour cells or tissue either under the skin, or surgically into the organ from which they were originally derived. Analgesia will be provided prior to and following a surgical procedure. Successful tumour engraftment and growth will then be carefully monitored. Mice bearing established tumours will subsequently be anaesthetised and undergo a non-invasive imaging investigation, taking around 1-1.5hrs. Once fully recovered and eating/drinking normally, awake mice may then be administered with drugs designed to treat the tumour, or be anaesthetised and have their tumours irradiated with X-rays, taking ~30 minutes. Having fully recovered, mice may undergo additional non-invasive imaging investigations to assess tumour treatment response, performed under anaesthesia. At study end, mice will be euthanised and tumours excised for ex vivo analysis.

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

As tumours grow, mice may appear distressed (hunched posture, anorexia, dehydration, reluctance to move), show muscle wasting or a loss in body weight. Such adverse effects may develop over weeks and months.

Following treatment, mice may develop reddening of the eyes, a staring hair coat, hunched posture or loss of body weight. Such adverse effects may develop over days or 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)?

The severity limit for the majority (~90%) of the mice used in this project is moderate. The project includes surgical procedures to initiate tumour growth that will result in mice likely

to experience short- term moderate pain or distress (~90%), or long-lasting mild pain or distress with therapy causing moderate impairment of their well-being or general condition (~70%). Any suffering will be controlled and monitored by effective peri-operative analgesia and care.

A severe limit will apply to non-tumour bearing mice (<5%) used to establish well-tolerated dose regimes for drugs being developed for the treatment of cancer, particularly for agents that have not been previously investigated in vivo and whose toxic effects will be unpredictable. Any distress or suffering will be minimised by close monitoring after drug administration.

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

- Killed

A retrospective assessment of these predicted harms will be due by 21 April 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?

Cancer is a disease of unmet clinical need, with 166,000 associated deaths annually in the UK, so the benefits of cancer research are very clear. Human cancers develop in three-dimensional space within specific tissues in the body, each providing a unique environment for a tumour to grow. Imaging investigations of tumour progression, drug delivery and therapeutic response can only be assessed using animal tumour models in situ, which take into account the tumour-host interaction, the complexity of the environment in which the tumour develops and grows, the role of accessory cell types that are also present within a tumour, and its three-dimensional architecture and function.

It is not ethical and often not possible to test new treatments on patients without first establishing that they are both effective and safe in animals.

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

Whilst alternative methods of performing cancer research are available, mainly through the use of cultured tumour cells and tumour spheroids grown on plastic dishes in the laboratory, there are a number of questions in cancer research that can only be addressed using solid tumours in animals.

Why were they not suitable?

Cultured cancer cells and spheroids are provided with constant levels of oxygen and nutrients, and are all growing at the same rate. This is rarely the case for cancers in the body, and variations in nutritive blood supply can significantly influence tumour growth and treatment response. The process of metastasis, by which tumour cells from a primary cancer must access the blood circulation, spread around the body and colonise new anatomical sites, is exclusively an in vivo phenomenon. Similarly, drug efficacy must be tested in vivo to determine that adequate levels are delivered to a tumour and that any adverse effects on normal tissues can be assessed and minimised.

A retrospective assessment of replacement will be due by 21 April 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?

Estimates of the numbers of animals to be used has been guided by review of i) the annual record of animal numbers submitted to the Home Office over the lifetime of the current project licence, ii) research project planning for which funding has either been secured or being applied for, and iii) project licence holder experience.

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

The use of non-invasive imaging means that each animal / tumour acts as its own control, allowing the use of paired statistical tests, harnessing statistical power from smaller groups of animals without compromising the scientific objectives of the study. Imaging studies are inherently sequential, so they lend themselves to sequential experimental designs, which utilise fewer animals / tumours to achieve the same statistical power as conventional designs.

Experience has also shown that involvement of a statistician at the outset of study design can significantly assist the reduction of animal procedures. All experiments are analysed using appropriate statistical tests to provide meaningful assessment of the imaging data, and statistical advice sought as necessary.

Where possible, studies will be designed to achieve several objectives using the minimum number 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?

Where a new tumour model is being utilised, a pilot study will be performed in a small cohort of mice to establish successful tumour engraftment and take rate, and gain familiarity with the subsequent tumour growth rate.

A retrospective assessment of reduction will be due by 21 April 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 the lowest species that are appropriate for in vivo imaging and cancer research studies, and are widely used for this purpose. This project will use inbred mice, and mice without a fully-functioning immune system in order to allow the growth of cancers derived from human tumour cells i.e. to prevent rejection. Mice will be housed in ventilated cages with sterile bedding, food and water, and also with cage toys and/or nesting materials to provide environmental enrichment. All procedures are performed in special cabinets using strict aseptic techniques to avoid infections. Animals will be inspected daily and, if necessary, advice obtained from a veterinary surgeon who is on call at all times. Suffering will be minimised by keeping tumour burdens within tolerable and acceptable limits, and according to national guidelines.

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

Less sentient animals are unable to support appropriate tumour models with which to accurately monitor, image and inform on the effects of cancer progression and therapy on the host.

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

Procedures will be performed by trained and competent personnel that have experience working with animal tumour models and are familiar with the effects of treatments.

Anaesthesia and analgesia will be used to minimise stress and suffering prior to, during and after procedures.

Additional welfare monitoring will be provided through the use of non-invasive imaging, particularly useful for mice bearing tumours in deep-seated anatomical locations.

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

Best practice guidance will be followed in accordance with the United Kingdom Coordinating Committee on Cancer Research (UKCCCR) guidelines for the welfare of animals in experimental neoplasia (doi:10.1038/sj.bjc.6605642), and the recently updated Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines 2.0 (https://doi.org/10.1371/journal.pbio.3000410).

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

Advances in the use of the 3Rs will be provided via regular communications received from the National Centre for the Replacement Refinement and Reduction of Animals in Research (NC3Rs) (https://www.nc3rs.org.uk/), and also through attendance at relevant meetings organised by the NC3Rs, the Laboratory Animal Science Association (LASA) and the Institute of Animal Technicians (IAT).

Any relevant advances that emerge will be effectively implemented working in conjunction with experienced technical staff within our institute.

A retrospective assessment of refinement will be due by 21 April 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. Applying regenerative neuroimmunology to chronic spinal cord injury research

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

Neuroimmunology, Chronic spinal cord injury, Spinal Cord-Immune Interactions, Regenerative Medicine, Therapy

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?

The aim of this project is to identify and manipulate key mechanisms governing the interactions between the immune system and the spinal cord in the context of chronic spinal cord injury. The ultimate goal being to foster the intrinsic repair capabilities of the spinal cord in a way that halts the secondary damage associated with injury and stimulates mechanisms of neuroplasticity through the regeneration of the injured cord.

A retrospective assessment of these aims will be due by 20 April 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 United Kingdom experiences more than1200 new patients with spinal cord injury (SCI) every year, the majority due to traumatic events with the remainder resulting from disease, such as the formation of non-cancerous tumours. Following SCI, the immune system is activated and results in the infiltration of immune cells into the damaged spinal cord tissue. Initially, the inflammatory immune cells are beneficial in the clean-up of cellular debris and damage, as well as forming a scar around the wound site to prevent the spread of inflammatory immune cells into the surrounding healthy tissue. However, the continuous presence of inflammatory immune cells damages the underlying nerves, meaning that messages travelling along the nerves become slowed, disrupted, or permanently halted. This causes a wide range of potential symptoms, including problems with bladder control, sensation, and partial or complete paralysis of arm and/or leg function that depends on the location in the spinal cord where the injury occurred (i.e., injuries to the spinal cord near the head effect the arms and legs whereas injuries near the waist only effect the legs).

The main function of the immune system is to protect its host against a wide range of pathogens, including bacteria, viruses, and parasites. However, there is an increased recognition that the immune system also plays a main role during brain and spinal cord development by controlling the generation of new nerve cells and that of the cells making the insulation of nerves (myelin), as well as that of new connections between nerve cells. Ultimately, cells and molecules of the immune system have a main function in prolonging inflammation in the spinal cord that contributes to the extensive tissue damage observed in SCI.

Neuroplasticity is the ability of networks of neural cells to adapt and change through (re)growth and reorganization, and it also implies protection from cell death, generation of new nerve cells and support cells, and changes in the amount of scar tissue.
Recent evidence has now shown that immune cells can be redirected towards a beneficial function, as seen in brain development, that could be harnessed to stimulate mechanisms of tissue plasticity and improve the clinical outcomes of patients with chronic SCI.

Previous work in the lab has in fact identified new ways to turn bad immune cells into good and promote the healing of the injured spinal cord in laboratory animals.

We have shown that the transplantation of a type of stem cells that is obtained from brain tissue into mice is able to interfere with the actions of the immune system in a way that lessen the damage in the persistently inflamed spinal cord. In addition, when brain-derived stem cells were transplanted into mice with SCI, these brain-derived cells could turn bad inflammation into good and promote the re-growth of damaged nerve fibres and reduce the amount of scar tissue. These experiments have clearly shown that modifying the interaction between the immune system and the spinal cord could be key in inducing neuroplasticity in chronic SCI.

With this license, we wish to further develop and explore novel ways to manipulate the interactions between the spinal cord and the immune system, to therapeutically manipulate inflammation and promote the regeneration of the damaged spinal cord. Amongst a number of potential mechanisms, we will focus on disentangling the way immune cells, which normally respond to bacteria and viruses, impact the spinal cord tissue after SCI. In fact, preliminary evidence suggests that altering immune cell activity can shift the cells and molecules found in the SCI from a toxic, cell death-promoting environment, to an environment that encourages regeneration. As such, this is a promising new approach to treat chronic SCIs. With this license, we will further investigate hypotheses such as these. Here, our ultimate goal will be to identify new and key molecular and cellular mechanisms of spinal cord-immune interactions that can be targeted with novel experimental molecular therapies. We envision these therapies will, ultimately, promote neuroplasticity in chronic SCIs through tissue regeneration in follow-up clinical trials.

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

The main output of this project will be new biological information on the function and therapeutic potential of mechanisms controlling persistent spinal cord inflammation. Additional outputs will include the publication of experimental data in scientific journals, and generation of accessible datasets.

Further products of this project will be patents protecting the main discoveries, tools to address mechanisms or deliver therapeutic agents, and technologies to identify effects.

Ultimately, this project will translate into new therapeutic approaches capable of modifying inflammation in chronic SCI and promote neuroplasticity via modulation of spinal cord-immune interactions.

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

In the short term, the main beneficiaries of this project’s outputs will be researchers from academic institutions and from industry. In the medium and long term, the outputs generated by this project will help the NHS and the patient community in the advancement of treatments for chronic SCI. These treatments will be aimed at preventing persistent inflammation of the spinal cord and promote neuroplasticity through the regeneration of the injured cord.

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

The outputs of this work will be disseminated to academic scientists throughout the duration of the license. We will communicate regularly with organized research networks and laboratory groups in the field of spinal cord injury research. These networks will allow us to share workloads and ideas, which depend on the expertise of each group, avoiding duplication of the experiments and accelerating the progress in these fields.

Preliminary data will be shared at national and international conferences and/or workshops in order to gain valuable feedback of the work from peers. This will also provide us the opportunity to build new collaborations locally and internationally to improve the quality and rigor of our research for the duration of the licence. We will also present our findings ahead of publication on preprint servers (such as arXiv, bioRxiv, or PeerJ).

When finalized, all our data will be published in peer-reviewed scientific journals. These data will include gene, protein, and metabolic throughput screen datasets, which we will also make available on appropriate databases for other research groups to access freely.

We are committed to regularly publishing both positive and negative results to increase awareness and inform the community of how our findings fit into the wider field and suggest which experimental outputs are worth progressing and which we feel would not be worth further exploration.

Finally, we will ensure that the published results are open access to maximise their impact and increase global awareness to both the public and fellow scientists.

Species and numbers of animals expected to be used

Mice: 1000

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.

Up to five days old newborn mice that have been produced on a different licence will provide us with cells for use in modelling certain aspects of their responses and function in a lab dish.

Unfortunately, it is extremely difficult to accurately and fully model the complexity of SCI using cells in a dish. Therefore, we are using mice as they are widely used in pre-clinical research because their genetic, biological, and behavioural characteristics closely resemble those of humans, thus allowing the study of many of the pathologies (or part of these) seen in patients, including formation of scar tissue, presence of inflammatory immune cells, and damaged of nerve fibres. In particular, the possibility to use genetically

modified mice in which the expression of specific genes can be monitored and/or induced/reduced allows the study of target proteins and pathways involved in brain and spinal cord injuries and repair.

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

Mice will be fully anaesthetised and fixed in a device to maintain a stable body position. Then, a laminectomy will be performed, which is a surgical procedure where a small portion of the thin membrane covering the spinal cord is exposed to reveal the underlying thoracic tissue (i.e., the middle portion) of the spinal cord. From here, a computer-assisted impactor, which allows fine control of tissue damage and measurement of the actual impact force, will be placed over the exposed spinal cord tissue. The impactor then launches a weighted object that hits the exposed spinal cord tissue resulting in a contusion (i.e., bruising) SCI. Alternatively, some mice will receive a sham surgery (i.e., laminectomy only) but with no injury to the spinal cord tissue. This surgery is required to generate non-injured controls to allow for the comparison of cellular and tissue responses in the injured versus non- injured spinal cord. Following contusion SCI, mice will be left to recover until fully awake and responsive. The weight and overall body condition of the injured mice will then be monitored daily throughout the length of the study.

Contusion SCI mice may receive additional injections. These injections can be intravenous (e.g., into a vein), intraperitoneal (e.g., into the abdomen), subcutaneous (e.g., under the skin), intrathecal (e.g., into the fluid filled spaces of the spinal cord), intracerebroventricular (e.g., into the fluid filled spaces of the brain), intraparenchymal (e.g., into tissue), and local into the spinal cord. Injections will include either substances (such as drugs, beneficial small molecules, and agents to induce gene modification), viruses as a vehicle to artificially carry foreign genetic material into cells of the mice, and cells, which are all are meant to foster the interactions between the immune system and the spinal cord in a way that results in a more robust regeneration, or less degeneration of the spinal cord.

Finally, to validate and identify biomarkers of SCI damage and their prospective value in the context of the regenerative process, we may also collect bodily fluids from live mice. These include blood and cerebrospinal fluid, which is a clear fluid that surrounds and cushions the brain and spinal cord from injury, for verification or discovery research using high-throughput screening technologies. Blood is obtained from a surface vein observable to the naked eye through a small prick using a fine needle, while cerebrospinal fluid is obtained via a quick, minimally invasive surgical procedure.

Experiments using contusion spinal cord injury may be as short as 1 day and/or may last up to 60 days (8 weeks or 2 months) post injury to study both immediate and delayed mechanisms of neuroplasticity.

The duration of each experiment is determined prior to the use of any mouse study and is variable in length. At the end of the experiment all mice will either be humanely killed, or tissues and organs 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.

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

Within the contusion spinal cord injury protocol, mice display complete paralysis of both hind limbs initially but spontaneously recover within the first 2-3 weeks post-surgery and regain some degree of ankle movement. Mice are also expected to experience transient early pain for 24 hours after surgery along with some weight loss for one week. Since SCI affects the voluntary control of the bladder, mice are not able to express the bladder in full during the first days after surgery but will recover within one week. During this time, we will make sure the bladders are manually expressed by applying appropriate gentle abdominal pressure at least twice a day starting on the day after surgery, until mice recover the reflex (approximately 1 week).

In mice receiving additional injections, there could be transient pain and discomfort for a maximum of 48 hours (depending on the route of injection). Sampling of blood through the superficial tail vein will not lead to major adverse effects except transient discomfort for the animal. Sampling of cerebrospinal fluid may result in changes in food/liquid intake or in normal weight gain and/or transient (max 48 hours) local pain.

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: Moderate 10%
- Mouse: Severe 90%

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

Killed

A retrospective assessment of these predicted harms will be due by 20 April 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?

Spinal cord injury in humans leads to permanent and irreversible damage to the spinal cord resulting in lifelong clinical dysfunction, affecting the quality of life of patients and their carers. The development of therapies able to reduce the impact of persistent inflammation on the human spinal cord, while promoting its regeneration, is a main unmet clinical need for chronic SCIs. Since no experimental molecular therapy can be tested in humans without appropriate validation in relevant animal disease models, concepts developed using cells grown in a lab dish (i.e., cell culture) have to be tested and refined in animals where the complex environment of the adult nervous system is present and where functional recovery can be measured.

Over the course of the last two project licenses, our group has established a publication record and a pipeline of druggable pathways and molecules. We have also developed a strong understanding of models of inflammation and regeneration using immune and stem cells extracted from mice and stem cells generated directly from skin cells of humans using refined cell culture systems. Our functional and screening assays are based on the use of immune and brain stem cells, due to their role in controlling both the damaging as well as the regenerative responses in the spinal cord in the context of chronic SCIs. Indeed, under non-injured and non-diseased conditions in mice and humans, these cells help to maintain the optimal functioning of the brain and spinal cord. Hence, when the spinal cord is damaged, the response of these cells is important for re-establishing a normal function of the spinal cord.

To validate our cellular findings and new therapeutic treatments, we need to use rigorously validated and widely accepted pre-clinical mouse models of SCI. This allows us to test whether the immune responses we observe in cell culture and the effect of treatments are conserved effects within the very complex injury context. The immune response in injury is indeed very different in a mouse compared to a plastic dish. In a mouse, there are many physiological responses (e.g., inflammation, formation of scars, etc.) which directly impact the ability of the spinal cord to recover, as well as the efficiency of the treatments. Further, SCI also affects many other cell types present, such as nerve cells, which are challenging to study altogether in a dish. For these reasons, mice are widely used in pre-clinical research because their genetic, biological, and behavioural characteristics closely resemble those of humans, thus allowing the study of many of the changes seen in patients, including tissue scar formation, presence of inflammatory immune cells, and damaged nerve fibres. In addition, the possibility to use genetically modified mice in which the expression of specific genes can be monitored and/or altered allows the study of target proteins and pathways involved in central nervous system (CNS) injuries and repair.

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

Over the years our team has refined and improved upon our models of cells grown in lab culture dishes that allow us to test the tolerance and efficiency of our treatments before testing them in a mouse.

Additionally, we have developed a new model of maintaining and expanding human immune and human stem cells in plastic dishes that does not involve the use of mice. This new system allows us to

(i) capture the response of human cells in a dish and (ii) design/perform experiments to test ideas about

the response of cells without having to extract these cells from mice beforehand. After the identification of targets, we will then proceed to test our results in our mouse models of disease or lesion.

As an additional replacement strategy, we will be applying data mining. This is the process of uncovering patterns and other valuable information from large data sets. The creation of next generation technologies allows us to analyse the responses and behaviours of individual cells at an extremely detailed level. These analyses are being carried out by several groups across the world, including my own team. In fact, large datasets are made accessible to the public even before publication. These public datasets are stored on dedicated websites (i.e., biorepositories) or on websites where the manuscript is stored prior to review by other scientists (i.e., pre-preprint servers). Everyone can then access these public data and perform their own analysis for targets that are important for experimental studies. Data mining has improved upon organisational decision-making through analysis of these freely available data sets. The data mining techniques that will be used in these analyses can be divided into two main purposes. One, they can either describe the target dataset. Two, they can predict outcomes through the use of machine learning algorithms. Here, a small list of targets can be created to test hypotheses without having to perform the same set of experiments, including animals, that these large studies have already completed. This way, the need for the repeated collection of cells from animals can be extensively bypassed.

Why were they not suitable?

Immune and stem cells grown in lab culture dishes are useful for studying some aspects of SCI, however they cannot replicate the complex changes that occur in the cells and tissues that support the function of the spinal cord. These includes changes to the supporting cells of the spinal cord (called glia, from the Greek for ‘glue’) that can lead to the formation of scar tissue, the recruitment of immune cells into the injury site, and damage to nerve fibres that is typical of chronic SCI. Immune and stem cells grown in culture also behave differently to those found in a living organism, showing loss of cellular heterogeneity (i.e., the unique identity of individual cells) and loss of communication with other cell types.

It is therefore necessary to use animal models to assess the complexity of biological and behavioural responses in an animal, both following an injury and after therapeutic treatment. There is also a requirement to demonstrate that a treatment is safe and effective in animal models before progressing to human application.

A retrospective assessment of replacement will be due by 20 April 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?

Animal numbers were estimated based on a previous project licence and a combination of the retrospective review, annual return of procedures, and estimated animal usage for the duration of the project. With the new mouse colony management system (MCMS) in use, we are now afforded unparalleled access to total animal usage year-over-year. Our review of the 2020 data showed that a total of 13 mice were used for SCI studies. These data were severely affected by the current COVID-19 pandemic, therefore in the next 5 years we are expecting at least a significant increase in the SCI numbers per year leading to approximately 200 mice/year for SCI experimental studies for a maximum total of 1000 mice.

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

The National Centre for the Replacement Refinement & Reduction of Animals in Research (NC3Rs) experimental design assistant is a tool which we constantly use to help the design and further refine our experiments.

According to our lab standard operating procedures (SOPs), experiments are constantly assessed at the pilot stage first (i.e., a first experiment is conducted with a reduced number of animals to adjust some key parameters before running the full experiment). This ensures that the correct number of mice necessary to achieve robust statistical results is used only when experiments are ready to be conducted in full. Mice are then placed in the experimental groups randomly, which helps to ensure case and control groups are homogenous. Treatments are given ‘blind’, which means that either the operator injecting

a treatment (or a vehicle, as control) or the surgeon performing the injections have been given no access to the information related to the treatment they are giving. Blinding is also applied to post-mortem tissue and molecular studies. Unblinding is the responsibility of the principal investigator and it is done only after the experiment is concluded and results are analysed, to avoid any bias in the generation of the results.

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 always perform pilot studies before undertaking a full experiment to ensure that larger studies are as accurate as possible. These pilot studies allow us to assess the experimental design and identify potential problems, as well as implement improvements early on in the licence. We are also coordinating 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.

A retrospective assessment of reduction will be due by 20 April 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 that undergo contusion SCI temporarily suffer and are in pain. However, the contusion SCI mouse model is the most refined for the purpose to cause the least pain, suffering, and lasting harm to the mouse and is a widely- and consistently used mouse model of SCI that has undergone continual refinements over decades of research.

The computer-assisted impactor used to induce injury provides calibrated and reliable readouts and allows fine control of tissue displacement and measurement of the actual impact force. This will allow us the flexibility to further refine our model to induce the minimum injury necessary to reach the experimental outcomes.

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

We are extremely limited in the use of invertebrates (e.g., worms), fish, or amphibia (e.g., frogs) as they are not fully suitable for the development and testing of clinically translatable new treatments for humans with chronic SCIs. In fact, while some preliminary work on regenerative biology is done in non- mammalian species, the complexity of the interactions between the immune system and the spinal cord in the context of tissue damage and regeneration can only be studied in mammals, as they possess striking anatomical and immunological similarities with the human spinal cord and immune system.

We also cannot solely rely on mice that have been terminally anaesthetized, as we need to assess the long term behavioural and pathological outcomes of our manipulations and interventions in promoting regeneration of the damaged spinal cord. Therefore, we need the mice to remain alive for several weeks-to-months after the onset of disease.

Finally, we need mice with cells that have reached a mature stage of development as representative of the cellular make-up present in adult human SCI. Therefore, we will use adult mice for the pre-clinical mouse model of SCI. The use of mice during the immature stages of life will be restricted to the collection of cells for cell culture studies using lab dishes where indicated and appropriate.

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

Before starting any study plan, we will discuss all experimental methods with the appropriate staff within the animal unit to ensure that all the necessary equipment is in place and that we are able to perform procedures under optimal conditions and/or supervision for the best health and welfare of the animals. Prior to running studies, we will determine if the necessary staff and expertise is available to successfully run the whole study, which skills are outstanding for the success of the study, and which relevant equipment is available to process samples under optimal conditions.

Once the study has started, we will rely on our established step-by-step care packages to ensure that the harm to the mice is as minimal as it can be, and the welfare of the mice is never compromised.

Over the years we have made significant refinements to our contusion SCI mouse model in order minimize the pain, suffering, and distress of the mice. These efforts have culminated in a dedicated and comprehensive standard operating procedure (SOP) that provides a detailed step-by-step care package. Refinements to this model are centred around the post-surgical care and housing of injured mice and the daily care and monitoring of the mice.

Several refinements were made to the housing of injured mice. For example, we provide bedding that does not inhibit the free movement of injured mice and does not get caught in the surgical clips used to keep the surgical wound closed.

We keep the mice in heated chambers for at least the first night post-surgery, and then attach heating pads to the outside bottom of the cages to maintain stable core body temperature.

We also provide unrestricted access to wet mashed food and supplemental edible hydrating substances to encourage eating and hydration, respectively.

For the daily care and monitoring of the mice, we have increased the number of daily checks to ensure the health and welfare of the mice is maintained.

We have incorporated the use of pain medications immediately pre-surgery and then as needed for at least 24 hours post-surgery to ease disease complications.

We perform fluid replacement through subcutaneous (i.e., under the skin) injections if signs of dehydration are present. After SCI, mice lose their spontaneous urination reflex. Thus, to refine this harm we manually express the bladder by applying appropriate gentle abdominal pressure at least twice a day starting on the day after surgery, until mice recover the reflex (approximately 1 week).

Having a dedicated SOP ensures that the highest quality of care is provided to mice throughout the course of this study and aims to significantly minimize the suffering and improve the welfare of paralysed mice. This SOP has been generated and further refined following recommendations from animal technicians, named animal care and welfare officers, and the named veterinary surgeon.

In the case of surgery, we have put in place post-operative assessment sheets specific to our model of injury to help the technicians to monitor better the recovery of the mice after surgery. This post-operative assessment sheet is constantly refined during our work, depending on our observations and in collaboration with the animal unit staff. Incidence of pain during the post-surgical recovery period will be controlled by the administration of pain killers, as directed by the named veterinary surgeon. Since surgeries will inevitably cause transient pain, which resolves by 48 hours, this will be minimized by the use of pain medications. Adult mice undergoing surgical procedures will rarely have complications 48 hours post-surgery. Regular discussion with a named veterinary surgeon will allow us to improve the management of pain if any new and more suitable recommendations appear during the work. In the case mice suffer from complications after surgery, appropriate guidelines are in place for humane endpoints.

Full training will be provided to new technicians who are unfamiliar with these procedures, as we have filmed previous study procedures to show how we expect our mice to recover. This helps new technicians to learn how to assess our mice correctly and in turn, means that mice recovering in our experiments receive the same high quality and consistent level of monitoring and care they need.

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

We plan our experiments in accordance with the guidance provided in the Planning Research and Experimental Procedures on Animals: Recommendations for Excellence (PREPARE) guidelines. This will guarantee we use the minimal number of animals to answer our objectives and ensure our results are both robust and reproducible. We will follow the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines when preparing our data for publication. In so doing, we will ensure our published findings are complete and clearly presented and easily accessible to other groups. This will lead to a reduction in the unnecessary duplication of animal experiments.

Excellent information is available on our establishment website, which is routinely updated with new 3Rs information. The National Centre for the 3Rs (NC3Rs) website will be regularly consulted to be sure that we are applying the latest recommendations for the refinement of our experiments. The Laboratory Animal Science Association (LASA) website provides updated information, especially regarding best research practices to perform aseptic (i.e., germ free environment) surgeries. We will also consider any new publications in a peer-reviewed journal relevant to our field offers refinements to our protocols.

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

Our establishment offers continuous training and recommendations via the animal facility and from animal care staff located within. We will keep informed of any changes to animal welfare guidelines by regularly consulting the website they provide to ensure that maintain compliance should any new updates be posted.

The National Centre for the 3Rs (NC3Rs) will be the main reference to assess whether our experiments match the highest standards of 3Rs, and we will adapt our protocols if the recommendations evolve throughout the duration of this project. Regular consultations on the latest practical guidance from Laboratory Animal Science Association (LASA), Institute of Animal Technology (IAT), and the Royal Society for the Prevention of Cruelty to Animals (RSPCA) will provide additional sources of new recommendations and advances in animal techniques and clinically applicable models.

Training records for all personal licence holders will be kept up to date using a centralized database. Senior group members will provide extensive training on the relevant regulated surgical or non-surgical procedures to all new lab members who will be working with mice as part of the ongoing project. Further, new lab members will be informed of the (optional) training services available to them. This will guarantee that general practices are firmly adhered to, which will ensure the welfare of the mice is consistently applied.

As a licence holder, it is my own responsibility to stay updated on published best practices by consulting information for licence-holders provided by our establishment and by speaking to other project licence holders.

A retrospective assessment of refinement will be due by 20 April 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. Retinal disease mechanisms and therapeutics

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

Eye, Retina, Macular degeneration, Uveitis, Therapy

Animal types	Life stages
Mice	adult, neonate, juvenile, pregnant, aged, embryo
Other species	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 the project is to develop treatments for eye diseases in order to prevent blindness.

A retrospective assessment of these aims will be due by 30 January 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?

An estimated 1.9 million people live with poor sight in 2013 and is predicted to rise to 2.7 million by 2030 (RNIB sponsored report) as eye diseases increase with the ageing population. This places a large economic burden on the UK (estimated at £22 billion in 2008). Aside from socio-economic considerations, it has been reported that after cancer, people fear losing their sight more than any other medical condition. This fear reflects the major impact of vision on the quality of life, including independent mobility, risk of injuries, social interactions, mental health and income. Treatment options for major causes of sight loss remain limited. Therefore, there is a high clinical need to better understand the mechanisms of eye diseases and develop new treatments.

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

The main output of the project is the development of new and improved treatments for eye diseases. The project is expected to improve our understanding of the mechanisms of eye diseases. Findings will be published, thus contributing to the expanding body of knowledge in the field. The results will support human clinical studies. Ultimately, new treatments would benefit patients and society.

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

New and improved treatments will benefit patients with eye diseases, researchers and the biomedical industry. Work in animal models have been essential for understanding the causes of disease in this complex organ (the eye) and for assessing the viability of new treatments.

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

Outputs from this work will be maximized by adoption of the latest scientific techniques and technological advances. The efficacy and safety of new therapeutic approaches will be validated using the appropriate disease models. To maximise rate of progress, we have support from expert collaborators locally and internationally. These will enable refinement of experimental design, technical support and feedback (e.g. to avoid duplication of work). All team members will be given close supervision to deliver the 3Rs and disseminate research findings for feedback from the research community.

Species and numbers of animals expected to be used

- Mice: 21,000
- Other species: 20

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 of lowest cognitive ability that are suitable for eye research and are highly appropriate because they are well established model organisms and numerous

model strains with inherited eye diseases already exist. Some eye diseases present at birth while others present with advanced age, therefore animals at different ages will be used to model the relevant human conditions. A small number of other species from UK licensed suppliers will be used to assess new treatments that need to be tested in eyes that are more anatomically similar to humans. The results will provide essential safety and efficacy data to facilitate human applications. The treatment will be delivered using minimally invasive human surgical techniques under anaesthesia and the effects assessed using non-invasive techniques.

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

Typically, an animal will receive injection of a therapeutic substance into the eye using minimally invasive technique under general anaesthesia. The animal will then be monitored regularly using non-invasive methods (e.g. imaging) to assess any treatment effects.

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

General anaesthesia is usually well tolerated and not associated with any adverse effects. Ocular treatment may rarely cause visual impairment, which is usually transient and not expected to impact on the animal's welfare or ability to thrive.

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: (i) 95% mild, (ii) 5% moderate.

Other species: (i) 90% mild, (ii) 10% moderate.

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

- Killed

A retrospective assessment of these predicted harms will be due by 30 January 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?

Animals must be used for this project since the development of therapies requires an assessment of efficacy and safety in live animals. The eyes of lower, non-mammal species

are quite different from those of humans and so do not make good models to use to test potential clinical therapies in.

Wherever possible, treatments will be initially investigated in non-animal alternatives such as cells and tissue from human donors.

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

Where possible, we have considered using human cells or tissue as alternatives. Some experiments may be conducted in retinal cells or organoids, but these do not model eye anatomy and the body's complex immune responses.

Why were they not suitable?

Whole living animals must be used for testing the efficacy and safety of many new treatments before they can go into human clinical trials. For instance, successful animal testing and approval of anti-VEGF therapies have transformed the treatment of wet AMD, a major cause of blindness. In the case of many advanced biological therapies, the whole body's immune response to the treatment plays a vital role in determining their effects. Therefore, a whole organism-based model is required to study the mechanisms of eye diseases and test new therapies.

A retrospective assessment of replacement will be due by 30 January 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 the breeding and maintenance of transgenic mice, we would maintain up to 10 different genetically altered lines. Each line typically results in the generation of ~300 mice/year, or 15,000 mice over the 5 year project. In addition, we would expect to maintain 5 knock-out/knock-in/transgenic lines with multiple alleles, which need greater number of animals to be bred to obtain the necessary genotypes (~1,000 mice/year/strain or 5,000 over the 5-year project). Finally, wildtype mice will be purchased from supplier to use as controls in some experiments (~200 mice/year or 1000 over the 5- year project). In total, 20,000 mice bred on this protocol plus 1000 mice purchased from the facility lead to a total of 21,000 mice used.

The number of other species (limited to 20) has been reviewed and approved by the Home Office and research funding bodies. This will provide sufficient number required to meet the experimental objectives and produce meaningful results to support future clinical trials.

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

To reduce the number of animals used, we will maintain close oversight of all animal lines and plan (with sign off) all experiments in advance to ensure that appropriate sample sizes are used. Weekly meetings will be held to reduce the number of animals bred and used as well as optimising tissue usage. We will cryopreserve lines to minimise maintenance breeding of lines that are not in active use.

Prior to testing any new treatment in animals, testing will be performed in cells to ensure safety. Small scale pilot study will be performed prior to testing in large cohort studies or non-rodent studies.

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

We have reduced animal use by using human cells and tissue. This will never be able to fully replace in vivo studies and does not completely remove the requirement for animal use, as the mouse line/s used to generate tissue must be maintained. However, procedures on live animals are reduced and several retinal tissue preparations can be made from one eye, allowing for experimental replication and/or multiple treatments to be assessed.

Using non-invasive imaging techniques, we can longitudinally assess eye health in the same animal over time and evaluate the effectiveness of therapies designed to prevent cell loss. Longitudinal measurements dramatically reduce the number of animals used compared to histological techniques and do not suffer from inter-animal variability. Furthermore, we can often apply treatment to one eye and use the other eye as the untreated control. This reduces animal numbers by abolishing the need for a separate cohort of control animals.

A retrospective assessment of reduction will be due by 30 January 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 will be used during this project, which are the most widely used animal model for human diseases. These small mammals are easy to manage in a laboratory environment, and numerous mutant strains with retinal degenerations already exist. Safe and minimally invasive techniques for delivering drugs into the eye or body are well-established as well

as non-invasive methods for measuring treatment effects (e.g. by imaging). A small number of other species may be necessary to provide pre-clinical data on novel therapeutic agents and techniques in order to support human clinical trials. Where surgical procedures are required for the delivery of therapeutic agents into the eye, these will be performed using similar instrumentation and techniques as for human surgery.

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

For treatments to have biomedical relevance and applicable to humans, a mammalian model organism must be used. Mice are the species of lowest cognitive ability that are suitable for eye research and are highly appropriate because they are well defined model organisms and many well-characterised disease model strains with inherited eye diseases already exist. Occasionally, other species may be necessary due to more similar eye anatomy and immune response to those of humans, thus providing meaningful safety data for supporting human applications.

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

We have established a minimally invasive injection techniques to reliably deliver treatment into the eye while minimizing adverse effects. As further refinement, we use clinical grade microscope, equipment and imaging devices that have been optimised for animal use. Aseptic surgical technique and appropriate analgesia are applied to prevent infection and make the procedure painless. Any research personnel conducting animal procedures is adequately licensed, highly trained and supervised.

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

- The Laboratory Animal Science Association (LASA)
- The guidance on aseptic surgery (LASA)
- Guidance on the Operation of the Animals (Scientific Procedures) Act 1986
- NC3Rs ARRIVE guidelines
- NC3Rs responsibility in the use of animals in bioscience research guidelines

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

We aim to stay up to date with and follow guidance on the 3Rs from the NC3Rs website. Also, at a local level, we regularly attend animal meetings where different aspects of the applications of 3Rs are discussed with experts in animal research and welfare.

A retrospective assessment of refinement will be due by 30 January 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?

35. Immune regulation and modulation in transplantation therapies

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

Key words

Organ transplantation, Cellular and non-cellular therapy, Immune regulation, Immune intervention, Tolerance

Animal types	Life stages
Mice	adult, embryo, neonate, juvenile, 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 understand the immunological contribution to organ transplant rejection and use this knowledge to design novel immune interventions both cellular and non-cellular to induce immune tolerance.

A retrospective assessment of these aims will be due by 29 April 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?

Transplantation is a highly successful approach for the treatment of end stage organ failure. Despite this, the average survival time of transplanted tissue (graft), such as the heart is 10 years.

Consequently, this puts pressure on the NHS as the number of patients waiting for a transplant outnumbers the number of donors. Loss of graft function is mainly due to the rejection of transplanted tissues in transplant recipients. Two factors contributing to this graft injury and dysfunction are the recipients' immune system and the side-effects of immune suppressive drugs used to control these cells. These drugs can directly damage the transplanted tissue as well as increase the susceptibility of these patients to develop cancers The importance of our research is two-fold, firstly, we will address the underlying immunological factors involved in graft rejection/loss and secondly develop novel therapies to prevent this. Our overall goal is to prolong the life span of transplanted tissue by manipulating the immune system of the transplant recipient such that there is no need to use damaging drugs. This will ensure the longevity of the grafted tissue and ultimately improve the lives of those receiving a transplant.

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

By the end of the project the following outputs will have been achieved.

1. **New Information:** New information on how immune cells contribute to tissue injury and to the allograft rejection to aid in the development of novel strategies to preventing organ rejection.

2. **Publications:** We expect to publish our findings in scientific journals as well as present the data at conferences and on social media.

3.**Novel cellular and non-cellular products for clinical trials:** The research supported by this licence is focused on producing novel immune based therapies for transplant tolerance in part by providing pre-clinical data to facilitate the translation of novel cell therapies into kidney and liver transplant patients.

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

Beneficiaries of our research will be:

1.**Transplant patients (long term):** In vivo studies will allow the development of data packages to support clinical trial applications. All experiments will contribute to better understanding of disease, novel therapeutics and new technologies for the treatment of transplant patients.

2. **Scientific Community (short term):** Our project will directly contribute to the field of transplant immunology; providing a greater understanding of mechanisms of tolerance, regulatory T cell (Treg) biology and new therapeutics to modulate these responses.

3. **Industry (long term):** Generation of novel therapeutics for transplant patients.

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

1) **Dissemination of knowledge :** publications and scientific meetings. The findings of our research will be disseminated at national and international meetings such as The British Society of Immunology, British Transplantation Society, and The Transplantation Society. We will also publish our findings in high impact transplantation orientated journals.

2) **Dissemination of knowledge:** Via MRC Centre/ Infection and Immunity Facebook/Twitter pages. To reach a wider audience we will highlight our finding using social media that exists at KCL.

3) **Collaboration** with other academic institutes and industry. Under this license, Quell Therapeutics will perform pre-clinical validation of cell therapy before transferring to the clinical trial setting. More specifically, safety and efficacy will be evaluated in animal models prior to clinical development.

- **Species and numbers of animals expected to be used**

Mice: 20,000

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.

The work outlined will use adult mice around the age of 6-12 weeks old. Mice in this age range represent a good model to study transplant injury and rejection due to the similarity of the mouse and human immune system. Moreover, the wide availability of laboratory reagents for use in mouse tissues increases the amount of useful data that can be collected from each experiment.

Manipulated mice made to lack or express proteins of interest of the immune system (transgenic and knockout mice) provide the opportunity to investigate the contributions of individual immune components to tissue injury, transplant rejection and the induction of tolerance.

NSG mice have been genetically manipulated to lack an immune system (absence of T cells, B cells and NK cells) allowing these mice to be engrafted with human cells. in a process called humanisation. This model allows a unique opportunity to investigate the mechanism of action, safety and efficacy of human cell therapy products before their use in transplant patients.

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

The animals will be used for the following,

1) Breeding:

Mice under this protocol will be used for breeding for 3 to 6 months.

2) Blood sampling for genotyping:

Tail blood will be collected via a superficial vein using a needle, which is a common procedure to collect blood for genotyping. There may be a mild discomfort during this procedure.

3) Tissue Donation:

Some mice will be used as tissue donors. Either harvesting skin grafts following sacrifice or harvesting heart grafts under terminal anaesthesia.

4) Injections and murine heart transplant Surgery:

Maximum duration of experiments = 100 days.

Mice may receive cells or compounds before surgery, including immune depleting reagents, such as monoclonal antibodies. Mice will receive a donor heart under general anaesthesia. To achieve this an incision will be made to expose the abdominal cavity and the donor heart transplanted, blood supply connected and the wound sutured . Some mice will receive cells or compounds following surgery as well as have blood samples taken. Some mice will receive a second graft, such as skin transplantation.

5) Injections and murine skin transplant surgery:

Maximum duration of experiments = 80 days.

Mice may receive cells, cellular components (EVs) or compounds before surgery, including immune depleting reagents, such as monoclonal antibodies. Donor skin transplant will be performed under general anaesthetic. In this procedure fur is removed and the surgical area disinfected using ChoraPrep® cutaneous solution. Then, a 1cm tail skin graft will be embedded into a graft site of the same size created on the recipients back. Skin grafts will be glued or sutured into the graft bed, treated with gelonet and held in place with a waterproof plaster. This will be removed 5 to 7 days following surgery. Some mice will receive cells or compounds following surgery as well as have blood samples taken. Some mice will receive a second graft, such as an additional skin transplant.

6) Injections and human skin transplant surgery:

Maximum duration of experiment = 50 days.

Mice may receive cells or compounds before surgery, including immune depleting reagents, such as monoclonal antibodies. Donor skin transplant will be performed under general anaesthetic. In this procedure fur is removed and the surgical area disinfected using ChoraPrep® cutaneous solution.

Then, a 1cm of a human skin graft will be embedded into a graft site of the same size created on the recipients back. Skin grafts will be glued or sutured into the graft bed and treated with gelonet and held in place with a waterproof plaster. This will be removed 7 days following surgery. Some mice will receive cells or compounds following surgery as well as have blood samples taken. Some mice will receive a second graft, such as an additional skin transplant.

7) Ischemia Reperfusion injury:

Maximum duration of experiment = 30 days.

The myocardial ischemia/reperfusion injury model will be performed under general anaesthetic. The skin area of the chest will be disinfected using ChoraPrep® cutaneous solution. In these mice the arteries [Left Coronary Artery (LCA) or proximal part of left anterior descending branch of the Left Coronary Artery (LAD) near its origin] providing blood supply to the left heart will be clamped for a pre- determined time to initiate tissue damage. The incision will be sutured and monitored daily for 7 days following surgery.

8) Viral and tumour challenges

Maximum duration of experiment = 30 days.

Mice may receive tissue transplant, cells or compounds before the application of viral or tumour challenge by injection into the skin or blood stream.

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

- **Pain (1-3 days):** Possible pain during/after surgery will be minimized using appropriate analgesia, as recommended by the NVS or NACWO. The animal will be monitored continuously until it has fully recovered. They will be allowed to recover in a warmed chamber not exceeding

32°C. Where signs of distress are observed (lack of mobility, discomfort, hunching or piloerection) via video/physical observation post-surgery, analgesia will be administered as required.

- **Hypothermia (24 hours):** Hypothermia is a common adverse effect of general anaesthesia for surgery in rodents. Therefore, all anesthesia will be performed while keeping the animal on a thermally-regulated pad or in a thermally- regulated chamber. After anesthesia, animals will be observed until they are awake, in a heated recovery box before being returned to their cage to prevent such an effect.

3. **Distress (24 hours):** Mice may show signs of distress immediately post-surgery; they will be assessed closely one hour after surgery, at the end of the day, and on the following day. If they are still showing signs of distress, we will consult with the NVS and NACWO.

4. **Wound Infections (1-7 days):** Complications including infection and wound dehiscence may occur as a result of surgical manipulations, although rarely. This will be minimized by using aseptic techniques. Frequent observation will be undertaken following the procedure to ensure that wound healing and recovery is free from bleeding and infection. In the unlikely event of wound breakdown, the mice will be culled using a Schedule 1 method. Any evidence of bleeding during surgery will necessitate gentle compression until bleeding is stopped. In the rare event of bleeding from the wound or signs of infection, mice will be removed and killed under Schedule 1 method. Topical antibiotic cream with or without systemic antibiotics will be applied in such instances to treat infection. Any animal that develops ulceration or skin infections that cannot be effectively treated inside 3 days, or is in distress, will be killed using a Schedule 1 method.

5.**Tumor growth:** Mice can suffer stress and discomfort due to size of tumour. Mice will be monitored for reduced mobility and provided with wet food as necessary and euthanised via Schedule 1methods if the humane endpoint is reached.

6. **Weight loss:** Mice reconstituted with human cells may undergo graft vs host diseases which will cause them to lose weight. Mice will be weighed daily to check for changes in weight and euthanised if they pass the humane endpoints of the study.

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: 25% of mice will undergo procedures with a mild severity

Moderate: 72.5% of mice will undergo procedures with a moderate severity

Severe: 2.5% of mice will undergo procedures with a severe severity

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

Killed

A retrospective assessment of these predicted harms will be due by 29 April 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?

Animals are required to achieve our project aim as they allow us to studying complex immune cells and the complete array of immunological factors in-situ. Transplant rejection is a complex immunological and non-immunological event which cannot be reproduced outside the body. It is not a single cell driven process but rather involves a combination of different cell types and factors forming a specific environment. Removing cells from this environment poses the risk of changes in their function which may result in false interpretation and understanding of these immune responses in a transplant setting.

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

An in vitro system which has been considered and performed is an MLR (mixed leukocyte reaction) where T cells from one individual are co-cultured with cells from another donor. Recognition of antigen between two unrelated donors provokes an allogeneic immune response. For preliminary studies, it is a reliable system, however it does represent what is occurring in vivo. a complete picture when it comes to transplant rejection.

An in vitro hypoxia system to study the functional alteration of a particular cell type under hypoxic conditions has also been considered. However, as mentioned above, this system does not allow us to study the multi factorial effects of ischaemia which exist in the body and does not truly represent what happens in vivo.

Why were they not suitable?

Unfortunately, we cannot accurately replicate the complex interaction of immune cells and immune factors in damaged tissue using in silico or in vitro technologies.

A retrospective assessment of replacement will be due by 29 April 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?

Previous experience from inducing tolerance in mice to skin grafts with regulatory T cells or regulatory B cells, indicates that cellular therapy can alter mean day of rejection by ~4 days for skin. Thus, for skin transplantation animal group sizes of 10 (2x5) will allow the detection of a statistically significant difference in graft survival outcome when p=0.05, with a statistical power of 83.8%.

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

The sizes of experimental groups and the number of repeated experiments will be kept to a minimum while ensuring that reproducible results are obtained with clear biological significance. When preliminary data is available, power analyses will be used to determine the minimum numbers of animals and repeated experiments that are required to meet statistical significance.

Prior to conducting any studies, a systematic review of the literature will be done in order to fine-tune the number of cells and techniques prior to animal testing.

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

Although experimental mice will not be used on multiple protocols (other than breeding), to reduce mice usage, mice may be used to supply tissues for multiple experiments. For example, a mouse culled by a schedule 1 method to collect donor skin for skin transplant studies, will also be used as a cell donor, or spleens and bone marrow for in vitro assays.

Our group has been carrying out mouse transplant surgery for over a decade and has, in collaboration with the NVS, refined these techniques to minimise the numbers of mice lost due to surgical errors. We have also refined the humanised mouse models to maximise the number of mice that engraft with a human immune system.

Breeding of the transgenic colonies will be done in collaboration with the BSU and a minimal number of breeding pairs will be kept to avoid unnecessary culling of unwanted animals.

We are planning to publish our results according to the ARRIVE guidelines.

Prior to any new studies a feasibility study (n=3), will be done to test logistics and gather information prior to the proposed larger study, in order to improve the latter's quality and efficiency. This will be used to reveal deficiencies in the design of a proposed experiment or procedure and these can then be addressed before animals, time and resources are expended on large scale studies.

A retrospective assessment of reduction will be due by 29 April 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.

This project aims to breed transgenic mice for use in biomedical research. These animals are needed to investigate the role of different immune cells and the influence of genetic factors in a transplantation setting. The mouse models used here are important, not only in providing new insights into the processes that lead to rejection, but also as models in which to study novel transplantation therapies.

For our studies, we routinely use well-established models of skin and heart transplantation in the mouse, to represent what happens in the clinic as closely as possible. Both these procedures are performed by an experienced microsurgeon which allows for a high success surgery rate (94% for heart transplants and almost 100% for the skin) reducing

technical failure. Procedures are always carried out under strict sterile conditions eliminating the risk of infection, whilst the use of analgesia perioperatively (Comfortan) targets pain in the recipient mice. Mice are regularly monitored post-transplant (up to 3 times a day) for 48 hours to ensure their well-being. Straight after the procedure, they are allowed to recover in an incubator at 28°C to control their body temperature.

Induction of myocardial IR injury is a severe model. Extra care is taken to increase the survival rate such as performing the precise intubation and controlling the inhalation of oxygen and isoflurane which results in quick recovery. The incision length of the chest is minimal to reduce the breathing difficulty caused by thoracotomy.

Regarding surgery, animals that fail to do so or exhibit signs of pain, distress or of significant ill health will be humanely killed by a Schedule 1 method. Any animal not fully recovered from the surgical procedure within 24 hours (eating, drinking and return to normal behaviour) should be humanely killed. The humanised mouse protocols developed to study transplant rejection have been selected as they allow the engraftment of human tissue, as previously demonstrated by others and us. This model utilises NSG mice which although they are considered “the holy grail” of the immune-deficient mice, the process can still be challenging. In our hands, we have managed to achieve almost 100% of engraftment of human cells in these immune-depleted mice. Combined with the successful engraftment and substitution of human cells, the refined humanised mouse model can provide an important bridge to study in vivo efficacy of transplant tolerance induction strategies in humans without the need of using non-human primates. Using these mice, we also have optimised a model of Graft versus Host Disease (GvHD) to assess the effects of cells which attack immune deficient recipients-in this case human cells to the immunologically “empty” mice. We have an accurate scoring system for monitoring all the signs of the disease including weight loss,

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 using published guidelines on minimal severity.

In conclusion, these are well established models in our group that have been practiced and refined over a number of years to minimise suffering and lasting harm to the mice.

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

The mammalian immune system of adult mice shares a close similarity with the human immune system. For these reasons, mice are the most frequently used animals in studies of the human immune system.

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

We will liaise regularly with the NVS and the NACWO regarding the welfare of the experimental animals and breeders as well as to discuss any refinements to protocols.

In addition, we will undertake the following

1. **Daily Monitoring:** Animals will be monitored post-surgery every day until the end of the experiment by the researchers as well as BSU staff.

2. **Post-operative care:** Mice undergoing surgery will be housed at 28oC to allow then to recover from anaesthesia. All mice receiving surgery will be given wet food for the first day and regularly monitored for any signs of infection, especially during the first seven days.

3. **Pain management:** Mice will be given pain management during and post operatively.

4. **Other refinements:** Pilot studies will be performed to refine the experimental methods and reduce animal numbers and suffering.

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

I am planning to follow the Prescott MJ, Lidster K (2017) Improving the quality of science through better animal welfare: the NC3Rs strategy. Lab Animal 46(4):152-156. doi:10.1038/laban.1217 and any future publications by the NC3Rs.

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

We will stay informed by attending seminars and webinars by www.NC3rs.org.uk the IAT and received the NC3Rs newsletters.

We will attend the annual NC3Rs/IAT Animal Technicians' Symposium.

A retrospective assessment of refinement will be due by 29 April 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?

36. Regulation of insulin secretion and glucose homeostasis in vivo

Project duration

5 years 0 months

Project purpose

- Basic research

Key words
diabetes, pancreatic islets, metabolic tissues, insulin, glucose homeostasis

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

Our main aim is to understand the cellular and physiological mechanisms that drive the development of diabetes so we can improve the treatment of this disease.

A retrospective assessment of these aims will be due by 20 April 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?

Over 8.5% of the world's adult population suffer diabetes, which causes annually more than 1.5 million deaths. The underlying biological causes that lead to diabetes are not well understood and current treatments rarely halt or reverse the progression of the disease. Beta cells are the cells within the pancreas in charge of secreting insulin in response to increasing levels of glucose in blood following a meal. These cells are grouped with other cells (including the alpha cells, that secrete glucagon under conditions of low glucose to counteract the effect of insulin) in the islets of Langerhans within the pancreas. It is well established that failure of beta cells to secrete enough insulin contributes to the development of diabetes and that different metabolic tissues (i.e. liver, fat, muscle, brain...) interact to make sure that the levels of glucose in the whole body are maintained within narrow, adequate limits. Diabetes is characterized by too high levels of glucose in blood ("hyperglycaemia) which can lead to life-threatening diabetes complications such as ketoacidosis (which could lead to diabetic coma), severe dehydration and, in the long-term, permanent damage in the eyes, kidneys, nerves and/or blood vessels, between others. A better understanding of the processes leading to diabetes, as well as the mode of action of current diabetes treatments is vital for the development of new and/or better therapies for this disease.

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

This project will provide new knowledge into the mechanisms that control how the different organs in the body work together to maintain an adequate level of sugar in the blood and why they fail to do so in diabetes

Specifically, the main specific outputs of this project are:

- Identification of new molecules ("made from specific pieces of the DNA, or "genes") that are important for the control of glucose levels and the development of diabetes

- Understanding why those genes are important and whether they are involved in the efficacy of certain diabetes treatments

- Better understanding of how specific treatments for diabetes work. This includes some commonly used drugs (i.e. exendin-4) or surgical treatment (bariatric surgery)

- Publications that will provide wide-access to this new knowledge

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

In the short term, this research will benefit the scientists working under this licence and the people that collaborate in their research, as well as their trainees (PhD students, Postdocs, Research technicians). The whole scientific community will benefit following the publication of our studies and the presentation of our data in scientific meetings (as early as 6 months after the start of the studies). The genetically modified animals generated will be made available to other researchers. Genetically modified animals will be bearing a small piece of DNA generated in the lab which will make them have more or less amount of the protein (or other biological molecules such as RNA) of interest. In this projects these molecules of interest will be those suspected to be important for the development of diabetes. The techniques used here will also be shared with the scientific community. This is important since, for example, one of the protocols described within this project provides the ability to monitor the development of diabetes and the function of the pancreatic islets over time in a non-invasive manner, in the living mouse. This means that the same animal can be studied at different time points with minimal disturbance and allows the reduction of the number of laboratory animals used, since regular killing at serial time points to assess the same parameters is not required.

The research proposed here is basic, or also called fundamental research, with the aim of improving scientific theories for better understanding on why diabetes occurs and how we can treat it so we don't anticipate a benefit to the pharmaceutical industry in the short term. Nevertheless a better understanding of the mechanisms contributing to the development of diabetes, as well as the benefits of bariatric-surgery to improve this disease will, in the medium-longer term, favour the generation of better, more refined treatments for diabetes and other metabolic diseases. This will therefore benefit the pharmaceutical industry and the patients suffering these diseases. The American Diabetes Association guidelines accept that bariatric surgery causes diabetes re-emission in 80% of cases but this is not without significant changes in the patients style of life. Our research may contribute to create more specific drug targets that could allow us to replicate the effect of bariatric surgery in a less invasive way (e.g. Injection of gut-derived peptides). We will also be working closely with human bariatric surgeons and endocrinologists to see how our findings can contribute to optimise patient surgical and medical treatment in Type 2 Diabetes.

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

As mentioned above, we will disseminate our data by publishing it in peer-reviewed journals. We will also generate and publish reviews and in-detail methods that will allow others to use the techniques optimized by us under this project licence. This will allow us to also showcase our means for troubleshooting and unsuccessful approaches. We will also share our research and new knowledge in local and international meetings and we will be available to train members of our own teams, and others within our institution. This will boost the establishment of new collaborations.

When possible, we will store tissues obtained with these studies to distribute to different researchers to ensure maximum effect and animal reduction. For example, in experiments

performed with bariatric surgery, the kidneys can be used for glucose transport research, stomach for bile acid research, liver for hepatic insulin resistance research, etc.

Species and numbers of animals expected to be used

- Mice: 15000
- Rats: 800

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 lowest vertebrates in which genetic manipulation can be successfully achieved and where diabetes studies are well documented. Rats give a better yield of blood and tissues per animal than mice and could be preferred if the relevant strain is available.

Both species are well acclimated to live in cages and laboratory conditions.

Adult animals are used in all experimental protocols since full control of glucose levels in the body is observed in adult animals. All life stages (adult, embryo, neonate, juvenile, pregnant) are required to generate transgenic mice in order to fulfil the objectives of this project in the breeding and maintenance protocols.

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

Most animals in our project will be genetically modified. In some cases, the genetic modification will alone promote the development of mild diabetes, defined as non-fasting glucose levels in blood > 13mM and characterized by excess urination and weight loss but, often, we will need to promote the development of the disease (to see whether our gene of interest delays or worsens diabetes). To do so we will administer "diabetogenic substances" which are chemicals that promote the destruction of the cells that produce insulin (beta cells) and therefore produce defects similar to diabetes type 1 (T1D) or, more often, we will feed the animals a high fat, high sugar diet. This diet mimics a westernized diet and promotes obesity and type 2 diabetes. The animals will be monitored for the development of diabetes by using standardized methods, including glucose tolerances tests and blood sampling, similar to what occurs to humans in the clinic. In some cases, we will perform imaging protocols which will allow us to monitor in more detail the function of the cells that produce the insulin.

Another typical process involving animals in this project will be the transplantation of islets in their cornea, so these islets can be imaged to assess their function while alive without using any invasive techniques. This will involve islet transplantation in the eye of the

animals, feeding the animals a high fat diet to promote diabetes and imaging the islets under a microscope while the mice are under anaesthesia.

Finally, a small percentage of our animals (~3%) will undergo bariatric surgery, a procedure that is performed in the clinic to humans to treat obesity and diabetes. Bariatric surgery consist in surgical removal of part of the stomach so the animals don't eat as much and loss weight. Due to weight loss and/or other mechanisms that are still unknown, diabetes remits. Animals will undergo this surgery and then will be evaluated for the development of diabetes by using the standardized methods mentioned above.

At the end of the studies, animals will be humanely killed and the tissues will be extracted post-mortem for further studies.

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

The vast majority of our animals will undergo very low adverse effects (defined as "mild"), mostly temporary discomfort related to the specific procedures performed (for example, a small cut to extract a drop of blood) or to the development of diabetes. The clinical symptoms associated with diabetes that we might observed in these rodents are minor weight loss and excessive urination. Some animals will undergo these procedures several times and thus we consider these low adverse effects to add up and call this level of severity "Moderate".

Animals undergoing gastric bypass (~3%) may present more severe adverse effects with a mortality rate of up to 30% by humane killing due to the adverse effects including bleeding inside the abdomen or stomach, intestinal obstruction or leaks and small hernias. The symptoms for this include dark faeces, hunched posture, erection of the hair of the skin ("piloerection"), social isolation, failure to groom, failure to feed/ drink, dark pigment discharger under eyes and nose and enlarged abdomen.
These symptoms will be monitored very closely.

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: Mild: 64.6% Moderate: 35% Severe:0.4%

Rats: Mild: 15% Moderate: 77.5% Severe:7.5%

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

- Killed

A retrospective assessment of these predicted harms will be due by 20 April 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 maintenance of normal blood glucose levels requires the coordinate action of several metabolic tissues (i.e. liver, fat, pancreas, etc) that secrete and respond to hormones performing actions that regulate the use of glucose by the body. Also the gut and the brain play very important roles in sending signals in response to food availability to control the production and response to hormones of the other tissues. These complex interrelations cannot be precisely reproduced in a petri dish (in vitro) and require a whole living organism.

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

In our work we use many different types of experiments to answer our scientific questions. Cell lines will be used whenever possible, to reduce the number of animals used in this project. For example, cell lines will be used to obtain preliminary data that help us identify genes that are likely to be involved in the development of diabetes.

We also use insulin-secreting cells (pancreatic islets) from human cadaveric donors whenever available and we will obtain human samples from patients undergoing bariatric surgeries that can be used for some of our studies.

We also use computational programmes to predict gene function and to simulate scenarios and analyse data whenever possible

These approaches will always be used to replace experiments on animals if at all possible

Why were they not suitable?

It is not currently possible to mimic the interplay between all metabolic organs, including the pancreatic islets and the brain, for the study of metabolism and diabetes. For example, it has been demonstrated that the hormonal changes that occur as a result of partial gut removal during bariatric surgery don't act directly in all other tissues, but can have several indirect effects thought several tissues: for example gut hormones affecting liver function that affects pancreas function to control insulin secretion. It is therefore impossible to fully facilitate cell lines and isolated tissues to replicate the effect of bariatric surgery in vitro

without an initial in vivo assessment. Also, our research will require extensive access to post- operational intestinal tissue in specific days during the study, which is not feasible with human subjects who undergo bariatric surgery.

A retrospective assessment of replacement will be due by 20 April 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 used statistics to predict how many animals will be needed in each of the different protocols so we can fulfil our objectives in the five year period. The data used to perform this statistical analysis has been obtained from similar experiments performed by us in the past or from similar published work done by others.

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

We have performed power calculations, which are mathematical calculations that predict how many samples (and in this case, how many animals) need to be use in a specific type of experiment so that when comparing different treatments or conditions, we are likely to detect an effect that is not due to chance.

Power calculations have been done with specific software such as Gpower and with other available tools (such as the Nc3R's experimental Design assistant) to determine the minimal number of animals required to obtain reliable results, and to ensure that no more animals than the strictly required are used in experiments. These tools will be used by all researchers working under this licence throughout the duration of the project to reduce the use of animals as much as possible.

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 take all reasonable steps to reduce the number of animals used in our project. Most of the animals used will be from genetically modified colonies which we will breed carefully at expert facilities in ways that minimise waste and ensure that every single

animal can be used in experiments. We will collect as much information as possible from every animal for example, making many measurements from the same animal over time. We will also collect tissues from all our animals, and share with other researchers, to perform experiments in the laboratory so no additional animals are required.

A retrospective assessment of reduction will be due by 20 April 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 mice and, occasionally, rats (see justification below)

Most of our mouse models will be genetically modified so we can study the function that specific genes play in the development of diabetes and its treatment. We will also use diabetes models. Type 1 diabetes is characterized by the loss of the cells that secrete insulin (pancreatic beta cells within the islets of Langerhans) while type 2 diabetes, which comprises 90% of the cases, involves malfunctioning of these cells and the lack of response to insulin of other metabolic organs. We will use models to mimic these types of diabetes (more often, T2D). For T1D we will inject substances that produced a controlled degree of beta cell death and, for T2D, we will feed our mice a "westernized" diet, consisting of high sugar and/or high fat content, which is well tolerated but leading to obesity and type 2 diabetes over time. We use these models cause they mimic very well the human disease and they are easy to control, infringing the minimal suffering in the animals.

We will use several experimental methods to monitor diabetes. The most commonly used method will be blood sampling to measure glucose and hormones (such as insulin). Very often, we will perform glucose tolerance tests where we will administer a bolus of glucose to the animals and we will measure, via a small cut in their tail vein, the concentration of glucose in their blood at different times after. This will tell us whether the animals are capable of control the levels of glucose in their blood, which they will not if they are diabetic. Additional methods will aim to understand how the genes that we have

manipulated, or the treatments or diabetes-inducing substances that we have given work in more detail. Examples are the use of imaging techniques (i.e. MRI) that will allow us to observe organ morphology without harming the animals.

All the procedures in this licence except the bariatric surgery are classified as either mild or moderate, meaning that the vast majority of our animals will undergo very low adverse effects (defined as "mild"), mostly temporary discomfort related to the specific procedures performed (for example, a small cut to extract a drop of blood) or to the development of diabetes. The clinical symptoms associated with diabetes that we might observed in these rodents are minor weight loss and excessive urination. Some animals will undergo these procedures several times and thus we consider these low adverse effects to add up and call this level of severity "Moderate". Also, procedures are done under local, general or terminal anaesthesia where appropriate to minimise stress and suffering of the animals.

Bariatric surgery is classified as severe due to the high mortality rate (Up to 30%) even in trained hands. Given the increasing prevalence of obesity in the general population, this technique is now frequently performed in the NHS to bring major health benefits for patients and help reduce healthcare costs in the long term. However, the physiological changes that occur after this procedure are complex and still poorly understood and animal models provide a unique opportunity to test the underlying mechanisms associated with the effects of bariatric surgery on glucose metabolism. This procedure will only be performed in a small proportion of animals.

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

We will use mice and, occasionally, rats. This is because mice are the lowest vertebrates in which genetic manipulation can be successfully achieved and where diabetes studies are well documented. Rats give a better yield of blood and tissues per animal than mice and could be preferred if the relevant strain is available. We will use adult animals in the experimental protocols since at earlier stages the control of blood glucose is not full and can't fully mimic that of humans and the development of diabetes.

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

We have carefully chosen the most refined models that can deliver as much scientific relevant data as possible with minimum amount of suffering. We will make all efforts to make animals comfortable whether or not they are undergoing an experimental procedure. This includes giving them a comfortable environment to live and handling them adequately so we don't cause them stress. Animals will also be handled often between experiments to reduce their levels of stress during experiments.

Bariatric surgery has been refined so we use optimal surgery materials (i.e. sutures, tools) and only highly trained scientists will be performing this technique. During any experiment, we will closely watch all animals for any sign of discomfort and distress and either stop the experiment or humanely kill any animals suffering unexpectedly. For example, animals

undergoing bariatric surgery will be monitored, post anaesthesia recovery, every 2 hours during the day and every 4 hours overnight in the first 72 hours as a minimum (more frequently if necessary) and daily thereafter. Advice will be sought from NAWCO (who is the Named Animal Care & Welfare Officer responsible for overseeing the day-to-day welfare of the animals at our institution), technicians and vets for keeping up to date with best practises and we will use all available guidance (see below) to continue the refinement of our approaches whenever possible.

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

We will follow the LASA (Laboratory animal science association) and the NC3R's (National Centre for the Replacement Refinement and Reduction of Animals Research) published guidelines including PREPARE (Planning Research and Experimental Procedures on Animals) and ARRIVE (Animal Research: Reporting of In Vivo Experiments), the "Responsibility in the use of animals in bioscience research", "Colony management best practice", "Minimizing the use of GA mice" and the topic-specific resources such as "Anaesthesia", "Handling and restrain".

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

We will be in continuous communication with the veterinary staff in our facility that will, in the case of Bariatric surgery, overlook our studies, and we will provide best advice for keeping up to date with best practises.

As project licence (PPL) holder, I will interact with all named persons and animal technicians at our institution to review current approaches and whether there are any new 3Rs opportunities. I will continue subscription to the NC3Rs (National Centre for the Replacement Refinement and Reduction of Animals Research) e-newsletter. Myself and/or the other researchers working under this licence will attend selected NC3Rs courses/workshops

A retrospective assessment of refinement will be due by 20 April 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?

37. Repairing the damaged spinal cord

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

spinal cord injury, central nervous system, axon regeneration, neuroscience, loss of function

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?

This project aims to understand why the nerves of the central nervous system (CNS), and in particular the spinal cord, fail to regenerate after injury, with a view to developing therapeutic agents to counteract this and ultimately preserve function.

A retrospective assessment of these aims will be due by 08 April 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?

Spinal cord injury affects around 1,000 people every year in the UK and an estimated 100,000-1.263 million new cases worldwide, with survivors experiencing life-long loss of function and reduced mobility. Currently, there are no therapeutic agents that promote axon regeneration after injury and the loss of function that ensues, leaving an urgent medical need for effective therapies. We wish to identify and test therapeutic targets and agents that will promote the regrowth of axons, counteract the negative effects of injury to the spinal cord and ultimately preserve/promote useful function.

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

The primary outputs of this work will be the information on identifying new therapeutic targets to not only prevent the negative consequences of injury to the spinal cord but also to develop strategies aimed at promoting the regrowth of injured nerves. A major potential benefit from the work proposed will be the acquisition of new knowledge for dissemination in peer-reviewed journals. Functional outcomes after spinal cord injury are poor at best and presently only palliative drug treatments are available for patients. At the moment nothing is available for patients that can reverse the damage to nerves caused by the injury. Therefore, there is an urgent unmet clinical need for new therapies aimed at enhancing functional repair responses of damaged spinal nerves.

Specific academic outputs will be to publish the findings. This is important for the development of the project but also to provide a knowledge base for other academics working in this field.

Other product outputs will be to support the development of the new and existing intellectual property which will allow us to facilitate translation into a clinically and commercially viable proposition.

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

In the short term, the benefits would be to provide high impact publications. This work will also help develop information fora clinical trial of several classes of drugs that are currently under study.

In the longer term this work has the potential to have an immense impact on the lives of patients with spinal injuries and other related conditions such as traumatic brain injury,

which shares the problems as spinal cord injury but affects up to 60 Million people worldwide. There is currently no treatment that reverses the pathological effects of spinal injury and the functional loss that occurs as a result is permanent. Our discovery strategies would provide key information on the pathological processes after spinal cord injury, allowing us to identify opportunities to test and develop therapies aimed at reversing or protecting against the negative effects of injury. As a result, we will preserve or repair neuronal function.

Examples of other potential beneficiaries of the success of this work are traumatic brain injured or stroke affected patients who will benefit through the development of new treatments that are neuroprotective, pro-regenerative and anti-scarring, since the same pathological processes occur in these groups of patients. Additional beneficiaries could include health care providers, particularly the NHS.

Spinal Research commissioned a report which estimated that the average lifetime cost of health and social care for a person with a spinal cord injury in the UK is £1.12 million (2016 prices). Therefore, the average cost to treat 1,270 patient/year in the UK is estimated at £1.43 billion/year. Effective therapies that protect spinal neurons from death and promote their nerves to regenerate, if successful, would relieve the pressure on the NHS and significantly reduce the cost burden of treating spinal injured patients.

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

We will maximise the outputs of the work by collaborating with othersworking in this field to maximise the use of the data we obtain. We will rapidly disseminate the outcomes of the tests, whether negative or positive, to inform the academic community and support other researchers developing technologies in this area. We are already working with several small and large pharmaceutical enterprises which we will seek to attract after proof-of-principle experiments have been successful. Some of the drug agents including those we wish to re-purpose will need to be provided by companies collaborating with us, however, we will always file re-purposing patents before approaching pharmaceutical companies, and hence there should be no problems with the freedom to publish our results and without restrictions. However, many companies require vetting of each publication prior to submission, but we will ensure this is completely in a timely manner.

Species and numbers of animals expected to be used

- Mice: 500
- Rats: 1500

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 rats for most of our experiments since adults do not regenerate their nervous system effectively. In addition, rodents closely mimic the human molecular responses to spinal cord injury. Although mice do not faithfully reproduce all of the molecular characteristics of injury as well as rats, mice do offer the opportunity to assess our objectives in readily available knockout strains, where the impact of a particular gene to aspects of neuroprotection, axon regeneration and scarring after spinal cord injury can investigated.

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

All animals will undergo a surgical procedure to cause different degrees of injury to the ascending spinal axons, a group of axons which are mainly sensory. Typically, animals undergo a moderate severity injury (spinal cord injury) and once our therapies have been validated, a small proportion of animals will undergo a severe injury (spinal contusion injury). The more severe injury closely resembles the human condition and guides our development of therapies towards the clinic. Animals will receive injections of therapeutic agents via appropriate routes and are followed up for 6 weeks with tests for sensory and locomotor function. Animals will be killed humanely before collection of tissues for histological/protein/mRNA analysis.

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

Spinal cord injury: Our moderate model of spinal cord injury causes minor neurological deficits affecting their movement and behaviour. They feed and drink normally, and have normal bladder function. Sometimes (<20%), animals may show weakness in one or both hind limbs, but these usually resolve within the first 3 days. In the severe model of spinal cord injury, animals lose bladder function and hindlimbs are paralysed for at least 2 weeks. During that time, animals are cared for extensively by us including, twice daily manual bladder expression, provision of soft bedding and careful monitoring of signs of distress.

Spinal cord contusion: This is graded as severe since many neuronal tracts are injured as a result. Animals will be lose the use of their back legs and require manual bladder expression for at least 2 weeks after injury. Animals do recover some bladder control during the first 2 weeks and enough to ensure automatic voiding. Limited mobility of the back legs also returns over time. Despite the provision of appropriate bedding pressure sores may develop in affected limbs. Autophagia of the back legs may be observed (in <30% of animals) during the first few weeks, in mild cases some animal nibble their toenails without damage to the flesh. To avoid animals nibbling on the insensitive limb, we coat the appendages with ‘Stop & Grow stop nail biting’ solution. At the first signs of this being ineffective, animals are killed humanely.

Pre-conditioning sciatic nerve lesions: In some animals, it is expected that pre-conditioning sciatic nerve lesions will cause loss of hindlimb function in the affected limb. Animals will recover over the first 2 weeks after injury. Despite this, animals are still able to move

around and feed normally. To avoid animals nibbling on the insensitive limb, we coat the appendages with Stop & Grow stop nail biting solution.Substances administered by injection: Stress due to restraint and transient discomfort from needle insertion is likely in 100% of animals. These are minimised by selection of appropriate (minimum possible) sized sterile needles and syringes.

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 animals will experience a moderate severity (80%). Some animals will experience a severe model of spinal cord injury (20%) but this will only be used after validation of targets in the moderate severity model.

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

Killed

A retrospective assessment of these predicted harms will be due by 08 April 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?

Whilst some elements of spinal cord injury can be modelled in cell culture, the complex, clinical picture and interaction of the whole-body systems, including in particular the immune and nervous systems cannot all be currently modelled in cell-culture or computer-based models. The use of live animals is therefore unavoidable and essential for discovery science, drug discovery and to demonstrate the activity of drugs in a situation relevant to the human condition. Neurons are not present outside the animal kingdom and so an animal is required. Only mammals have a sufficiently developed immune-system to readily compare to humans, and rodents are the animals of lowest neurophysiological- sensitivity required to achieve the scientific aims. It is not ethical to conduct experiments on humans with spinal cord injury, especially where those experiments require the removal of parts of the immune or nervous system for ex-vivo investigations. Zebrafish or other lower sentient animals cannot be used since these species spontaneously regenerate and are therefore not representative of the human condition. Therefore, there is no feasible alternative that

can entirely replace the use of a living animal that would allow the objectives to be met. However, we will use in vitro and ex vivo work to inform our animal studies.

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

There are currently no alternatives to animal work for the spinal cord injury model.

No cell culture- or organoid-based models exist that encompass all of the aspects of disease for any of the models described in this project. However, individual aspects will be modelled in vitro and ex vivo. For example, we regularly use in vitro neuronal cultures to detect therapeutically useful molecules in terms of neuroprotection and axon regeneration to then take forward into animal studies. Only if agents show efficacy in our cell culture-based models do they proceed to in vivo animal studies.

Why were they not suitable?

The fundamental reason why the use of animals is required is to understand these complex processes that occur in a damaged spinal cord, which at present no in vitro methods can model the complexities of the systems involved in this condition. It is difficult to use primary cells to culture all of the different types of cells since they require different growth mediums and factors for survival. Indeed, the reason why many new drugs fail between cell culture and in vivo studies is in the inability to full recapitulate the in vivo environment. Technologies are being developed to address this gap, including the development of 3D cultures. However, none of these model systems are yet able to phenocopy the integration and interplay between the numerous cell types that constitute spinal cord injury.

A retrospective assessment of replacement will be due by 08 April 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?

Numbers of animals have been based on pilot data, in-house data and published data from our own similar studies.

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

We used the NC3Rs EDA system to calculate animal numbers to be used for this project. We used sd values from several of our own and other's published data to facilitate power calculations and reduce animal usage.

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 seek to refine protocols, such as the development of other quantitative measures for injury such as associated biomarkers that can predict potential injury severity and recovery, which will facilitate “reduction”. Experiments will be planned so that they can be published in accordance with the ARRIVE

2.0 guidelines.

Wherever possible, we will use archived samples and share control groups across different experiments wherever possible.

As part of good laboratory practice, we will write a protocol for each experiment including: a statement of the objective(s); a description of the experiment, covering such matters as the experimental treatments, the size of the experiment (number of groups, number of animals/group), and the experimental material; and an outline of the method of analysis of the results (which may include a sketch of the analysis of variance, an indication of the tabular form in which the results will be shown, and some account of the tests of significance to be made and the treatment differences that are to be estimated). We will make appropriate arrangements to randomly assign animals to experimental groups and blind studies.

At the end of the experiment, we will harvest the maximal possible number of tissues. Tissues not immediately analysed will be archived and will be made available to other researchers working on similar questions.

A retrospective assessment of reduction will be due by 08 April 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.

Due to the nature of the scientific question, the only appropriate model is spinal cord injury (SCI). In the human condition, all spinal cord injuries are severe and cause neuropathic pain and loss of function.

However, pain in the animal model is rarely observed in the moderate severity SCI model and is controlled well by analgesia in the severe contusion model for up to 2 weeks after contusion, by which time the pain resolves naturally. Spinal cord injury in the rat has unavoidable adverse effects with the contusion model exhibiting greater levels of adverse effects. These are unavoidable in order to mimic the human condition and find a therapy which does not exist in spinal cord injury and is an urgent medical need. However, we have made every effort to ensure the approaches cause the least possible pian, suffering, distress or harm withing this scenario by applying a number of refinements. These include using the moderate severity SCI model to establish the benefits of potential treatments and then only when validated, using the severe contusion model in a small number of experiments for final validation and relevance to the human condition. Other refinements include the use of soft bedding to minimise pressure sores and the use of anti-nail biting solution to discourage biting of denervated limbs. We also have over 20 years' experience of all of the models described in this application and are able to identify adverse signs as early as possible.

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

We cannot use less sentient species (e.g. zebrafish) for this work, because unlike mammals, they are able to regenerate their CNS spontaneously. Rats are typically used for our experiments since they share similar pathophysiology to humans after injury to the CNS. Mice are also used since there is established and reliable transgene technology, and established models of spinal cord injury. There are a large number of genetically modified mutants available and there is extensive amount of work that has already been performed and published using mouse and rat models of spinal cord injury.

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

All therapeutic agents are evaluated and optimised in vitro prior to in vivo application. We keep our experimental time points in longitudinal studies to a minimum and use archival control results where possible. Multiple analyses are done on harvested tissues. We use the minimum number of interventions and minimal volumes for drug delivery during experiments and continually seek methods to reduce these by studying alternative drug

delivery strategies. e. All animals are picked up using refined handling techniques to minimise distress and the technical staff are well versed in recognising the signs of distress in these animals as they have many years' experience in handling and caring for our spinal cord injured animals. We use bespoke welfare sheets that include body conditioning scores to monitor animals post-operatively, soft mash is placed at the bottom of the cage along with soft furnishings to minimise injury to the affected hindlimbs. The contusion model of SCI is extensively supported, especially in the first 2 weeks including twice daily manual bladder emptying, extensive monitoring (minimum of 5 times during any 24-hour period), soft mash at the bottom of the cage and

longer spouts for drinking water. All of these refinement steps significantly reduce the animal welfare burden.

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

Prior to all experiments we will consult the PREPARE guidelines checklist to ensure that valuable data will be generated in the experiment.

Experiments will be conducted in accordance with the guidelines published by the Laboratory Animal Science Association (LASA).

The resulting data will be published in Open Access Journals wherever possible and in accordance with the ARRIVE 2.0 guidelines.

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

We will stay informed of advances in the 3Rs through attendance of seminars and conferences, as well as discussions with the NVS and NACWOs.

We will review each experiment on completion to determine any refinements that can be applied to future experiments.

Continued review of the scientific literature will be undertaken on a regular basis in order to identify any newly emerging technologies and models that could be potentially adopted in order to replace in vivo animal use.

We will also stay up to date with guidance published by LASA on the most refined experimental methods. We are already signed up to receive the NC3Rs newsletter and will attend local events such as conferences and follow advice in webinars hosted by NC3Rs.

A retrospective assessment of refinement will be due by 08 April 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?

38. Modelling fibrosis in multiple organs to understand 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

Scar, Fibrosis, Diagnosis, Therapy, Chronic disease

Animal types	Life stages
Mice	adult, juvenile, pregnant, neonate, aged
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 aim of this project is to provide a greater understanding of how scarring (or fibrosis) occurs in chronic diseases that can affect any organ in the body. In some instances fibrosis can even lead to multiple organ failure and/ or cancer. The goal is find better ways of diagnosing fibrosis early enough to reverse the disease process and develop new medicines to reduce or remove the scar and improve disease outcome.

A retrospective assessment of these aims will be due by 05 April 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?

Fibrotic diseases (or scarring) are increasing and a major cause of death worldwide. In some cases, end-stage diseases can be treated by transplantation; however, there is a huge shortage of donor organs; significant side-effects from medicines (to suppress rejection by the body); and focus on end- stage disease is too late. Urgent development of novel diagnostics to determine stage of disease and anti-fibrotic medicines are needed. This requires a better understanding of the underlying process of fibrosis to develop hypothesis based approaches to newly identify factors that are present specifically during certain stages of disease, allowing highly targeted strategies for therapeutic intervention.

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

We anticipate new information will be published in scientific journals. However, we also expect some experiments will lead to novel discoveries for patient benefit. We hope much of our work will lead to new products (such as diagnostics) or for development as new treatments to improve scarring in disease.

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

Short term benefits of this project will be to the broad scientific community investigating chronic disease with new data on how fibrosis occurs. Long-term benefits will see our data being applied to health care as has been the case already for our studies. We have recognised that the transition from discovery in cells, through to animal models and toward human / patient benefit can take up to 10 years. However, we have found this to be a reality and our work is already being applied as novel tests to diagnose liver disease in the clinical setting. Our goal is to ensure all organ fibrosis we investigate as part of this project is eventually translated to human disease and patient benefit.

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

We collaborate broadly with the national and international scientific community. This will continue with personal dissemination of knowledge. More broadly our work will be published in high quality scientific journals as happens now to capture the widest readership. Even where our work is at preliminary stages, we present this at scientific meetings (with published abstracts) meaning that even approaches that may not be

successful in our hands will be of use to the scientific community in this area. Through progressing our work for patient benefit we are also engaged with patient and public involvement (PPI) groups currently for liver fibrosis, lung fibrosis (including from COVID19 infection) and multimorbidity fibrosis (affecting any organ).

Species and numbers of animals expected to be used

- Mice: 30450
- Rats: 1500

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 project intends to use rats and mice. For experiments with a primary focus on the use of ex vivo tissues or cells, the use of rats will be considered where this will significantly reduce the number of animals needed to generate sufficient material. The ability to use material originating from a single animal across several different conditions will provide increased statistical power to experiments by limiting variability and allowing paired approaches to analyses. Moreover, the use of rat where possible provides a closer genetic link to human - i.e. rats share more DNA in common with humans than mice do, so represent a better match. However, the use of mice is unavoidable as it provides a well-recognised species for work involving genetic alterations and there are standard protocols, methods and reagents used that have been optimised for this species with acknowledged benefits for use. As an adult disease - we will use adult animals to induce fibrosis by various methods depending on the organ under study.

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

In any organ, fibrosis (or scarring) occurs through tissue damage. Depending on the organ under study, this will be achieved through injections or inhalation of tissue damaging compounds, through dietary changes or by surgical procedures. To understand the initial phase of disease duration of experiments may only last up to 72 hours. However, to understand the long-term progress of disease experiments may last several months. For example some chronic fibrosis models can take 12 weeks to fully replicate the human disease and for progression to cancer (which can occur in the liver) this can take up to 60 weeks.

In some cases, where we have discovered new ways to treat and improve fibrosis, we will use medicines to treat the animals with fibrosis. From our previous experience this usually takes place in the middle of the disease model so that we see the real effect of medicines in halting or reversing the scar.

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

In most instances, following completion of each project animals will be humanely killed and tissue from animals will be removed for studies in the laboratory. In some instances, animals will be treated with agents that cause fibrosis. Although transient discomfort may occur at the time of administration the animals appear normal soon afterwards. Similar to humans, animals can sustain fibrotic injury for a long period of time with no apparent symptoms. In the rare scenario that an animal shows signs of organ failure the animal will be humanely killed to ensure the animal does not exceed the severity limits set out in the project. Some animals will undergo surgery to induce fibrosis, but these are not life-threatening procedures. For surgical induced models animals receive post operative care including pain reducing agents. For some chronic disease models associated with scarring, the most common adverse effect is weight loss usually toward the end of the experiment. However these are typically within the shorter time periods of experiments (up to 2 weeks) and generally, aside from this, animals typically appear well. In lung fibrosis, shortness of breath can occur but is unlikely in the timescale of our experiments (up to 28 days). For the liver, liver fibrosis commonly leads to liver cancer in human.

We have an experimental model to investigate this in mice using an injected chemical. These animals will develop tumours specifically in their liver typically towards the end of the 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 fibrosis models have been selected as they are generally well tolerated in rodents. The expected overall severity for the majority of this project is moderate and animals will be closely monitored for any adverse affects.

Approximately 50% of rats and mice involved in this project will experience mild severity limit, 40% moderate severity limit and 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 05 April 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?

Despite progress in understanding the biology of scarring (fibrosis) in chronic diseases, many discoveries have been unsuccessfully translated for patient benefit.

In addition, fibrotic disease and cancer are complex processes that progress and resolve over many weeks. This involves not only the affected organ, but also the immune system, production of factors that promote the disease process and cell-cell interactions during the periods of tissue damage and repair involved in fibrosis. For this reason, it is not possible to study these events in an in vitro/ ex vivo system as studying various cell types in isolation in vitro (cells / tissues in a dish) would not provide us with any insights into complex cell interactions within the body system during fibrosis.

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

There are several possible non-animal alternatives that we have considered. These include:

- Established cell lines as a useful starting point for assessing the efficacy and tolerability of therapeutic interventions and genetic manipulation. These can also be used in initial molecular studies prior to animal work.

- Human tissue obtained from people undergoing invasive diagnostic sampling (e.g. biopsy tissue). Tissue can be used as a pre-screen prior to animal studies.

- Computer modelling using published datasets.

Although some aspects can be investigated directly in cells and tissues, unfortunately we cannot understand fully the complex process of fibrosis. Current models in human tissue are typically limited to taking the tissue or specific cells into a dish. We use this but it is very limited to a short time frame and cells/tissues rapidly lose function when taken from the body.

Moreover, where specific genes have been identified as critically important in driving the disease process we are unable to manipulate these well enough in the currently available systems in human.

Despite these limitations with the current non-animal alternatives, where possible we will use the above as partial replacement as much as is practically possible

Why were they not suitable?

Immortalised cell lines deviate from the normal behaviour of cells over time and are therefore not a robust model in isolation. They also do not capture the complex interplay between different cell types. Where appropriate, preliminary investigations will be performed using established cell lines in vitro before progressing into use of primary cells or tissues ex vivo and through to in vivo studies.

The currently available models that use humans cells or tissues cannot fully recapitulate the whole body environment involved in disease progression. This involves a complex interaction of different cell types and the damaged tissue / scar environment. The models are also short lived so do not replicate the process involved in fibrosis which occurs over many years in human.

We will continue to ensure all animal studies and discoveries are translated to human tissue as we already have in the past.

A retrospective assessment of replacement will be due by 05 April 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 carried out statistical calculations for our models and have a great deal of experience having published in peer reviewed journals of the numbers required to ensure significant results are produced using both in vitro (cells in a dish) and in vivo (in the animal) experiments. For the majority of our work this typically requires 5 animals in 4 groups (to include control measure for both those undergoing fibrosis induction).

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

In designing our project we have used expertise from statisticians who are part of the NC3R's Experimental Design Assistant (EDA). For any new models that require experimental design we will seek similar advice and in addition consult the NC3Rs EDA.

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

From our current (and previous) project licences we have gained a lot of experience and information to instruct our experiments. This includes detailed calculations and real experimental outcomes for number of animals required. In many cases we have archived control tissue from previous experiments so we can reduce animal numbers by only carrying out the treatment (for example only inducing fibrosis). For many of our experimental models we can only use male animals (many female animals are protected from fibrosis in several organs). However, in this broad project we can now use females for many of our experiments including all cell preparation and even some in vivo fibrosis experimental models. Where possible we make use of human cells and tissue to further reduce the need for animals. In addition, we are very well organised as a group and routinely make use of several tissues from one animal such as liver, lung, kidney and heart. These structures already in place in the group ensure we limit the number of animals used for this project.

A retrospective assessment of reduction will be due by 05 April 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 the case of cellular studies, we will use rats as this allows a greater analysis of the mechanisms associated with the disease process compared to mouse, due to closer genetic proximity to human. However, for in vivo studies, mice will be necessary based on the use of genetically modified strains.

To investigate the therapeutic potential of our findings in fibrotic disease in different organs from multiple insults, it is necessary to use more than one model of injury. We have chosen established models of organ fibrosis that have good comparison with the human disease and have been refined over many years in labs worldwide. In all cases the models used are well tolerated.

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

The experiments proposed will use the simplest possible animal system. The mouse and rat are the only systems we can use as they have good replication of the human disease process (induced by fibrosis) and the mammalian system provides all cellular interactions (for example with an intact immune system and endocrine system). Moreover, the mouse is the only mammalian system that allows broad genetic manipulation of specific discoveries as potential therapies in disease. The use of terminally anaesthetised animals would not allow the course of disease to be studied.

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

As evidence of limiting animal experimentation through refining our models, improved technical skills and post-operative care we have reduced the mortality of a surgical model to induce liver fibrosis (bile duct ligation) from 30% to ~10-15%. We have also successfully implemented warming cabinets into any post operative care regimes to greatly improve welfare from any surgery induced fibrosis. We will seek to refine all other protocols wherever possible (most are much less severe). Such measure have included adding non-invasive monitoring of lung function during models of lung fibrosis to ensure animals welfare.

We are also part of an extensive national fibrosis network on broad organ fibrosis where all involved are open to discussion over how to improve methodologies in practice. This has often resulted in shortened experiments in recognition that the same outcome can be achieved in a much reduce time span. This will continue and is an excellent platform.

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

Prior to any animal experiment, details are described in detail and discussed with our unit to ensure best practice is taking place within the licence. We have standard operating procedures in place for complex experiments such as those requiring anaesthesia and surgery. We will follow LASA guidelines (https://www.lasa.co.uk/) including animal welfare, administration of substances and aseptic technique. We will also ensure our personal research areas are up-to-date through monthly literature searches to refine any experimental models we use.

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

Our unit provides weekly newsletters that include updates from the NC3R website. This includes specific insight into any specific areas researchers have an interest in. However, we will also ensure we consult the 3Rs website regularly for any advances relevant to our work that can be implemented. This is a requirement for any individuals carrying out animal research.

A retrospective assessment of refinement will be due by 05 April 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?

39. Metabolism and pharmacokinetic studies of pharmaceuticals

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
  - 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

Metabolism, Pharmacokinetics, Rodents, ADME, Pharmaceuticals

Animal types	Life stages
Rats	neonate, juvenile, adult, pregnant
Mice	neonate, juvenile, adult, pregnant
Hamsters	Adult
Guinea pigs	Adult

Animal types	Life stages
Rabbits	Adult
Beagles	Adult
Minipigs	Adult
Cynomolgus macaques	Adult
Goats	Adult
Chickens	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 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 perform metabolism and pharmacokinetic studies to support the development of Human and Veterinary pharmaceuticals. These tests, commonly referred to as ADME studies, are designed to investigate the Absorption of test substance related material (how substances get into the blood), Distribution around the body, (including into blood and urine), pathways of Metabolism (how the body breaks substances down), and routes and rates of Excretion (how the body removes the substance), by harvesting samples (blood, plasma and other bodily fluids, tissues and excreta) at different times after administration.

These studies will be performed for both regulatory (to ensure test materials are safe, for example) and for discovery (to look at the metabolic profile of drugs earlier in their development path, for example) purposes.

A retrospective assessment of these aims will be due by 01 April 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 primary benefit of work carried out under this licence will be to allow regulatory authorities (who are totally independent from the commercial interests behind every marketing application) to come to decisions regarding the marketing of new compounds based upon safety data generated in these studies relating to exposure (pharmacokinetics) and ADME (metabolism) characteristics of the test compound. This will provide data supporting the rate and extent of absorption; distribution into tissues; key enzymes involved in metabolism; and the rates and routes of elimination; to assist in scaling safety margins of exposure and toxicity into man.

The introduction of new pharmaceuticals and veterinary pharmaceuticals is beneficial because of associated improvements in the health and welfare of humans and animals.

There is also a major benefit when including screening experiments, as the most promising compounds for further development will be selected and then fully tested so that they can reach the market sooner. This will ensure subsequent benefits in both the health and welfare of humans.

The use of the experiments described in this licence may indicate major safety limitations with the development of the substance under evaluation at an early stage thus precluding requirement for additional experiments after these screening studies. This can greatly

reduce the number of animals required in a programme of work. In addition, scientific knowledge gained in one programme of work will often be applied to future experiments in order to reduce animal numbers and/or reduce pain and stress to those animals used in the subsequent work, or to target investigations to a particular organ or tissue during toxicity testing or clinical trials.

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

The project will provide high quality, peer reviewed data for regulatory authorities, that will allow governments to decide whether the materials tested are safe for the public to take (pharmaceuticals) . It will also enable drug developers to make decisions about their potential new drugs, in terms of their metabolic profiles, and enable them to decide whether or not to continue developing them, or stopping work on them.

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

The primary benefit of work carried out under this licence will be to allow regulatory authorities (who are totally independent from the commercial interests behind every marketing application) to come to informed decisions, based upon data generated in these studies, regarding the risks and/or benefits when humans are exposed to medicinal products.

Achievement of the objectives of this Licence will enable medicinal products to progress into clinical testing and onwards to marketing authorisation and ultimately improving the health and welfare of humans. Without these pre-clinical studies, progression of new medicines to early human studies and on to patients/marketing could not occur.

Sponsors will benefit from work undertaken within this project licence, by obtaining data which allows them to make decisions on the development of the drugs and to support regulatory filings.

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

Where confidentiality permits, data, study design and best practice will be openly shared at conferences, workshops, webinars, blogs and publications.

As 3R’s benefits are also realised under this project licence, these will be shared more widely with other establishments.

Species and numbers of animals expected to be used

- Beagles: 600
- Cynomolgus macaques: 500
- Rats: 16500
- Mice: 16500
- Guinea pigs: 60
- Rabbits: 300
- Minipigs: 500
- Goats: 20
- Other birds: No answer provided
- : 150

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 majority of animals used during the course of this licence will be rodents (mice and rats). Adult animals will be used in all protocols; additionally neonate and juvenile rats may be used in specific exploratory studies. Scientific opinion, including that of the regulatory agencies (who are independent of governments), indicates the use of one rodent and one non rodent species for many of the metabolism and pharmacokinetic studies that are required, to allow drugs to be properly assessed for safety and for potential testing in humans.

The most appropriate non-rodent species (minipig, dog or non-human primate (NHP)) will be selected with reference to studies performed in rodents, evaluating cross-species metabolism and toxicity studies in test tubes (similarities in effects between rodents and minipigs/dogs/NHP), and pilot pharmacokinetic and safety assessment studies. The species selected based upon this information is the one which is predicted to be most similar with human in terms of sensitivity, pharmacokinetics and metabolism. Where scientifically justified the minipig will be used in preference to dog or NHP.

The most widely used and best understood second species for metabolism studies is the dog. When considering veterinary products, the drug may be specifically aimed at dogs, in which case it is obligatory to study the target species. Dogs are only used where the purpose of the programme of work can only be achieved by their use.

Studies involving non-human primates (NHP) are only initiated where no scientific or feasible non- human primate alternative exists. Primates will only be used in the testing of pharmaceuticals for use in life threatening or debilitating clinical conditions in humans. It should be noted that in life and test tube testing will sometimes indicate that primates are the only practical animal model that can be used in metabolic studies to give an indication of what may be expected in man, in order to fulfil the requirements of international regulatory agencies for the purpose of investigating life-threatening or debilitating clinical conditions in humans.

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

Typically, on this project, animals are dosed over a period of time with test materials, and sampled (e.g. blood or urine) before having tissues taken after they have been humanely killed. Most studies would last a matter of days (much less than a month) although some, occasionally may last for longer than that.

Most studies will involve animals being dosed with a test material, and a series of sequential samples will be taken. These will usually be bodily fluids such as blood (generating plasma or serum) as well as urine/faeces and even expelled air. Dosing of animals is commonly done orally using a flexible tube, or by injection using a syringe and needle, maybe directly into a vein, or into a muscle into the arm or leg, or just under the skin.

Blood samples are usually taken from easily accessible veins in the neck, leg or the tail. We are limited to how much blood we can take at once or over a month. If we need a large blood sample, we would do this when the animal is anaesthetised and we would not let them recover consciousness.

Some animals will have to be confined for periods in special cages (metabolism cages) to collect urine/faeces/air. They will usually have access to food and water, and adapt to these new surroundings well. To dose and bleed animals we often have to restrain them for their own safety for short periods, but this won't harm them.

In some studies, we also have to surgically prepare animals for testing. These surgeries are needed to get specific samples (e.g. bile) or to implant a device that supplies drugs constantly. We only surgically prepare animals when there is no other option to dose or collect a specific sample.

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

When dosing an animal by injection or taking blood, the amount of pain an animal feels is similar to what a patient would feel having an injection done by a doctor. If we have to repeatedly inject animals using a needle and syringe, we would choose different sites to do this where possible. If we can take blood samples when an animal is deeply unconscious then we do. If we need to take repeated blood samples or need to dose repeatedly then we try and use different sites. Of course everyone who performs these procedures are trained to a high standard.

Animals undergoing surgery receive the same sort of care as a patient would in hospital. We discuss their pain relief and use of antibiotics with a vet before we start. We administer drugs as necessary and give them plenty of time to recover from surgery before we use them in experiments.

We often need to take a urine/fecal sample for analysis, so we would then put an animal into a special metabolism cage which is smaller than their normal cage. The animal can still move around however, and we'd normally introduce an animal to this cage to acclimatise them to it. Virtually every animal will get used to their new cage within about 15 minutes and are fine.

Dosing with pharmaceuticals may cause adverse effects in some studies, although this is rare. We do observe our animals at least twice a day, and the people who do this know the signs when an animal is ill. If an animal is ill, we would check it more frequently, and get more senior staff involved in its care for advice, including vets. We also help sick animals out by giving more bedding, more heat and special food to make them more comfortable.

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)?

On the last project, about 52% of animals were classified as having displayed moderate severity. This is because legally, all surgical procedures carried out on an animal must be classified this way, and on occasions, there were prolonged periods of dosing and

sampling required to get the information we needed. The rest of the animals were classified as having displayed mild severity.

It's impossible to predict the proportion of severities expected on a service licence, as this will be dependent on what study types we are asked to perform.

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 01 April 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?

Metabolism is a very complicated process, and although there are metabolic tests that can be performed in-vitro (without the use of animals), there are no adequate models to replace the whole animal experimental model, as the complexity of the human body cannot be fully replicated in a test tube.

In the case of new pharmaceuticals, the regulatory authorities are obliged to protect human volunteers for clinical trials by requiring use of proven test systems and accepting use of animals until an in-vitro alternative has been demonstrated to be reliable and reproducible. This includes the study of absorption, distribution, metabolism and excretion of these substances. Information on distribution and elimination is necessary in all cases where such data are indispensable to determine the dosage for humans.

Investigations are continually evolving into pharmacokinetic modelling from in-vitro data, thereby allowing better prediction of human responses and reducing the number of animals needed to generate the required data, but currently these remain limited in their reliability to go beyond indicating trends, due to the nature of complex metabolic processes in-vivo.

In minipigs, investigatory work is currently underway to provide better characterisation, which may allow the replacement of dogs and possibly non-human primates by this species. Concurrently with this, alternative bleeding techniques (ear or saphenous vein sampling) continue to be refined & developed that will provide a less stressful method of sampling this matrix from this species.

In many cases the protocols listed in this Project will be used later in the life cycle of a test substance and in many cases, in-vitro tests will have been conducted previously (often by

the Sponsors) to ensure the drugs do what they are meant to, before we expose them to testing in animals.

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

There are some studies that can be carried out in-vitro that can be used to support ADME work including tests that assess metabolism and absorption of substances, and how well they bind to key proteins in the blood. Predictive software can also be used.

Why were they not suitable?

None of these tests can yet model the complex and integrated mechanisms governing the ADME of pharmaceuticals fully and hence, animal testing is still a requirement. Work is on-going to refine the prediction of human pharmacokinetics from in-vitro data, although this is not currently at a stage where it can replace the animal models.

A retrospective assessment of replacement will be due by 01 April 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 used on this project is estimated from those used under previous projects and after consideration of regulatory trends, and in review of future services being developed and offered for the screening and selection of new molecules for regulatory development studies.

Studies will be designed under this licence such that the minimum number of animals will be used in order to obtain the maximum information, whilst the scientific objectives of each study are met, in accordance with regulatory requirements and agreed standard practices.

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

We have a wealth of experience and knowledge regarding regulatory requirements and the industry standards used, hence we are in an ideal situation to influence study designs when discussing study requirements, and will always consider ways of reducing animal numbers. We also have available professionally trained statisticians to help design studies, especially where the test compound is a biological and use of statistics can provide a guide as to the best number of animals required to determine if a novel new compound is a statistically acceptable biosimilar to an original molecule.

Standard study protocols are reviewed by the AWERB against known guidelines and the Company's ethical compliance policies.

In order to fulfil the requirements of the regulatory agencies, animal group sizes for metabolism studies are variable, depending on the test substance being used, the specific type of study being undertaken and the strain of animals being used. For instance, for most studies involving pharmaceuticals, group sizes will not generally exceed 3, unless a sound scientific rationale is provided.

Furthermore, reduction in animal use continues in study designs where a number of candidate compounds (each in trace amounts) may be co-administered in a single (cassette) dose with consideration to avoid drug-drug interactions. Also, in the case of certain screening studies, colonies of animals (dogs and primates for instance) may be used to allow comparative data generation. These approaches minimise the total number of animals required for pharmacokinetic studies.
Notwithstanding the above, studies will be subject to ethical review and approval by the Project Licence Holder.

Alternative blood sampling techniques (eg saphenous vein sampling) have allowed a reduction in the total number of animals required to generate the necessary data in minipigs in particular. Also, increased sensitivity of analytical techniques has allowed us to investigate a number of procedures that have allowed micro-sampling of blood in several species. These changes allow a more comprehensive profile to be generated from single animals (hence reducing the overall number of animals used), or by allowing a cross-over design to be used on smaller animals (typically rodents).

The re-use of animals, under carefully legally controlled conditions, under the supervision of a vet, also means the overall number of animals we use is reduced.

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 where a new substance is being tested in animals for the first time, we would often test that in a small group of animals (usually 3-5) to give us confidence that the dose levels we chose are safe, and the substance affects the system its designed to, without making an animal ill. These are called pilot studies.

We will try and get as many outputs as we can from a single animal where possible, without adversely affecting its welfare. So if we need to get a blood sample, or if we need to find the levels of a substance in say urine, for example, we will often do that in the same animal, rather than use separate ones, when possible.

A retrospective assessment of reduction will be due by 01 April 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 ADME study designs used are regulatory driven and we have been submitting data and reports to all of the major regulators for more than 30 years. Scientific opinion, including that of the regulatory agencies, indicates the use of one rodent (e.g. mouse or rat) and one non-rodent species (pig/minipig, dog or primate) for many of the metabolism studies that are required. Options are often limited for non- rodent species due to supply constraints, need for species with achievable husbandry requirements and need for background knowledge of the metabolic performance characteristics of the animal. The most widely used second species for metabolism studies is the dog (although the minipig will be used in preference where relevant to achieve the study aims). When considering veterinary products, the drug may be specifically aimed at dogs, in which case it is obligatory to study the target species.

In some studies, a low dose of radioactivity is used (incorporated into the test material) as a marker to allow us to track the distribution of drug in the animal. This is only used on specific short term studies (mainly in rodents), and the radioactivity levels are designed to be safe to both animals and humans, and to cause no harm on their own.

Other species (hamsters, rabbits, guinea pigs, goats and chickens) may be periodically used under this licence, where their use is specifically indicated (for metabolic or pharmacokinetic purposes, or that they are a continuation of previous work already carried out in that species).

In order to minimise animal suffering, all dose levels used under this licence will be expected to cause only mild to moderate effects on animals. Trained animal technicians observe all animals (after surgery and/or post-treatment) at least twice daily and at pre-set post-dosing intervals. Senior technicians and vets are available to assist the Personal Licence holder in documenting reactions to treatment and deciding when and how to intervene to prevent further suffering. In the case of surgically prepared animals, these will also be observed at least once a day during the post-surgical period.

Dog and primate metabolism cages have been modified to allow dual or triple housing (where appropriate), thereby introducing companion animals within the experimental space. Previously animals were housed singly, and by having companion animals it reduces stress for all of the animals

Refinements for blood sampling include the development of a non-restraint dependent method of bleeding in rats (via a peripheral vein). The increased sensitivity of analytical techniques has led to micro sampling of blood (in several species) has reduced the volume of blood required to be sampled from animals and allowed better data to be generated.

We have an ongoing programme that investigates refinements involving husbandry and enrichment for all species.

Animal welfare is of utmost importance and Good Surgical Practice will be observed for any animal undergoing surgical procedures. Surgery will be conducted using aseptic techniques (to prevent infection) which meet at least the standards set out in the Home Office Minimum Standards for Aseptic Surgery. Before we start surgery, we agree with a Vet what pain killers or antibiotics the animals need both before and after the surgery. When recovering from surgery, we give the animals extra heat and monitor them closely until they start behaving normally again. We then check them at least twice daily before they go on study.

In some tests we use animals that are genetically altered, to mimic metabolic conditions seen in humans we are investigating. Sometimes we alter the existing conditions of animals by introducing a drug, or changing their diet. This doesn't cause harm on its own, but the consequences of these may cause a mild degree of suffering to the animals.

Periodically, investigations may be undertaken to develop additional refinements for animal welfare or sample collection. These will only be introduced following approval by the AWERB, and following conduct of a validation study if appropriate.

All protocols in this licence fall within the moderate severity limit.

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

Rodents will be used in most of the studies conducted under this licence. Rodents are considered to be the species with a similar enough brain/nervous system and physiology that will allow us to achieve the study aims and are considered suitable for the predicting what's likely to happen in humans.

For other species, there would need to be specific scientific justification provided for them to be used. This may be, for example, that other studies investigating a particular test substance have already been carried out in that species, or there is a particular metabolic pathway that is only available in that species, or say that is the target species for the actual test substance.

The use of dogs and primates will be driven by the need to fulfil regulatory requirements and to provide data to support clinical trials in humans. Primates will only be used where no other large animal species is suitable, and for studies to support clinical trials. Where scientifically justified the minipig will be used in preference to dog or primate, however, there are limitations in the metabolic characterisation of this model. For example the liver enzymes and transporter mechanisms for xenobiotics are yet to be established.

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

Many of the procedures used in this licence are standardised, well defined and already well refined over many years. We will continue to assess any future possible refinements over the duration of this licence.

Where animals do show adverse clinical signs after dosing or surgery, we will increase the frequency and length of observations, and provide supplementary interventions (like extra bedding/food/heat) where needed, until the signs resolve.

Similarly, if after discussing with a vet and senior technician, we decide an animal is not recovering from procedures, and there is no prospect of them doing so in the near future, we will humanely kill them to prevent further suffering.

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

For any surgical interventions, then the LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery (2017) will be followed.

For blood sampling and dosing then the following guidelines/literature will be followed:

First report of the BVA/FRAME/RSPCA/UFAW joint working group on refinement, Laboratory Animals, 27, 1-22 (1993).

Diehl et al (2001).A Good Practice Guide to the Administration of Substances and Removal of Blood, Including Routes and Volumes, Journal of Applied Toxicology, 21, 15-23.

Regulatory guidelines
Testing of pharmaceuticals for human health are performed in order to generate PK and ADME data to satisfy non-clinical requirements in drug development - ICH M3(R2) Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorisation for Pharmaceuticals (ICH, 2010); US EPA: Health Effects Guidelines, OPPTS 870.7485, (August 1998); FDA Guidance for Industry on the Safety Testing of Drug Metabolites (MIST), March 2020

Testing pharmaceuticals for animal health are performed under the guidance of EU Directive 2001/82/EC and subsequent amendments.

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

This will be achieved by regular discussions with our Named Information Officer, colleagues in Animals Technology, and by attending appropriate training courses and conferences, or getting feedback from such events.

A retrospective assessment of refinement will be due by 01 April 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?

40. Toxicity in macaques by inhalation administration

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

Regulatory, Pharmaceutical, Primate, Inhalation, Toxicology

Animal types	Life stages
Cynomolgus macaques	adult, juvenile

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 purpose of this project is to provide safety data on pharmaceuticals for use in human clinical trials, using non-human primates (small monkeys called Cynomolgus macaques). These pharmaceuticals will usually be delivered by inhalation and simulate administration by an inhaler, mask or ventilator in humans.

A retrospective assessment of these aims will be due by 03 May 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?

Regulatory agencies around the World (who approve the use of pharmaceuticals for public use) need to make sure that pharmaceuticals that are administered to human are safe before dosing them in clinical trials. The data from studies in this project will allow regulators to make those decisions.

The nature of some of the pharmaceuticals we test mean that sometimes we can only use primates instead of other more commonly used species like rats and mice. And in fact, the law will not allow to use primates unless we can prove there is no other suitable species we can use to test these pharmaceuticals.

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

The overall benefit of this project is that it supports the development of safe, new medicines to improve the health and quality of life of human patients by generating high quality data that is acceptable to regulatory authorities and enables internal decision making for others.

Achievement of the objectives of this licence will enable safe development candidates to progress and will also help to remove unsuitable candidates from the development pipeline at an early stage, thus saving animals and resources.

Study reports will be included in regulatory submissions to allow regulatory authorities to make judgements on whether to permit clinical studies or to licence a drug. Guidance issued by the Home Office in 2005 and 2014 recognises that the justification for animal-based regulatory toxicology and safety testing is the need for regulatory authorities to have sufficient information to assess the risks to which humans are exposed to by new drugs.

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

Patients will benefit from these studies as this work will contribute to the development of new drugs that help alleviate human conditions. These new drugs may work better in the clinic, relieve or cure diseases and have better side effect profiles. We may, by our work, also contribute to better knowledge and understanding of these types of drugs, and that knowledge may be used to develop further new drugs.

In addition, the models on this project may be used to assess the safety or other in life properties of a new drug, and find a dose that causes no adverse effect. This is important when planning future trials in humans, to make sure any starting dose in a clinical trial is safe for the patients taking it.

Others will also benefit, as the data we generate will allow them to progress their new drugs into clinical trial, or otherwise if they are found to have adverse side effects.

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

The work will be shared with others who will use it to determine their future strategy, or for submission in documents required by regulatory authorities. Whilst we have no direct control over what happens to the data after we have shared it, we trust from information given to us that it is used for regulatory purposes or to support regulatory purposes (e.g. to support drugs progressing to clinical trials, or to show that a certain chemical is safe for human exposure). Previously however, we have collaborated with others and shared data we have produced in the form of Scientific publications that are in the public domain.

Species and numbers of animals expected to be used

- Cynomolgus macaques: 1100

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 small non-human primates on these studies, both adult and juvenile. We only use non- human primates when other species (like rats, mice dogs and/or pigs) are unsuitable to get the answers we need from the studies. Most studies would be carried out in adult animals, juveniles would only be used for specific studies for pharmaceuticals targeted at juveniles.

This is often because for the type of pharmaceuticals we are testing (for example 'biologics', peptides or antibodies) we can only see any toxic effects if we use primates, maybe because the biological target of the drug is only present in a primate, or maybe because the requirements of the study mean we can only use primates to get the results we need that will satisfy global regulatory bodies.

We are not allowed to use primates by law unless there is no other animal species that we can use that will give us the results we need to satisfy the regulatory authorities.

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

Animals usually receive repeat doses of drug by inhalation and routinely undergo, clinical observations, body weight measurements, and have blood samples taken for analysis, clinical pathology (including blood analysis and urinalysis), and then are killed humanely and undergo extensive pathological examination. Blood sampling procedures are similar to what you would experience when you have a blood sample taken by a medical professional. In much fewer cases it may be necessary to dose by more than one administration route, like intravenous or even orally.

Animals undergoing surgery receive the same sort of care as a patient would in hospital. We discuss their pain relief and use of antibiotics with a veterinary surgeon before we start. We administer drugs as necessary and give them plenty of time to recover from surgery before we use them in experiments.

These surgical procedures are carried out only for essential purposes.

Infrequently we may include body temperature measurement by rectal thermometer, ophthalmoscopy (checking the structures of the eye), ECG (checking the heartbeat and rhythm is normal), and measuring blood pressure. Although the animals may be restrained whilst doing this, it will be for as short as possible and because it safer for everyone, and no more than for a set period of time per day. The animals are trained using positive reinforcement (treat rewards) to move about the cages for handling/procedures, and to sit in restraint chairs.

Rarely the animals will undergo specific repeat investigations to investigate particular findings related to concerns highlighted in earlier work: Because these investigations are targeted for a particular body system where a concern is suspected, it is considered unlikely that more than one of these investigations would be necessary on the same animal/study (e.g. semen collection, vaginal swabbing).

At the end of the studies, if animals are not humanely killed to examine their internal organs, to check for any damage or irregularities, they may be kept alive for potential re-use, subject to specific legal conditions, and only after being checked that they are in good health by a veterinary surgeon.

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

When dosing an animal by injection or taking blood, the amount of pain an animal feels is similar to what a patient would feel having an injection done by a doctor. If we have to repeatedly inject animals using a needle and syringe, we would choose different sites to do this where possible. If we can take blood samples when an animal is deeply unconscious then we do. If we need to take repeated blood samples or need to dose repeatedly then we try and use different sites. Of course everyone who performs these procedures are trained to a high standard.

Routinely we take a urine sample for analysis, so we would then put an animal into a special cage which is smaller than their normal cage. The animal can still move around however, and we'd normally introduce an animal to this cage to acclimatise them to it. Virtually every animal will get used to their new cage within about 15 minutes and are fine.

Generally, if we have to use any equipment to help us get the results we need, we acclimatise our animals to it so they get used to it, and it doesn't upset them too much when we start dosing them. So we carefully introduce them to things like facemasks and restraint gradually, for short periods at first, and usually they accept it after a while. And if they don't acclimatise, we take them off the studies, to stop causing any harm.

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)?

On the last project, about 80% of animals were classified as having displayed moderate severity. This is because these studies can last between a few weeks to up to a year, and although the individual procedures are usually mild in nature on their own, the cumulative effects make them moderate overall.

It's impossible to predict the proportion of severities expected on a service licence like this, as this will be dependent on what study types we are asked to perform.

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

- Killed
- Kept alive

A retrospective assessment of these predicted harms will be due by 03 May 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?

All of these studies are performed for regulatory agencies, or to support regulatory submissions. The regulations we follow mandate us to use animals, and won't allow decisions to be made on how safe pharmaceuticals to be used in humans in a non-animal system.

In addition, the systems we are investigate cannot be fully explored in non-animal systems (e.g. in test tubes) because test tubes can't match the complex physiology found in animals.

In many cases the work performed in this Project will be used later in the life cycle of a new drug and in all cases, tests in test tubes will have been conducted previously (often by others) to ensure the drugs do what they are meant to, and are safe, before we expose them to testing in animals.

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

There are no other non-animal alternatives for the work being undertaken on this project. The regulations we are following will not allow safety decisions to be made on non-animal systems alone.

Why were they not suitable?

Although there are test tube tests that can model some parts of how drugs get into our bodies, and how our body deals with them, and can identify undesirable effects, for example, there is no series of test tube tests that brings all these complex happenings together, like we see in animals and humans.

That's why we need to test the new drugs in animals, as they have similar physiology and processes as humans, and that testing gives us a good idea what may happen if they were ever tested in, or exposed to humans.

A retrospective assessment of replacement will be due by 03 May 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 we have used are based on figures of previous usage from previous projects, or a projection thereof (based on estimated incidence) based on requests received from others in the past. It is, however, impossible to accurately predict the number of studies that may be performed, in the circumstances.

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

Studies are designed to provide maximal data and statistical power (where appropriate) from the minimum number of animals considering that it is better to increase the number of animals used to achieve the objective that to use too few animals and risk having to repeat the study. For regulatory studies, guidelines require the number of groups and animals per group to be adequate to clearly demonstrate the presence or absence of an effect of the test substance; core study designs are based on international guidelines where these exist. Otherwise reference is made to standard study designs with input from the Department of Statistics, where appropriate, to identify the optimum number balancing the need to achieve study objectives while avoiding excessive animal use. These internal designs are reviewed and updated in line with changing external guidelines and internal refinements that either minimise numbers or reduce severity.

Scientists within the department are actively involved with cross-industry initiatives aimed at sharing best practice and the application of the 3Rs in toxicology and this feeds into review of study numbers and practices (e.g. Sparrow et al, 2011, Opportunities to minimise animal use in regulatory toxicology; a cross-company review; poster presentation at Society of Toxicology).

Whenever possible, common control groups will be used in order to minimise the numbers of groups used. In addition, the regulatory test guidelines used for testing, stipulate group sizes.

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

Where possible, if the primary objective of the study will not be compromised and the animals' overall experience will not exceed the severity limit, additional endpoints may be added to assess pharmacokinetics , immunology, safety pharmacology or efficacy (particularly for biologically derived pharmaceuticals) so that a separate study will not be needed. It is anticipated that the total number of animals for each compound's development programme will be decreased overall by the use of additional investigations during the toxicology programme.

A retrospective assessment of reduction will be due by 03 May 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.

This project will use both adult and juvenile non-human primates (macaques). We only use non-human primates when other species (rats, mice and other large animals like dogs and/or pigs) are unsuitable for scientific reasons.

The models we use are the least invasive procedures for the least amount of time we need to do them to get the information we want. they are carried out using standard and recognised techniques by fully trained staff. We also have veterinary help on hand for advice and on the rare occasions we have to anaesthetise the animals.

For situations involving restraint procedures (e.g. in a chair or in a metabolism cage) the animals are habituated to this equipment starting with short periods, then building up. Most animals habituate fine to this equipment, but if they don't (rare) we remove them from the study.

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

Primates are only used when no other species is suitable to get the information we need-in fact we have to prove that the primate is the only species that will give us the answer we need (instead of rodents or dogs or pigs) that will translate to the effect we would see in man.

Most of these studies require repeat dosing for days, weeks or months, to assess potential adverse effects in man, so it is not practical to perform them under terminal anaesthesia.

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

Animal welfare is of utmost importance and Good Surgical Practice will be observed for any animal undergoing surgical procedures. Surgery will be conducted using aseptic techniques (to prevent infection) which meet at least the standards set out in the Home Office Minimum Standards for Aseptic Surgery. Before we start surgery, we agree with a Veterinary surgeon what pain killers or antibiotics the animals need both before and after the surgery. When recovering from surgery, we give the animals extra heat and monitor them closely until they start behaving normally again. We then check them at least twice daily before they go on study.

We have introduced a newly designed restraint chair for animals which provides sufficient restraint but allows more natural movement, and comfort. We use this type of restraint during inhalation dosing, and it is limited on a daily basis.

During dosing and restraint, animals are constantly and closely watched for signs of distress. We also monitor carbon dioxide levels within the inhalation system throughout, so they do not reach dangerous levels.

All procedures are subject to ongoing assessment and technique improvement, and we participate in cross-company working parties on best practice. Animals are regularly reviewed for general health and veterinary staff are on call at all times to assess and relieve any adverse events.to improve the animals experience include but are not limited to group housing, environmental enrichment, including novel toys and foods, human interaction, acclimatisation and training to procedures, to move around the cage and to leave the cage voluntarily as required, forage opportunity and calming measures such as stroking/gentle talking are used to help animals have a better experience of restraint.

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

Regulatory guidelines

Testing of pharmaceuticals for human health are performed in order to generate PK and ADME data to satisfy non-clinical requirements in drug development - ICH M3(R2) Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorisation for Pharmaceuticals (ICH, 2010).

ICH S6 (R1) (Preclinical Safety Evaluation of Biotechnology-derived Pharmaceuticals), which gives guidance on the type and design of studies for biological compounds.

EMEA/CHMP/BMWP/42832/2005 Rev 1 Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues.

For blood sampling and dosing then the following guidelines/literature will be followed:

First report of the BVA/FRAME/RSPCA/UFAW joint working group on refinement, Laboratory Animals, 27, 1-22 (1993).

A Good Practice Guide to the Administration of Substances and Removal of Blood, Including Routes and Volumes, Journal of Applied Toxicology, 21, 15-23 (2001).

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

This will be achieved by regular discussions with our Named Information Officer, colleagues in Animals Technology, and by attending appropriate training courses and conferences, or getting feedback from such events.

A retrospective assessment of refinement will be due by 03 May 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?

41. Assessment and mitigation of chemical toxicity

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

Chemical Toxicity, Toxins, Medical Treatments, Diagnostics, Hazard Management

Animal types	Life stages
Rats	adult, neonate
Mice	adult
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?

The aim of this project is to generate new information about the toxicity of chemicals, pharmaceuticals and other potentially toxic materials to enable effective management of their hazards and to identify and assess ways to mitigate their toxicity.

A retrospective assessment of these aims will be due by 08 May 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 convergence of chemistry, medicine and biology has produced, and continues to identify, many new and potentially useful pharmaceutical and other chemical compounds, some of which could exhibit harmful and toxic effects in humans. Collectively these are also referred to as material(s) of interest in this licence and Non-Technical Summary. Identifying those materials that are toxic and understanding the nature and effects of their toxicity, through integrating in silico and in vitro methodologies with the ability to undertake experimental studies in animals, enables prediction of the biological effects in humans and allows assessment of mitigation strategies that could be used to prevent, reduce and manage the impact of exposure to any toxic materials. The ability to prevent, manage or treat toxicity will ultimately save lives.

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

1. There will be an increased understanding of the adverse effects and hazards posed by materials of interest.
2. Assessments will be made of potential therapeutics and other interventions such as decontamination, to prevent or treat the adverse effects of the materials of interest.
3. Potential biomarkers of exposure to new or existing materials of interest will be investigated and diagnostic approaches assessed for their potential benefit in mitigating the adverse effects of exposure to such materials.

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

New knowledge about the hazard posed by the materials of interest will provide benefits to those that may be exposed, including the civilian population, first responders and medical professionals responding to, or treating, the effects of exposure to, or overdose from, these materials. In the short term the outputs of this work will provide understanding of the potential hazards, allowing effective planning for avoidance of the hazard, and in the longer term these studies will provide benefit by driving further research and development to prevent or mitigate the adverse, hazardous, effects of exposure.

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

The outputs of this work will be maximised by continually maintaining focus on priority materials of interest and by ensuring timely exploitation of the new knowledge generated.

This is facilitated by the organisation's approach to project management and close working relationship with those commissioning the research.

Where possible, the outputs of this work will be published at conferences and in peer-reviewed journals. Outputs that are not published are recorded in the organisation's information management system as technical reports and where appropriate these are shared with collaborators to ensure that this knowledge is appropriately and effectively used to advance the aim of this work.

Where the long term output of this work is a variation to the currently licensed indications for use of a therapy (for example to add that the therapy be used in a new way to treat exposure to a material of interest) this will be disseminated via medicinal product information and will be accessible to healthcare professionals.

Species and numbers of animals expected to be used

- Mice: 406
- Rats: 1982
- Guinea pigs: 168

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.

Mammalian species (mice, rats and guinea-pigs) will be used for experiments conducted under this licence as the organisation has pre-existing data that informs experimental approaches and a good understanding of how such experiments can inform and predict responses in humans with respect to physiology, pharmacology and biochemistry and toxicology. In addition, these species have been, and continue to be, commonly used for toxicology assessments enabling comparisons to open literature and proprietary historic data sets to place new adverse effects data into context and to trigger assessment of treatment and mitigation strategies if necessary. Generally, adult animals will be used in these experiments, to reflect the human population most likely to be exposed to the materials of interest. Other developmental ages, for example neonatal animals, may be used where the model(s) have previously shown benefit in predicting therapeutic effects.

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

Most animals will receive a single administration of the material of interest and will be monitored to observe and measure the biological impact that the administration causes. In some cases, an intervention may be applied before or after the administration of the material of interest in order to assess a treatment or mitigation strategy.

In some cases, administration may require that the animal is restrained temporarily with the administration being given by an injection, by inhalation, or by application to the skin. For some experiments animals will be surgically prepared with a device that enables access to blood vessels via a sampling / injection port to facilitate administration of compounds and/or blood sampling whilst respiratory monitoring is taking place. Following exposure, further injection of a treatment or handling and restraint to decontaminate the exposure site may be necessary.

The response of the animal will be assessed by scoring of observed signs, measurement of respiration, behavioural assessment or survival outcome, or with combinations of these. Where scientifically appropriate, animals may be terminally anaesthetised for the exposure. In some cases the materials of interest may cause analgesia, sedation and anaesthesia and prior, additional terminal anaesthesia would not be used in these cases.

Typically, observations would extend for up to 24 h after exposure. For some behavioural assessments, periods of training may be required such that the whole procedure may require up to 14 days with exposure to the material of interest taking place of the final day.

Animals may have blood samples taken to measure the concentrations of materials of interest and medical treatments in the blood.

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

Animals exposed to materials of interest that have sedative or anaesthetic properties are expected to experience effects such as drowsiness, sedation, anaesthesia and respiratory depression. This respiratory depression may result in death, however, the animal will be anaesthetised by the material of interest if this happens. Animals that survive would be expected to make a complete recovery. These effects are expected to last for hours.

Where scientifically appropriate, animals will be terminally anaesthetised for exposure and unaware of the effects of the materials of interest. Where this is not appropriate, exposure to materials will cause life threatening toxic effects such as respiratory paralysis, respiratory depression and/or cardiovascular effects and neurological dysfunction. In the absence of treatment such effect will be short-lived 5-30 min and limited by imposition of experimental and humane endpoints where possible.

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)?

	Predicted % of animals at each severity catagory			
	Non-Recovery	Mild	Moderate	Severe
Rat	9	38	48	5
Mouse	62	0	8	31
Guinea pig	16	0	59	38

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

Killed

A retrospective assessment of these predicted harms will be due by 08 May 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 important to understand how materials of interest can cause injury so that appropriate controls and treatments can be identified where necessary. Exposure to the materials of interest can cause a complex range of effects with respiratory, cardiovascular and neurological changes that can be debilitating and, in some cases, life threatening. Effects may be acute or long-term. These effects result from disruption of the complex mechanisms and systems that maintain normal bodily functions and these complexities cannot currently be recreated in vitro. Therefore, such data cannot currently be gained without the use of animals to model the whole body response to the harmful materials.

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

Computer modelling, cell-based systems, organ-on–a-chip and simple model organisms, have all been considered as part of this work. All have a place in contributing to our understanding of the biological effects of harmful materials. Integration of these approaches may ultimately provide the ability to predict toxicity, identify treatments and help translate data from animal models to man; but this has not yet happened to such an extent that animal studies can be replaced. Aspects of this work already use these

approaches, where appropriate, to reduce the requirement for animal experiments and to refine in vivo experimental approaches.

Why were they not suitable?

Exposure to harmful materials results in disruption of the complex mechanisms and systems that maintain normal bodily functions. Multiple and complex signaling pathways and biochemical mechanisms are involved. Whilst computer modelling, cell-based systems, organ-on–a-chip and simple model organisms can model separate aspects of a response providing an understanding of the mechanism of harm and identification of potential treatments, these models are not yet integrated such that they can provide a whole body response that is predictive of effects in man. Moreover, although the use of simple model organisms such as worms, fish and flies, may provide some aspects of system integration, their evolutionary distance from humans means that they cannot currently replace the need for experiments in mammalian species.

A retrospective assessment of replacement will be due by 08 May 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?

Numbers of animals have been estimated based on previous experience in conducting similar studies under previous project licences. This information has been combined with the current and expected demand for this work, based on that which is currently planned and funded, together with a contingency of approximately 30% (sufficient to enable an additional complete study for each activity). This allows for flexibility in experimental plans, if this is necessary, as new data are generated by research.

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

Statistical advice on experimental design is sought at the planning phase of each experiment. This ensures the most appropriate experimental approach is taken for example in assessment of dose- response relationships, to ensure the minimum number of animals are used and that every animal contributes effectively to determination of the

relationship. Where appropriate, shared control groups are planned into studies to reduce animal numbers and maintain any duplication to an absolute minimum. Where possible, studies that rely on data from a pilot experiments will be designed such that some or all of the pilot animals can contribute to the main study groups.

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 experiments are integrated with in vitro approaches which enable down-selection to representative materials of interest. These approaches include cell-based systems, functional tissue preparations and/or simple model organisms. These studies reduce the number of experiments needed in animals by focusing effort only on those materials that need to be assessed in animals (i.e. the most potent, active materials).

For dose-response characterisation, pilot (dose ranging) experiments may be conducted in limited numbers of animals to ensure that the correct doses are targeted for subsequent experiments so that all animals contribute valuable data to the analysis. Where appropriate, low/no impact behavioural assessment may be incorporated into these experiments to provide data to help design effective behavioural assessments for the materials of interest in subsequent experiments.

A retrospective assessment of reduction will be due by 08 May 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.

Responses to materials of interest and mitigation strategies will be studied in mice, rats and guinea pigs. In many cases materials of interest have analgesic, sedative and anaesthetic properties such that exposure and biological impact is unlikely to be perceived by the animal. In other cases, effects may be examined under terminal anaesthesia or with suitable non-lethal endpoints indicative of adverse effects in humans. Use of lethal

endpoint will be avoided where possible with effective experimental endpoints selected based on behavioural or physiological effects considered to be relevant to mechanism of action in humans.

Some animals may undergo surgical procedures to allow administration of treatment and / or blood sampling over an extended period of time thus reducing the burden of multiple sampling procedures. These animals will be given analgesics during their recovery from surgery.

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

It is important to assess the adverse effects caused by materials of interest that might be distinct from overt toxicity, sedation or anaesthesia. To assess the biological impact of these materials and predict hazard effects and incapacitation in humans requires an animal model that expresses relevant and complex behaviours that can be observed and measured and that processes biochemistry and physiology similar to that in man. Laboratory rodents (rats and mice) are considered to be the most appropriate species for this purpose.

For some studies under this licence, the use of terminal anaesthesia is proposed, where scientifically appropriate, to reduce pain and suffering that might be otherwise be caused by the material of interest. In some cases, where the material of interest induces analgesic, sedative or anaesthetic effects, terminal anaesthesia would not be appropriate; however the effects of the materials of interest themselves, in inducing such effects will reduce pain and suffering.

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

Animals will be continually monitored following exposure until they have made a full recovery or reached an experimental or humane endpoint. Behavioural monitoring of animals after administration of a material of interest may be used to measure incapacitating effects rather than overtly toxic/lethal effects for certain materials of interest. Behavioural training will aim to use positive reward rather than food deprivation to motivate training and behavioural performance.

Surgical implantation of vascular access ports will facilitate administration of materials and treatments and facilitate blood sampling whilst limiting damage to vessels through repeated access. Analgesics will be given pre- and / or post-surgery. Animals will be monitored as they recover from anaesthesia and will be regularly checked post-surgery to ensure appropriate level of analgesia and monitor the progression of wound recovery.

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

All surgery will be done aseptically following the relevant LASA guidelines.

Available guidelines on the NC3Rs website regarding blood sampling, routes and volumes will be followed. The website will also be followed regarding the administration of substances.

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

Updates on advances in the 3Rs are regularly distributed by the Named Information Officer. The Licence Holder is actively engaged in the establishment's Animal Welfare and Ethical Review Body (AWERB). Any appropriate advances will be discussed with veterinary staff and, where appropriate and compatible with the scientific aims of the project, these advances will be incorporated. The Licence Holder and all staff working under this licence maintain their required annual CPD, attend relevant external scientific meetings and have meetings and teleconferences with international collaborators working within this field.

A retrospective assessment of refinement will be due by 08 May 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?

42. Musculoskeletal pain: mechanisms and treatment

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

Bone, Fractures, Pain behaviours, osteoarthritis, osteoporosis

Animal types	Life stages
Mice	adult, aged, juvenile

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?

Long-term skeletal pain is the most common complication of musculoskeletal disorders. We need to better understand the mechanisms involved in chronic skeletal pain to develop analgesics directed towards more restricted pathways involved in bone pain and this is the aim of this project.

A retrospective assessment of these aims will be due by 05 May 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?

Despite a huge increase in the burden of skeletal pain, there are to date no analgesics that target specifically this pain which impacts on the quality of life of millions of patients worldwide - and can eventually require orthopaedic joint replacement.

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

This research will first increase our scientific knowledge of the mechanisms of pain after osteoarthritis (OA) and fractures. This will lead to research publications, presentation of data at seminars, national and international conferences. We will present our data at targeted conferences which have clinical streams in order to facilitate interactions with clinical colleagues working on skeletal pain. We also hope that answers to our questions may provide indications for the use of new therapies targeting pain in musculoskeletal diseases. This proposal is also ideal for PhD training involving both in vitro and in vivo experiments and a large range of imaging, histological, molecular and pain methodologies.

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

The results from this project will be of interest to a broad range of scientific communities including skeletal biologists, neuroscientists and orthopaedic surgeons. This research is also of major interest for wider audiences concerned by fracture pain, OA pain and joint replacement surgery.

Due to the development of more standardized small rodent models of fracture repair, most research studies now use animal models to assess factors and treatments that affect fracture healing. Because pain is rarely reported in these studies, we hope that our studies investigating pain in these models will raise attention to the scientists performing fracture surgeries on rodents and help them to better choose post-operative analgesics. The development of tools to evaluate spontaneous and evoked behaviours which are indicative of pain in rodents, have rapidly expanded to classify the type of pain and quantify it to improve animal welfare. Those animal studies are transposable to humans and have clinical relevance in patients with musculoskeletal pain. This proposal will also look at treatments of skeletal pain, which has huge implications on animal welfare.

In the longer term, we hope that the results of this research project may be able to guide the selection of drugs that reduce skeletal pain. This may lead to clinical trials examining

the advantage of targeting specific signalling pathways for decreasing skeletal pain and this will benefit animals and millions of patients suffering from musculoskeletal diseases.

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

Important findings will be shared with the scientific community through publications, seminars, webinars and newsletters. We will make sure to publish unsuccessful results too as these are important to the bone research community and avoid repetition of experiments. The applicant is part of an EU training network and outputs from this project will be shared with Europe-wide bone research networks and platforms. This project will benefit from this network though extensive collaborations, share of innovative models, specific topic conferences, training courses and invited speakers as well as contacts with the industry for drug developments. We have used results from previous studies performed using the same models to improve the design of our experiments and inform the development of our new scoresheet. Similarly, findings of those studies will help us to refine them.

Species and numbers of animals expected to be used

- Mice: We estimate that we will use less than 1200 mice in total.

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.

Rodents are appropriate species because their fundamental skeletal biology is very similar to humans. Mouse models in particular have become of increasing interest for skeletal research because their life expectancy is only around 2 years, studying skeletal diseases associated with ageing such as osteoporosis and osteoarthritis is easier as these diseases develop earlier and the effect of treatments can be seen in a shorter time period. Also, a broad spectrum of antibodies and gene-targeted mice are available. We generally use young adult mice which are not growing anymore (12 to 13 weeks) to perform our studies. However, because fractures, osteoporosis and osteoarthritis are higher in the elderly population, in some conditions we may want to age mice until 40 to 60 weeks of age. These ages correspond to adult mature mice which are past development but not yet affected by senescence and do not show adverse effects in relation to ageing. Nevertheless, these adult mature mice show bone loss as it starts from 3 months of age and this is interesting for our project. In most of our studies, animal will be kept for 6 weeks after surgery. No animal will be kept until more than 66 weeks of age.

In this project we will use mainly female mice (more than 70%) as osteoarthritis and osteoporosis occur mainly in females. However, STR/ort mice develop degenerative changes of the knee joints resembling human OA, with the males being more severely

affected than the females, so we will also use male STR/ort mice. We may also want to compare pain behaviors in males and females, so we may use male mice in all our protocols.

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

One typical experiment would use around 40 female mice as our statistical power analysis requires us to use around 10 mice per group. We usually have 4 groups, one sham group, one with surgery (fracture or removal of the ovaries to induce osteoporosis) or submitted to mechanical loading of the joint to induce osteoarthritis, one group with surgery plus treatment and one sham group plus treatment. These treatments could be novel analgesics, drugs that target ionic channels, neurotrophins and bone resorption. They can be injected (from daily to one or two injections in 6 weeks) or administered in the food or by oral gavage. Mice are usually kept for 6 weeks after surgery, the maximum being 12 weeks after surgery. Pain-like behaviors are measured at baseline before surgery and weekly from one week after surgery. They include measurements of spontaneous pain such as nesting, burrowing, locomotion, general activity but also responses to stimuli such as pressure, heat and cold that measure mechanical and thermal sensitivities. In addition, when surgery is performed in one limb but not in the other one, it is possible to measure the weight put on each limb. Blood can be collected every two weeks. Mice are usually euthanised 6 weeks after surgery.

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

All the models described in this project are widely used by ourselves and other bone researchers in the world. All mice in this project will receive appropriate pain killers after surgery and post-operative care. Because this project involves measuring pain after bone loss and fractures, pain -like behaviors in our project will be only measured 7 days after surgery to make sure that mice are receiving adequate pain relief after surgery. In case of removal of ovaries (ovariectomy) and sectioning the sciatic nerve (neurectomy) which are two surgical models that induce the loss of bone, analgesics are given for a minimum of 3 days post-surgery. In case of fractures, our previous data have shown moderate pain after fracture surgery which is maintained during the process of bone repair which lasts several

weeks. We would like to study this chronic pain and assess the effects of new analgesics targeted more specifically at bone pain. Because pain after fracture surgery starts to decrease one week after surgery, analgesics will be given for 7 days after fracture surgery.

The possible adverse effects of surgery models (removal of ovaries, sectioning the nerve and fractures) are pain, wound infection (<1%) and risks of bleeding (1%). Wound infection and risk of bleeding are minimised by careful aseptic surgery. The animal can be expected to be at risk of mild to moderate degrees of spontaneous pain from the time of surgery to the terminal phase of the experiment. Pain will be indicated by reduced appetite and activity, poor demeanour and grimace observation.

There are specific adverse effects associated with fractures. Long lasting moderate pain could be present. Lameness is expected postoperatively but by day 2 animals should be ambulating freely with occasional limping and normal grooming. Weight bearing will be reduced but will increase daily and is expected to return to 70% of preoperative condition by 7 days postoperatively. Pin stability will be checked during surgery checkpoints.

The mechanical loading procedure (usually less than twenty five minutes) may also induce a temporary lameness, typically for no more than 30 minutes after the procedure has finished. Mild or moderate pain may also be recorded as indicated by in-appetence, lethargy, poor demeanour and grimace observation.

Occasionally, aging animals will be used (less than 5%) to mimic the loss of bone with age. Mice lose bone mass at 16-25 months. We wouldn't expect to see any associated harmful phenotype at these ages in our strains. Age-related conditions such as weight loss, lameness and respiratory difficulties will be carefully monitored for in these ageing animals.

Administration of substances by injection should only cause transient discomfort. Animals will be observed during the period immediately post-administration for any unacceptable signs of discomfort or distress. After gavage, animals will be observed for signs of stress and bronchial administration (<1%).

Some of the compounds injected may alter an animal's metabolism but should have no adverse effects on the welfare of the experimental animals. Animals will be observed during the period immediately post-administration for any unacceptable signs of discomfort or distress (such as decreased food and water consumption, rapid breathing, weight loss, hiding, self-mutilation, abnormal posture and appearance).

General anaesthesia: Anaesthetic complications are most uncommon (<1%) and will be minimised by correct dosing of injectable or inhalation anaesthetics, by accurate weighing and by good maintenance of body temperature. Post anaesthetic animals will be monitored to ensure they make a swift recovery and may have additional husbandry measures put in place such as additional warming, wet mash or high calorie diets placed on the cage floor.

The behavioral tests provoke very mild degrees of pain that do not persist after testing.

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)?

Our mouse models are mainly surgical (fractures, removal of ovaries, section of the sciatic nerve) or involve mechanical loading of the bone and joints and have been previously used and validated. All fall in the overall moderate severity level except for fractures that

fall under a severe protocol but this will represent no more than 15% of our mice. Some animals (50%) on moderate protocols may experience moderate suffering that resolves after a few weeks, usually around 2 to 3 weeks. Animals under severe protocol are expected to suffer moderate pain that is maintained for several weeks. We use appropriate protocols and end points to manage pain and infection. To stop infection, sterile surgeries will be performed and we will use antibiotics if necessary. Pain is relieved by pain killers administered before and after surgeries and other procedures.

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

- Killed

A retrospective assessment of these predicted harms will be due by 05 May 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?

Our project investigates the mechanisms of bone and cartilage repair and of skeletal pain and requires the use of several animal models as these complex physiological systems cannot be effectively modelled in vitro. The integrated physiological environment of the living animal is indeed still essential to elucidate the pathophysiological mechanisms of skeletal conditions and of their associated pain. In this project, we will use well-established models of osteoporosis (ovariectomised mice), osteoarthritis (Str/ort mice that develop spontaneous OA, mechanical loading of joints) and fractures (osteotomies and bone defects). To investigate pain in these models, we use a set of behaviours both spontaneous and evoked.

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

Many aspects of our work are however achieved through the use of in vitro cell culture (bone formation and resorption assays, chondrocytes assays, culture of dorsal root ganglia) and ex vivo imaging together with molecular analyses.

We also started clinical studies in human patients to correlate fracture pain with biomarkers measured in serum of these patients. These markers are also quantified in mouse serum after osteotomy.

Why were they not suitable?

There are suitable as these in vitro techniques allow identification of the most likely treatments to be validated in vivo and this replacement reduces the number of animals used. The clinical studies allow us to translate some of our findings in animal models. The integrated physiological environment of the living animal is however still essential to study skeletal pain. This is because pain involves both the peripheral and central nervous systems and this is impossible to model it in vitro. There are no molecular readouts of pain that are appropriate to assess the effects of novel analgesics on pain.

A retrospective assessment of replacement will be due by 05 May 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 often design our protocols in such a way that we have 4 groups, a Sham group, a disease group (surgery or application of mechanical loading) a sham group treated with a drug and a disease group treated with the same drug. Our experiments therefore often involve 40 mice as we usually have groups of 10 mice.

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

We always aim to reduce the numbers of animals we use. We adhere to PREPARE and ARRIVE guidelines to design our experiments and report our data. In this project, most of our experimental models have been previously validated and used and do not require pilot studies. However, pilot studies may be necessary to estimate the effects of drugs.

Fractures and Sham surgery are performed on one leg, allowing the use of the contralateral leg as a control when possible. This is also the case for mice submitted to mechanical loading of the bone which is performed on one leg only. Sham surgery includes the same surgical procedures as the fracture group except that there is no fixator placed and no generation of fractures. This allows to control for post-surgical pain unrelated to fractures. This is also the case when evaluating pain after removal of the ovaries and neurectomy.

In most of our studies, our main end point is the assessment of pain and the use of Sham animals is essential to differentiate between the pain induced by surgery and skeletal pain. However, in some experiments we will only assess the bone loss induced by hormonal and mechanical changes, nerve section and bone diseases. in these cases, non-operated animals can be used to minimize the number of animals used in sham surgery groups. In addition, if we repeat long series of similar experiments, we may be able to use information from historical controls from previous experiments to reduce our number of Sham controls.

We also aim to refine our power calculations after a series of similar experiments to make sure that our number of Sham mice is reduced to its minimum.

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

For completely new procedures, a pilot group (including only 4-5 rodents) will be used to determine the outcomes before generating all the data. All tissues that are potentially interesting for our research are collected from one single experiment in case we need them in the future.

A retrospective assessment of reduction will be due by 05 May 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 intend to use only mice and predominantly females as all our skeletal diseases models and our joint loading models were developed in this species.

The mouse is an appropriate species because its fundamental bone biology is very similar to humans, and, as mentioned previously, holds many advantages in terms of pathology, timescale for disease progression, space requirements and biological tools available. All the models described in this project are widely used by ourselves and other bone researchers in the world and have proven to be very useful for the advancement of clinical management and the prevention of musculoskeletal diseases.

Our research aims at investigating pain associated with these musculoskeletal diseases and mouse behavioural models have been important tools to increase our understanding of the mechanisms of pain as well as identifying novel analgesics. Despite intending to assess pain behaviours during the progression of skeletal diseases and fractures, our strategy aims to minimise the animal suffering and this would be also further lessened by the use of appropriate analgesia protocols, where possible, for example after surgeries. However, we will not be able to use analgesics during all the duration of our studies as we want to assess pain-associated with bone loss and bone repair. Novel analgesics will however be tested in our models. Our models of skeletal diseases and fractures are mainly surgical and have been previously used and validated. These fall in the overall moderate severity level, except for fractures where long lasting moderate pain can occur. Our models were designed not to produce excessive trauma or suffering. For example, our model of osteoarthritis (OA) is non-invasive compared to other models of OA which are surgical. Our fracture model is the most invasive but we will minimise its use.

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

Clinical skeletal pain is a perception that is influenced by psychological and experiential factors but, despite this, considerable progress has been made in modelling and quantifying nociception in animals, particularly in mice. Unlike the clinical setting where patients can verbalise their pain level, preclinical studies in animals rely upon expression of nociceptive behaviours to assess pain levels. Consistent animal behaviours in response to noxious stimuli are referred to as pain behaviours and have been extensively used in mouse studies. Skeletal diseases occur in the ageing population and this can’t be mimicked in younger mice. We also mainly use female mice to reflect the clinical situation where osteoporosis and fragility fractures occur in women.

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

Animal suffering will be limited in our studies by our strict monitoring of actual severity limits. We provide a number of supportive measures for an effective pain management regimen: Peri-operative analgesics will be administered for at least 7 days after fracture surgeries. Animals will be monitored for pain and will be given analgesia until they begin weight bearing on the fractured limb and are able to ambulate freely. Stress-relieving environment will be provided (eg nesting material, enrichment).

Nesting material has the potential to improve mice well-being. Cages will be filled with soft bedding. Easy to reach and soft palatable food and water will be provided.

To minimise variability and the number of animals used, most studies will only be performed in mice of the same sex, age and strain to limit variability in pain behaviours and bone healing. The same surgeon will perform all surgeries. Mice are habituated to pain measurements one week before the procedure and pain measurements are performed before surgery to measure pain baseline.

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

We adhere to ARRIVE guidelines for designing and conducting our experiments and LASA guidelines for substance administration. We also follow NC3Rs best practices for example for blood sampling. We attend training offered by our institution, the Home Office, UK academic institutions and EU collaborators working on bone pain.

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

We will attend 3Rs webinars and seminars as well as other training that is available to make sure that we stay informed. The NC3Rs website is a good source of information on 3Rs development and we will regularly consult it.

A retrospective assessment of refinement will be due by 05 May 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?

43. Tumourigenesis and development of antibody treatments

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

cancer, intracellular delivery, macromolecules, antibodies, 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?

We aim to mimic human cancers in mouse models and to treat these cancers through delivering novel anti-cancer therapies inside cancer cells. The novel therapies we will develop will use macromolecules to give an anti-cancer effect. Macromolecules, such as antibodies, are much larger than the small molecules traditionally used in anti-cancer therapies, and this larger size requires optimisation of the methods used for macromolecule delivery inside cells. By improving scientific knowledge of macromolecule delivery into cells we will then help to advance macromolecules towards clinical use.

A retrospective assessment of these aims will be due by 15 May 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?

Traditional small molecule anti-cancer therapies have been successfully developed to bind to a number of proteins inside cells which cancers use to survive and proliferate. However the range of proteins this traditional small molecule therapeutic approach can be developed against is limited by the requirement for deep pockets in the target protein that the small molecule can enter and bind, target proteins with large flat surfaces cannot be bound by small molecules. Macromolecules are less limited by this requirement as they too can have large flat surfaces able to bind target proteins with flat surfaces.

Macromolecules, such as antibodies, may also be conveniently produced against a variety of targets by molecular biology techniques. In contrast the medicinal chemistry development of small molecules is more laborious. The challenge of realising the potential of intracellular macromolecules delivery inside cells in a functional state. The biggest hurdle is that their size precludes passive diffusion across the cell membrane. The delivery of functional proteins inside cells is one of the biggest challenges in bioscience and the work performed under this PPL will help address this challenge.

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

The outputs from our work on generation of procedures for intracellular delivery of functional proteins will be publication in peer-reviewed journals, submission of data at international research conferences, public engagement forums, and patent filings. These will give as wide as possible visibility to our work. Since the desired outcome of our work is to develop effective anti-cancer agents against tumour targets which are under investigation by many other researchers, it is important to disseminate the information as widely and rapidly as possible. The successes or failures of this approach should form part of the communication programme in order to prevent global duplication of work and unnecessary use of mice. Finally, it is important to provide information through public engagement and scientific communication opportunities because realistic expectations of application of these studies must be transferred to non- scientific audiences.

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

There will be short-term and long-term benefits for the project. Short term benefits will be adding to the accumulated knowledge of macromolecule delivery methods. In the longer term this will help to advance anti-cancer macromolecules towards clinical use, which will be of benefit to patients suffering from tumours driven by targets that are currently "undruggable" by traditional small-molecule anti- cancer therapies. These targets include the intracellular proteins RAS and Myc. More than 30% of all human cancers are driven by the RAS family of genes (https://www.cancer.gov/research/key- initiatives/ras). High and/or aberrant Myc expression occurs in >70% human cancers and is related to poor prognosis and aggressive conditions (Wang, C., Zhang, J., Yin, J. et al. Alternative approaches to target Myc for cancer treatment. Sig Transduct Target Ther 6, 117 (2021). https://doi.org/10.1038/s41392-021-00500-y).

Taken together these targets contribute to a vast number of human cancers each year which have unmet clinical need. Research performed under this licence will contribute to meeting this clinical need and alleviating suffering across human cancer patients.

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

We plan to maximise the outputs by collaboration with other scientists who could benefit from aspects of the intracellular macromolecule delivery technologies we have developed. As we develop more sophisticated systems, we will disseminate this knowledge and plan pathways with our clinical colleagues towards implementation in patients.

Species and numbers of animals expected to be used

- Mice: 5250

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.

Rodents that lack a fully active immune system are a valuable research tool in cancer research enabling human tumour material to be grown. Genetically Altered (GA) mice are also a valuable research tool enabling mice with specific targeted genes to be bred and maintained for use in many areas of cancer research. Many of the studies performed under this PPL will involve the use of laboratory systems such as in silico computer modelling, in vitro cell culture and ex vivo human tissue assays (i.e. analytical procedures) to complement the animal work. However, laboratory-based assays cannot adequately replicate many of the complex molecular, cellular, physiological and behavioural properties necessary to fully understand how genetic modifications result in normal or abnormal tissue growth.

For this project adult mice will be bred in efficient colonies. The adult offspring of these breeding colonies will be used in other protocols in this PPL. Adult mice are chosen for this research to avoid the presence of continuing developmental processes which could confound studies, for example periods of rapid weight gain masking treatment-induced weight loss.

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

GA animals will be bred in efficient breeding colonies. The offspring of these breedings, or immunocompromised mice obtained from other PPLs, will then undergo the following general procedure:

A typical animal will be implanted with cancer cells to cause the formation of a tumour. The mice (typically, implanted mice will be 2-3 months old) will then have experimental anti-cancer agents (i.e. macromolecule delivery vehicles) administered. The effect of these will be monitored by blood withdrawal, tumour measurements and/or non-invasive imaging. At the end of a study, or at a humane endpoint, mice will be humanely killed, typically up to 30 days from the last dose.

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

Mating of mice is not typically expected to have any adverse effects, animals will be provided with an enriched environment.

Animals with implanted tumours will be closely monitored for typical clinical signs of pain or distress, as will animals who have been administered experimental anti-cancer agents. Animals that do not recover from severe clinical signs (e.g. persistent hunching, tremors/convulsions, vocalisation, labored respiration) within 10 minutes or moderate clinical signs (e.g. intermittent hunching, tremors/convulsions, vocalisation, abnormal respiration) within 30 minutes will be humanely killed.

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 used under this Project licence are GA mice.
- Less than 1% of animals are expected to experience severe severity.
- Approximately 55% of animals are expected to experience moderate severity.
- Approximately 45% of animals are expected to experience mild severity

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

- Killed

A retrospective assessment of these predicted harms will be due by 15 May 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?

As much as possible will be done in cell-based assays (growing cells in culture medium) and with biochemical/biophysical assays in the lab that do not involve mice. However laboratory systems cannot adequately replicate the complex molecular, cellular and physiological interactions necessary to fully understand how novel intracellular delivery systems will behave in animals.

Examples of some of these interactions which can be achieved in animals are the filtration of nano- sized delivery vehicles such as viruses and nanoparticles by organs such as the liver and kidney, the effect of blood components on these delivery vehicles when in systemic circulation and their ability to extravasate at desired disease sites (i.e. solid tumours). By studying these interactions in live animals we can engineer our delivery vehicles to provide the greatest therapeutic benefit and aid their translation to the next stage of development. Without using animals the rate of development will be severely hindered and the likelihood of failure to translate to clinical use is vastly greater.

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

Use of 3D cell culture systems and mixed cell culture techniques. These attempt to recapitulate some of the biological complexity of a living organism by mimicking tissue architecture and cell types.

Why were they not suitable?

There are a large number of variables which are relevant to the action of macromolecule delivery vehicles which are not adequately replicated in these cell culture systems including:

blood flow and the associated fluid dynamic forces this produces

variable tumour microenvironments including differences in pH, oxygen concentrations, presence of immune cells, presence of collagen and hyaluronan, extracellular enzyme activity

the filtering actions of the liver, kidney, spleen etc.

A retrospective assessment of replacement will be due by 15 May 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?

Research into the causes and subsequent treatment of a wide range of cancer types requires the use of specific strains of mice including those with impaired immune systems and animals with specific genetic modifications that are relevant to the genetic pathways now known to be associated with tumour development.

We estimate that we will breed approximately 200 Genetically Altered mice per year, use 100 mice per year in pilot/tolerability studies, and use 750 genetically altered mice per year (obtained from this project licence and others) in larger efficacy studies.

These figures are based on projected study sizes and frequencies. Required mouse numbers can vary based on factors such as progress of laboratory based work which precedes these studies in mice.

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

The NC3R's Experimental Design Assistant (EDA) will be utilised where possible to guide experimental design. This aids in obtaining the best data possible (i.e. minimising the requirement for repeats) from the least number of animals. The ARRIVE guidelines 2.0 Recommended Set will also be used as a framework to guide experimental design. The use of the EDA is complementary to the implementation of the ARRIVE guidelines.

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

Unnecessary production or import of genetically altered animals will be avoided by searching cryobanks and databases. Examples of resources available include:

NC3R's breeding and colony management: https://nc3rs.org.uk/breeding-and-colony-management

Animal Welfare Management Discussion Group (AWMDG): An email network for Named Persons to share ideas

RSPCA Animals in Science Resources: https://science.rspca.org.uk/sciencegroup/researchanimals PubMed: http://www.ncbi.nlm.nih.gov/

Web of Knowledge: https://wok.mimas.ac.uk/

Jackson laboratory: http://www.jax.org/ & http://jaxmice.jax.org/index.html

Home Office Technical Advice: https://www.gov.uk/guidance/animal-research-technical-advice

The strain used for generating a new colony will be carefully considered to avoid producing unwanted mice. Animals will only be bred if a requirement has been established, and the breeding programme will be subject to regular review to optimally meet anticipated demand. Spare animals will be made available for use on other scientific projects where permissions allow.

Breeding will be optimised, wherever possible, to produce only the genotype required.

In studies involving administration of therapeutic agents, e.g. macromolecule delivery vehicles, the methods of production of these agents will be made reliable and reproducible in order to minimise variability in mouse studies and subsequently minimise the number of mice required.

Substantial in vitro work will be performed to characterise the action of the novel therapeutic agents we produce in cells cultured in 2D and 3D environments. In the case where these agents are intended to target cells in systemic circulation, e.g. CD7 positive T cells found in human CD7 expressing transgenic mice, blood samples will be taken and the targeting of these cells by our therapeutic agents will be assessed ex vivo. These in vitro and ex vivo experiments will inform subsequent in vivo experiments, for example in choice of proposed initial doses for dose setting in pilot studies. Pilot studies will also be utilised to estimate effect sizes and variability to better inform group size decisions in larger therapeutic efficacy studies. Pilots will also highlight any deficiencies in proposed experimental designs before larger studies take place.

A retrospective assessment of reduction will be due by 15 May 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.

For novel targeted anti-cancer agents to be studied, animal models bearing tumours with specific cancer markers are required. For this reason immunocompromised and non-immunocompromised mice bearing human tumour markers, such as CD7 (a human blood cancer marker),will be bred and used in protocols within this PPL.

Transplantation of cancer cells with specific markers into immunocompromised mice is an efficient and simple method of producing solid tumour models bearing specific markers. Both human and murine cancer cells can be used with this methodology and it also allows for manipulation of the cancer cells in vitro (e.g. gene knockout or overexpression) before implantation to see how various genes regulate response to our novel therapeutic agents in vivo. This is much more physiologically relevant than in vitro studies of cancer growth.

CD7 is highly expressed in certain acute leukemias. Therefore by using human CD7-expressing mice we will be able to model macromolecule delivery to CD7-expressing cells in a more physiologically relevant system than in vitro.

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

Work under this PPL is designed to mimic disease in human patients and then assess the intracellular delivery of macromolecules as therapy for this disease. This can only be achieved in live animals as the tumours will only grow when complete body systems are supporting them, and the interaction of macromolecule delivery vehicles with these complete body systems must also be observed to determine the future therapeutic viability of these vehicles.

We plan to use adult mice. More immature mice cannot be used as ongoing developmental processes will add confounding variables to studies which may ultimately result in more mice being used. For example ongoing immune system development may alter tumour implantation properties and subsequent effects of treatment on these tumours. Less sentient animals, e.g. zebrafish, do not adequately recapitulate features of

the human anatomy and human cancers to permit relevant study of our therapeutic delivery vehicles in these models. Terminally anaesthetised animals cannot be used as the necessary studies take place over the course of days or weeks (to allow tumour growth, multiple doses etc.), too long a period to maintain anaesthesia.

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

The establishment has staff experienced in monitoring animals for clinical signs of pain and distress. Tube handling has been implemented at the establishment to minimise stress associated with handling. Animals will be allowed to acclimatise to any new environment (e.g. having just been imported from another establishment) for 10 days before any procedure is performed. During the acclimatisation period the animals will be handled to reduce the stress associated with handling during future procedures.

Following procedures, e.g. administration of novel compounds or anaesthesia, observation periods are stipulated within this licence to ensure animals either fully recover or are humanely killed if showing clinical signs given in the humane endpoints of each protocol. Analgesia will be used where animals appear to be in pain, pre-emptive and multimodal analgesia is preferred.

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

Published guidelines for best practice will be followed, including:

Refinement and reduction in the production of genetically modified mice; Laboratory Animals Vol 37, Supp 1 July 2003.

Breeding and Colony Management (NC3Rs) https://nc3rs.org.uk/breeding-and-colony-management

Assessing the welfare of genetically altered mice. Wells et al (2006) Laboratory Animals 40(2), 111-114

Home Office Technical Guidance: https://www.gov.uk/guidance/animal-research-technical-advice

ARRIVE guidelines: https://arriveguidelines.org/arrive-guidelines

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

Wherever we can we will replace the use of animals in research, refine experimental procedures and minimise the number of animals used in experiments. We will work closely with named personnel responsible for animal welfare at the establishment, who in turn work closely with organisations such as the National Centre for 3Rs (NC3Rs), to work on new approaches and technologies to minimise the use of animals and improve animal welfare.

A retrospective assessment of refinement will be due by 15 May 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?

44. Antibody production for research, diagnosis and therapy

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

Antibodies, Diagnostics, Research, Therapy, Contract Service

Animal types	Life stages
Sheep	adult
Goats	adult
Donkeys	adult
Domestic fowl (Gallus gallus domesticus)	adult
Alpacas	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.

Required at inspector’s discretion

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?

Farm animals (sheep, goats, alpacas and hens) and donkeys will be injected with minute quantities of proteins, peptides or haptens to stimulate the production of antibodies.

Smaller molecules e.g. peptides and haptens will be conjugated to carrier proteins to increase their immunogenicity. Blood donations will be taken and the:

- antibodies from the blood serum will be used for production of diagnostic kits and therapeutic treatment development
- blood cells will be used to produce recombinant antibodies via immune phage display libraries

A retrospective assessment of these aims will be due by 26 May 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?

It is important that we undertake this work to:

1. Produce antibodies against food pathogens, drugs, toxins, environmental factors/ pollutant, bacteria for the production of diagnostic kits

2. Produce antibodies against Clostridia, Covid-19, Ebola, toxins, venom, drugs for development and manufacture of therapeutic products

3. Produce recombinant antibody libraries.

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

Immunoassays are vital to clinical diagnosis and the monitoring of therapy. They also find a multitude of other related applications, such as screening for environmental pollutants. Because of the versatility and almost universal applicability of immunoassay, the potential analytes are almost endless.

The availability of immunoassay reagents fulfils the vital needs of clinical diagnostics and many other analytical applications. Immunoassays also provide the fundamental analytical basis for a vast range of activities in research and other investigational areas.

We expect to produce high quality antibodies that will be fit for their intended purpose as outlined by the customer or end-user.

Where the project is delivering antibodies for research, medicine or vaccine development the outputs may take a longer to deliver benefit, these outputs may take the form of publications and new information.

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

Direct benefits of this project include:

Production of antisera for research into immunotherapeutic studies to treat various diseases such as

C. difficile associated disease, Covid-19, Colchicine poisoning and assorted cancers.

It will develop and validate a novel approach to the treatment of diseases which is quick, effective, without side-effects, prevents recurrence and reduces overall costs. It will be based on the oral and intravenous administration of polyclonal antibodies directed against the target. The development of new antibody based therapies for treatment of disease has huge life saving potential.

Antibodies may also be useful for the development of sensitive diagnostics for early disease detection and to support research into disease treatments such as use of exosomes in cancer diagnostics and therapies. They are also used for development and manufacture of sensitive and accurate diagnostic tests for a range of targets including drugs of abuse, environmental contaminants and factors e.g.

Legionella; and food borne pathogens. Millions of patients worldwide benefit from accurate and timely diagnosis of disease, infection and toxicity by the application of immunoassays to direct therapy and screening.

Production of a new therapeutic treatment may take many years and requires significant investment. Regulatory bodies require substantial evidence of safety, and efficacy before licences are granted.

Expert opinion argues that polyclonal antibody based treatments will always have application in treatment of diseases such as snake bite, certain viral and bacterial diseases, where epitopes are multiple and treatments need to be developed quickly.

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

By use of larger animals such as sheep, goats, alpacas and donkeys in combination with careful and considered immunogen design, we aim to maximise the outputs of this work by producing high levels of specific antibodies that are fit for purpose from minimal numbers of animals.

The company collaborates with Universities and other research groups to develop antibodies that will support the research and development of novel and life-changing therapeutic and diagnostic solutions.

The company has an on-going research programme with partners to develop methods for sheep phage display antibodies through purification of peripheral blood lymphocytes from hyper-immunised sheep. It is expected that this programme will be expanded to include other species once established in sheep. These two projects have the potential to reduce the future number of animals as once immortalised antibodies can be produced in-vitro and the use of further animals is reduced or eliminated.

Species and numbers of animals expected to be used

- Donkeys: 4
- Domestic fowl (Gallus gallus domesticus): 25
- Sheep: 500
- Goats: 10
- 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.

Sheep and goats, with their contractile spleens can adapt to regular bleeding, with no effect on their health. Unlike many other species used for antibody production sheep and goats are genetically programmed for “affinity maturation” of the antibody response; leading to much higher antibody affinity than other species.

Antibodies from hens may be obtained by collecting their eggs, without need for bleeding, because hens' antibodies are found in their egg yolks as well as their blood.

Donkeys need to be used to produce the secondary reagents, i.e. donkey anti-sheep IgG, Fc, / Fab. These reagents cannot be produced in sheep as they would not be recognised as foreign and hence not produce an antibody response. Donkey secondary reagents are used in assays which employ a sheep antibody as the primary reagent.

Camelids are unique with having smaller functional single chained antibodies that consist of a single variable binding domain termed VHH and two constant domains know as CH2 and CH3 which allow for fast antibody discovery and large-scale antibody production. Alpacas are large animals which provides larger volume of antiserum at each blood donation to minimse the number of animals and their time on a project.

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

Only mild procedures are used for injection of the animals, comparable to vaccinations in humans. The collection of their blood is comparable to blood donations by humans. Antibodies from hens may be obtained by collecting their eggs, without need for bleeding, because hens' antibodies are found in their egg yolks as well as their blood.

The duration of the procedures may be from 15 weeks to the duration of the licence depending upon customer requirements.

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

Donkeys pose a problem because, like horses, a vigorous local immune and inflammatory response may lead to swelling and a sterile abscess at the immunisation site. Donkeys may also show wide individual variability and some may show substantial reaction to injections. Only Freund's Incomplete Adjuvant will be used in donkeys. Less than 1% incidence of systemic reactions, e.g. anaphylactic shock, is expected or anaemia. During the previous 5 years of the licence no systemic reactions have been observed.

Sheep and goats develop local swelling at the sites of immunisation. After one to two years' immunisation some local swellings may become larger and flabby. Blood removal may cause bruising/haemorrhage/haematoma at the collection site. From many years' experience the likely incidence is very low.

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)?

A mild severity level is expected in animals used in short projects and this can become moderate severity in animals used for longer projects. The severity levels in immunised sheep are expected to be 50% mild and 50% moderate. Moderate reaction is due to swelling at immunisation site.

In all other immunised animals the severity is expected to be mild in >95% of animals.

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

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

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

Polyclonal antibodies play an important role as the key reactant in a vast range of immunoassays. Despite the introduction of monoclonal antibodies, which may be produced in cell culture, polyclonals of animal origin have certain advantages and their use is unavoidable in some vital applications where increased specificity and overall affinity to the target is observed.

Other antisera, such as second-antibody separating agents for immunoassay application, may be required in greater amounts. This accounts, in part, for the well established use of donkeys to produce secondary antisera to sheep IgG or rabbit IgG, when fewer animals can produce the same quantity of high quality antiserum. Secondary antibodies cannot be produced in the same host as the primary antibody because they are native and do not elicit an immune response.

There are still applications for which animal derived antibodies are essential, e.g. antivenom production, where antibodies to all of the epitopes of the toxins and peptides are required: 10s to 100s. It has been proven that using large farm animals, e.g. sheep, for commercial production for human therapy of life threatening diseases is reliable and cost effective: products should be, safe, effective and affordable at their point of use

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

Phage display antibody production.

This establishment has been using phage display technology for a number of years. This technology is used with its sister company (in the USA), using technology licenced from a major pharmaceutical company. This technology does not use naïve libraries but relies on extraction of RNA from peripheral blood lymphocytes from hyperimmunised animals. The sheep are immunised for a short period – 20-24 weeks, the response is monitored and RNA purified from suitable responders. Continued production of antibodies for commercial diagnostic use uses Fab fragments produced in E.coli fermentation. This establishment is currently unable to offer this to external companies due to the terms of the licencing agreement with the pharmaceutical company. Our technology has reduced the need for long term immunisation of animals.

This establishment is currently undergoing a collaboration to develop and adopt phage display technology to offer our customers, which will replace some of the ongoing use of animals for long term antibody production to support diagnostic and in particular therapeutic manufacturing requirements of customers.

Why were they not suitable?

The phage display technologies are exciting and during the last 30 years have seen incremental development and commercial applications, mainly in the field of human therapy. Naive human libraries coupled with sophisticated antibody chemistry have

produced fully humanised antibodies against important human diseases where repeated administration is required e.g. rheumatoid arthritis.

Despite the potential for phage display the technology is still not widely adopted, particularly for early research and commercial diagnostic applications. Reasons are several fold:

- Lack of commercial companies offering the service
- Lack of broad expertise and facilities
- Intellectual property issues
- Timeliness in assembling the end reagents: particularly if several rounds of panning and or conversion to the whole Ig molecule is required (phage display typically ScFv or Fab fragments).
- Economics: essential for grant funded research projects and many smaller vital niche market diagnostic products, e.g. plant diseases, environmental monitoring, orphan diseases: £30,000 to

£50,000 for phage display versus less than £1,000 for polyclonal production.

- Naive libraries not as effective as libraries obtained from hyperimmunised animals.
- Whole “phage” antibodies are required to be produced in mammalian cell culture, which inevitably requires the use of animal derived products, e.g. serum, growth factors, etc.

A retrospective assessment of replacement will be due by 26 May 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 have been estimated based on usage in previous years.

About 100 sheep, 2 goats, 5 alpacas 1 donkey and 5 hens are estimated to be used each year.

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

The required volumes of the antisera, and hence the animal numbers required, depend greatly on their application. A very high titre antiserum produced in one or a few animals may serve to supply vast numbers of diagnostic tests. The animal numbers required will depend on the reviewed demand of the client.

The manufacture of therapeutic antibodies may require many hundreds of litres of the antisera starting material per year. However for developmental work carried out at this establishment, only small groups of sheep are envisaged. For example, typically three sheep will be started with each immunogen to allow for variability in the immune response between individuals.

Re-use, providing there are no scientific or welfare prohibitions, reduces the total number of animals used in regulated procedures.

The NC3R's Experimental Design Assistant (EDA) will be used.

Intelligent up front design: good immunogen design is used to prepare immunogens with antigens that are:

- immunogenic (or conjugated to proteins to increase their immunogenicity), specific to the required target
- In the case of peptides - unique leading to less cross reactivity with other parts of the antigen and manufactured to the optimum number of amino acids
- Selection of the antigen can have a large positive effect on the desired antibody response and thus limit the numbers of animals required in order to obtain the desired results.

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

Larger sheep are selected for production and are better suited to our environment. Larger animals are able to donate larger blood volumes thus reducing their time on a project and reducing the number of animals required.

Project outcome questionnaire is supplied to customers on the completion of each project to determine if their specific outcomes were reached.

This establishment has an on-going research programme with partners to develop methods for sheep monoclonals and phage display antibodies through purification of peripheral blood lymphocytes from hyper-immunised sheep. These two projects have the

potential to reduce the future number of animals as once immortalised antibodies can be produced in-vitro and the use of further animals is reduced or eliminated.

A retrospective assessment of reduction will be due by 26 May 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 phylogenetic distance of hens from mammals may lead to raising of superior antibodies against some mammalian proteins, which may have advantages for certain diagnostic applications. Hens offer various potential advantages. The chicken equivalent of mammalian IgG is IgY, which is selectively transported into the egg yolk. Egg collection is non-invasive. One hen will produce roughly as much IgY in its eggs as the IgG that may be collected from the serum of one sheep over the same period.Egg yolks are processed by separation of the IgY from the hydrophobic yolk lipids.

Second antibodies must be produced in a species distant from the host used to produce the primary antibody. Use of Donkeys is limited to secondary antibody production only, resulting in minimal procedures being carried out on donkeys. In addition, large volumes are obtained from each blood donation leading to shorter project times and thus minimal distress to the animal. Re-use of equines within this protocol familiarises the animal with the process which reduces stress. Equines are fed and reassured during the procedure and being used to handling are relaxed and confident. This confidence increases with re-use.

Sheep and goats provide the most effective and economical source of polyclonal antibodies for therapeutic uses. They may be bled regularly with no ill effects. Large sheep provide larger volume of antiserum at each blood donation to minimse the number of animals and their time on a project.

Polyclonal antibody production must be initiated in order to provide the B-cells or RNA required for in- vitro techniques such as monoclonal or phage display antibody production.

Re-use of sheep within this protocol familiarises the animal with the process which reduces stress. Sheep learn the routine and become familiar with the race handling, restrainer and trolley system and therefore are less stressed with each re-use.

Camelids are unique with having smaller functional single chained antibodies that consist of a single variable binding domain termed VHH and two constant domains know as CH2 and CH3 which allow for fast antibody discovery and large-scale antibody production. Alpacas are large animals which provides larger volume of antiserum at each blood donation to minimse the number of animals and their time on a project.

Total traceability and quality of our product is essential to the customer for their required use for the benefit of human health The re-use of an animal which is continually under veterinary supervision is essential and this historical health screening ensures a product of the highest standard.

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

The use of animals such as sheep, goats, alpacas, donkeys and hens permits the husbandry of animals under high standards of farming conditions; with grazing in fields in the summer and indoor housing with feed supplements in winter. Their useful life is generally longer than commercially farmed animals. All animals are under the day-to-day care of an animal welfare officer and are visited frequently by NVS.

Less sentient animals are unable to produce large volumes of high quality antibodies that are affinity matured compared to the animals listed.

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

Refined techniques include optimised emulsion preparation, targeted immunisation sites and breed selection (hybrids not pure breeds, larger breeds).

Use of animal conveyor system for handling, turning and weighing of animals: used for routine husbandry (vaccination, dagging, foot trimming), immunisation and blood sampling. Safe efficient and calm system for both animals and handlers. Use of the same systems for routine husbandry and procedures allows animals to acclimatise over extended period of time before procedures begin. Use of single needles when immunising. Weighing animal immediately before all blood collection to ensure volume calculations are correct before procedure started. Habituation of Donkeys to their handling system. Use of refined adjuvants - where Freunds Complete is used it is for primary immunisation step only and subcutaneous delivery of immunogen.

Extensive daily monitoring of animals undertaking procedures.

Continuous improvements for procedures and training for operators undertaking procedures to maintain a high level of performance and minimise effects.

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

The Home Office Supplementary Guidance (HOSG) on projects for the production of antibodies as a service provides principles for protocols of minimal severity, including advice on adjuvants, on immunisation routes and sites, and on removal of blood in accordance with the BVA/FRAME/RSPCA/UFAW Joint Working Group recommendations (2000).

NC3Rs: Single Use of needles - putting refinement into practice NC3Rs: Experimental Design Assistant

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

Subscription to the NC3Rs e-newsletter to keep up to date with the latest 3Rs developments. These monthly updates focus on funding opportunities, 3Rs events and publications.

Regular local and corporate animal welfare committees discuss 3Rs routinely with specific projects across the global organisation designed to implementing the 3Rs.

This establishment has the facility to browse a large and diverse range of publications via their parent company e.g. Journal of Visualised Experiments

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

45. Metabolism and toxicity in non-human primates

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

Pharmaceutical, Non-human primate, Regulatory, Toxicity, Absorbtion, Distribution, Metabolism and Excretion (ADME)

Animal types	Life stages
Rhesus macaques	adult, juvenile
Cynomolgus macaques	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.

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?

This project will allow us to evaluate the metabolism, immunology and toxicology of drugs in the non- human primate (NHP) (Cynomolgus and Rhesus macaques) for the avoidance, prevention, diagnosis or treatment of debilitating or potentially life-threatening clinical conditions or their effects in man.

These studies will provide data to satisfy regulators around the world that these drugs are safe for use. These drugs will be used to treat debilitating human illnesses (like cancer and diabetes for example) for which there is an unmet clinical need, or the need for more effective drugs. All tests on this licence are being carried out to meet global regulatory requirements (tests that governments require before they allow testing in humans).

We also may conduct the validation or investigative studies which enable the regulatory programme of work described above.

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

New medicines have the potential to benefit in new or improved disease treatments. Before potential new medicines are administered to humans their safety must be evaluated. This testing is a mandatory legal requirement and provides information on risks to people taking new medicines. Often, the new medicines we test on this programme will be highly specific for a molecular target or receptor, which often make them less likely to have side effects than traditional medicines.

At present there are no alternatives that don’t use animals that are scientifically, ethically or legally acceptable as replacements for systemic toxicity assessment. In addition, we only use NHPs when no other species is suitable based on the nature of the drug (so if a drug can be tested in say a rat or a mouse, or in a dog or a pig, and we would expect an equal outcome to the study then we would use them instead of a primate).

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

The overall benefit of this project is that it supports the development of safe, new medicines to improve the health and quality of life of human patients by generating high quality data that is acceptable to regulatory authorities and enables internal decision making for others.

Achievement of the objectives of this licence will enable safe development candidates to progress and will also help to remove unsuitable candidates from the development pipeline at an early stage, thus saving animals and resources.

Study reports will be included in regulatory submissions to allow regulatory authorities to make judgements on whether to permit clinical studies or to licence a drug. Global guidelines recognise that the justification for animal-based regulatory toxicology and safety testing is the need for regulatory authorities to have sufficient information to assess the risks to which humans are exposed to by new drugs.

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

Patients will benefit from these studies as this work will contribute to the development of new drugs that help alleviate human conditions. These new drugs may work better in the clinic, relieve or cure diseases and have better side effect profiles. We may, by our work, also contribute to better knowledge and understanding of these types of drugs, and that knowledge may be used to develop further new drugs.

One of the key benefits is the production of data that is required by regulatory authorities, to ensure medicines can be dosed safely to humans. These drugs that will be tested are for debilitating or life threatening human conditions, in some cases where there is an unmet clinical need to treat such conditions.

In addition, the models on this project may be used to assess the safety or other in life properties of a new drug, and find a dose that causes no effect. This is important when planning future trials in humans, to make sure any starting dose in a clinical trial is safe for the patients taking it.

Others will also benefit, as the data we generate will allow them to progress their new drugs into clinical trial, or otherwise if they are found to have adverse side effects.

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

The work will be shared with others who will use it to determine their future strategy, or for submission in documents required by regulatory authorities. Whilst we have no direct control over what happens to the data after we have shared it, we trust from information given to us that it is used for regulatory purposes or to support regulatory purposes (e.g. to support drugs progressing to clinical trials). Previously however, we have collaborated with others and shared data we have produced in the form of Scientific publications that are in the public domain.

Species and numbers of animals expected to be used

- Rhesus macaques: 630
- Cynomolgus macaques: 7150

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 small non-human primates on these studies, both adult and juvenile. We only use non- human primates when other species (like rats, mice dogs and/or pigs) are unsuitable to get the answers we need from the studies. Most studies would be carried out

in adult animals; juveniles would only be used for specific studies for pharmaceuticals targeted at juveniles.

This is often because for the type of pharmaceuticals we are testing (for example 'biologics', peptides or antibodies) we can only see any toxic effects if we use primates, maybe because the biological target of the drug is only present in a primate, or maybe because the requirements of the study mean we can only use primates to get the results we need that will satisfy global regulatory bodies.

We are not allowed to use primates by law unless there is no other animal we can use that will give us the results we need to satisfy the regulatory authorities.

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

Animals usually receive repeat doses of drug by their intended route of administration in the clinic, and routinely undergo, clinical observations, bodyweight measurements, and have blood samples taken for analysis, clinical pathology (including blood analysis and urinalysis), and then are killed humanely and undergo extensive pathological examination. Blood sampling procedures are similar to what you would experience when you have a blood sample taken by a medical professional.

Animals undergoing surgery receive the same sort of care as a patient would in hospital. We discuss their pain relief and use of antibiotics with a veterinary surgeon before we start. We administer drugs as necessary and give them plenty of time to recover from surgery before we use them in experiments.

These surgical procedures are carried out only for essential purposes.

Infrequently we may include body temperature measurement by rectal thermometer, ophthalmoscopy (checking the structures of the eye), ECG (checking your heartbeat and rhythm is normal), and measuring blood pressure. Although the animals may be restrained whilst doing this, it will be for as short a time as possible to cause less stress for the animal and because it is safer for everyone, and no more than for a set period of time per day. The animals are trained using positive reinforcement (treat rewards) to move about the cages for handling/procedures, and to sit in restraint chairs.

Rarely the animals will undergo specific repeat investigations to investigate particular findings related to concerns highlighted in earlier work: Because these investigations are targeted for a particular body system where a concern is suspected, it is considered unlikely that more than one of these investigations would be necessary on the same animal/study (e.g. semen collection, vaginal swabbing).

At the end of the studies, if animals are not humanely killed to examine their internal organs, to check for any damage or irregularities, they may be kept alive for potential re-use, subject to specific legal conditions, and only after being checked that they are in good health by a veterinary surgeon.

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

When dosing an animal by injection or taking blood, the amount of pain an animal feels is similar to what a patient would feel having an injection done by a doctor. If we have to repeatedly inject animals using a needle and syringe, we would choose different sites to do this where possible. If we can take blood samples when an animal is deeply unconscious then we do. If we need to take repeated blood samples or need to dose repeatedly then we try and use different sites. Of course, everyone who performs these procedures are trained to a high standard and hold a UK personal licence outlining their competency in the procedure.

Very occasionally we may need to take a urine sample for analysis, so we would then put an animal into a special cage which is smaller than their normal cage. The animal can still move around however, and we'd normally introduce an animal to this cage to acclimatise them to it. Virtually every animal will get used to their new cage within about 15 minutes and are fine.

Generally, if we have to use any equipment to help us get the results we need, we acclimatise our animals to it so they get used to it and tolerate the procedure when we start dosing them. So, we carefully introduce them to things like restraint gradually, for short periods at first, and usually they accept it after a while. And if they don't acclimatise, we take them off the studies, to stop causing any harm.

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)?

On the last project, about 80% of animals were classified as having displayed moderate severity, the rest were classified as mild. This is because these studies can last between a few weeks to up to a year, and although the individual procedures are usually mild in nature on their own, the cumulative effects make them moderate overall.

It's impossible to predict the proportion of severities expected on a service licence like this, as this will be dependent on what study types we are asked to perform.

All protocols on this licence are classified Mild or Moderate only, there is no intention to perform any procedures that are Severe in nature.

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 24 May 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?

Pharmaceutical testing is a mandatory legal requirement and provides information on risks to people taking new medicines. At present there are no alternatives that don't use animals that are scientifically, ethically or legally acceptable as replacements for systemic toxicity assessment.

In vitro and in silico methods (test tube work not using animals and computational methods) are used in combination with animal studies to inform study designs and assist in understanding of potential toxicity but cannot yet replace in vivo (animal) studies (except on some rare occasions) and only with approval from global regulatory agencies.

We maintain a constant awareness of regulatory guidance and ensure that where non-invasive methods exist which fulfil the regulatory requirement, they are used in preference to animal studies.

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

There are no other non-animal alternatives for the work being undertaken on this project. The regulations we are following will not allow safety decisions to be made on non-animal systems alone.

However, in the evaluation of biosimilars (protein based drugs of the same structure), which may be performed under this licence, a stepwise approach is taken prior to any testing in animals. This process would start with studies in test tubes to compare the characteristics of the new product (the biosimilar) with the reference product (usually already marketed) to assess differences in the properties of the two materials. Only if there are differences in the responses of the two materials in test tubes, or the test tube studies were not deemed extensive enough to predict any differences in humans, would we consider using an animal model.

If the biosimilar comparability exercise for the physicochemical and biological characteristics and the studies in test tubes are considered satisfactory and no issues are identified which would block direct entrance into humans, an animal study is usually not considered necessary, and would not be performed.

Why were they not suitable?

Although there are test tube tests that can model some parts of how drugs get into our bodies, and how our body deals with them, and can identify undesirable effects, for example, there is no series of test tube tests that brings all these complex happenings together, like we see in animals and humans.

That's why we need to test the new drugs in animals, as they have similar physiology and processes as humans, and that testing gives us a good idea what may happen if they were ever tested in, or exposed to humans.

However, in all cases we will assess whether data already exists or can be generated in other ways other than the use of animals, and that we will ensure that animal reduction, replacement or refinement strategies and alternatives provided in the regulatory guidance will be considered, and animal use avoided where possible.

A retrospective assessment of replacement will be due by 24 May 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 we have used are based on figures of previous usage from previous projects, or a projection thereof (based on estimated incidence) based on past studies. It is, however, impossible to accurately predict the number of studies that may be performed, in the circumstances.

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

Studies are designed to provide maximal data and statistical power (where appropriate) from the minimum number of animals on the consideration that it is better to increase the number of animals used to achieve the objective rather than using too few animals and risk having to repeat the study. For regulatory studies, guidelines require the number of groups and animals per group to be adequate to clearly demonstrate the presence or absence of an effect of the test substance; core study designs are based on international

guidelines where these exist. Otherwise, reference is made to standard study designs with input from the Department of Statistics, where appropriate, to identify the optimum number balancing the need to achieve study objectives while avoiding excessive animal use. These internal designs are reviewed and updated in line with changing external guidelines and internal refinements that either minimise numbers or reduce severity.

Scientists within the department are actively involved with cross-industry initiatives aimed at sharing best practice and the application of the 3Rs in toxicology and this feeds into review of study numbers and practices.

Whenever possible, common control groups will be used in order to minimise the numbers of groups used. In addition, the regulatory test guidelines used for testing, stipulate group sizes.

In the evaluation of biosimilars (protein based drugs of the same structure), which may be performed under this licence, a stepwise approach is taken prior to any testing in animals. Hence in some cases the overall number of animals used can be reduced, and studies in test tubes may be suitable.

This process would start with studies in test tubes to compare the characteristics of the new product (the biosimilar) with the reference product (usually already marketed) to assess differences in the properties of the two materials. Only if there are differences in the responses of the two materials in test tubes, or the test tube studies were not deemed extensive enough to predict any differences in humans, would we consider using an animal model.

If the biosimilar comparability exercise for the physicochemical and biological characteristics and the studies in test tubes are considered satisfactory and no issues are identified which would block direct entrance into humans, an animal study is usually not considered necessary, and would not be performed. This obviously then reduces the overall number of animals that would be used.

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

Where possible, if the primary objective of the study will not be compromised and the animals' overall experience will not exceed the severity limit, additional endpoints may be added to assess pharmacokinetics (PK), immunology, safety pharmacology or efficacy (particularly for biologically derived pharmaceuticals) so that a separate study will not be needed. It is anticipated that the total number of animals for each compound's development programme will be decreased overall by the use of additional investigations during the toxicology programme

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

This project will use both adult and juvenile non-human primates (macaques). We only use non-human primates when other species (rats, mice and other large animals like dogs and/or pigs) are unsuitable for scientific reasons.

The models we use are the least invasive procedures, for the least amount of time we need to do them, to get the information we want. They are carried out using standard and recognised techniques by fully trained staff. We also have veterinary help on hand for advice and on the rare occasions we have to anaesthetise the animals.

For situations involving restraint procedures (e.g. in a chair or in a metabolism cage) the animals are habituated to this equipment starting with short periods, then building up. Most animals habituate fine to this equipment, but if they don't (rare) we remove them from the study.

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

Primates are only used when no other species is suitable to get the information we need. In fact, we have to prove that the primate is the only species that will give us the answer we need (instead of rodents or dogs or pigs) that will translate to the effect we would see in man.

Most of these studies require repeat dosing for days, weeks or months, to assess potential adverse effects in man, so it is not practical to perform them under terminal anaesthesia.

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

Animal welfare is of utmost importance and Good Surgical Practice will be observed for any animal undergoing surgical procedures. Surgery will be conducted using aseptic techniques (to prevent infection) which meet at least the standards set out in the Home Office Minimum Standards for Aseptic Surgery. Before we start surgery, we agree with a Vet what pain killers or antibiotics the animals need both before and after the surgery. When recovering from surgery, we give the animals extra heat and monitor them closely until they start behaving normally again. We then check them at least twice daily before they go on study.

We have introduced a newly designed restraint chair for animals which provides sufficient restraint but allows more natural movement, and comfort. We use this type of restraint during infusion dosing, and is the period of time the animal is restrained in the chair is limited on a daily basis.

During dosing and restraint, animals are constantly and closely watched for signs of distress.

All procedures are subject to ongoing assessment and technique improvement and we participate in cross-company working parties on best practice. Animals are regularly reviewed for general health and a veterinary surgeon are on call at all times to assess and relieve any adverse events.

Refinements to improve the animals experience include but are not limited to group housing, environmental enrichment, including novel toys and foods, human interaction, acclimatisation and training to procedures, to move around the cage and to leave the cage voluntarily as required, forage opportunity and calming measures such as stroking/gentle talking are used to help animals have a better experience of restraint.

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

Regulatory guidelines

Testing of pharmaceuticals for human health are performed in order to generate PK and ADME data to satisfy non-clinical requirements in drug development – International Congress on Harmonisation (ICH) M3(R2) Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorisation for Pharmaceuticals (ICH, 2010) and associated legislation.

ICH S6 (R1) (Preclinical Safety Evaluation of Biotechnology-derived Pharmaceuticals), which gives guidance on the type and design of studies for biological compounds.

EMEA/CHMP/BMWP/42832/2005 Rev 1 Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues.

For blood sampling and dosing then the following guidelines/literature will be followed:

First report of the BVA/FRAME/RSPCA/UFAW joint working group on refinement, Laboratory Animals, 27, 1-22 (1993).

A Good Practice Guide to the Administration of Substances and Removal of Blood, Including Routes and Volumes, Journal of Applied Toxicology, 21, 15-23 (2001).

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

This will be achieved by regular discussions with our Named Information Officer, colleagues in Animals Technology, and by attending appropriate training courses and conferences, or getting feedback from such events.

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

46. Genes influencing autoimmunity of the central nervous system

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

multiple sclerosis, immune cells, autoimmunity, therapy, genes

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

The aim of this project is to investigate how genes affect the development of multiple sclerosis-like disease, and how these genes may be targeted for therapeutic benefit.

A retrospective assessment of these aims will be due by 09 June 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?

Multiple sclerosis (MS) is a chronic autoimmune disease that affects the brain and spinal cord, leading to serious physical disability. It affects approximately 1 in 1000 people in the UK and poses a major personal, social and economic burden: the average age of MS onset is 30 years – a time that is decisive for work and family planning – and about 25 years after onset 50% of individuals require use of a wheelchair. The risk of developing MS depends on both inherited and environmental factors, but little is known about how these factors trigger the disease. Currently available treatments cannot prevent disease development and nor can they cure it; instead they have a modest effect on reducing disease symptoms, but they cannot ultimately halt disease progression. Therefore, there is an unmet need to identify more effective approaches for treating MS.

Investigating the functional effect of genes associated with risk for and resistance against MS development and progression can help to better determine how molecules and cell types of the immune system interact to promote or protect against autoimmunity of the brain and spinal cord and how they may affect the severity of disease. Building on data obtained from analysing tissue from patients and healthy controls, the use of genetically modified models can enable an understanding of the drivers of MS-like disease as individual risk factors can be investigated in the context of an entire organism in the healthy state and during disease in a way that cannot be performed by other methods. Obtaining this information is critical for assessing genes as potential therapeutic targets – either for the development of new treatments or for the improvement of existing approaches. Thus, although this is a basic biological research project, it is anticipated that the information generated will have a future impact in helping to inform clinical practice.

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

If successful, this project will be one of the first demonstrations that it is possible to identify a genetic variant defined as disease-associated, through the analysis of genome-wide association studies and the UK Biobank population-based cohort, and convert this into a novel therapy using genome editing to replicate the effects of the naturally occurring variant. As such, it opens up a new pathway that enables precise advanced cellular therapies to be developed from naturally occurring variants identified by large scale genomics projects.

By the end of this project the anticipated outputs are new information relating to:

- technical aspects of genome editing disease-
- relevant pathophysiological mechanisms
- potential drug targets, and potential
- therapeutic approaches

This information will be disseminated by publication in high profile and high impact biomedical journals. The proposed work is also likely to directly result in new intellectual property, and patent applications will be filed to maximise the commercial potential of outputs.

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

Academic beneficiaries

This research aims to open up a new avenue for the treatment MS, and given the immune genes and genetic variants being studied, also has implications for other immune-mediated disorders, which collectively affect 10% of the global population. Thus, in the short term (3-5 years) the mechanistic insights related to the function of disease-associated genes will be of a broad relevance to researchers in biomedicine, immunology, genetics and genomics.

Patients

In addition to the immunological and technological insights obtained, the therapeutic potential of the project in the development of novel treatment strategies may have direct implications for MS patients, as well as patients with other immune-mediated diseases. If successful the outcomes of this work could lead to new therapeutic approaches in the longer term (5-10 years); moreover, this approach could have a higher efficacy than currently available treatments, and with fewer side effects.

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

Collaboration

This project already involves collaboration with other academic groups, and further collaborations will be sought to help maximise the academic impact of the work. Experimental protocols generated through the project will be readily shared with the research community, and training of collaborating research scientists will be provided as required. Materials generated by the project will be made available to the scientific community upon request subject to an appropriate material transfer agreement being put in place. Any software developed during the project will be formally released onto widely utilised sites/platforms for software sharing amongst the academic community, so that the analysis of data generated can be performed quickly and easily.

Dissemination of new knowledge

The data and technological insights – whether indicative of successful or unsuccessful approaches – will be published in peer-reviewed biomedical journals, in compliance with open access policies, for dissemination throughout the biomedical, immunology and molecular genetics community. The work will also be presented at specialist international and UK conferences in immunology, genetics, genome editing and medicine. Prior to significant publications, relevant press offices will be contacted for preparation of press releases to the media, so that the dissemination of the research extends to as wide an audience as possible. Significant publications will also be disseminated through social media platforms and key findings will also be presented on institute webpages. Publications will be accompanied by an appropriate summary in the public science arena including the public engagement and outreach websites. Web-based platforms will be monitored for the extent of webpage activity and link sharing as means of evaluating the dissemination impact to wider audiences. Further dissemination to the general public and to patient groups, will be performed through participation in specific public engagement events and science festivals.

Patents and license agreements

Given the likelihood that the proposed work will result in new intellectual property, patent applications to cover any arising intellectual property will be drafted and filed using available mechanisms available through the institution.

Species and numbers of animals expected to be used

- Mice: 9000

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.

Choice of animal type:

The mouse is being used for this project because:

- the mouse genome of the inbred strain of interest can be readily manipulated to create genetically altered mice to study genes of interest
- the mouse circulatory system of the inbred strain of interest can be readily manipulated to study
- the effects of the genes of interest specifically in cells of the circulatory system multiple sclerosis-
- like disease can be induced and studied using the strain of interest

- years of research with mice have enabled development of optimised, refined methods for manipulation of the mouse genome and circulatory system and for disease induction, relative to other species

Species that are less sentient than mice cannot be used for this project as they must show substantial conservation of genes relative to the human genome, have highly analogous diversity of immune cell types and similarity in the structure and cellular composition of the brain and spinal cord), and have the capacity to develop a disease upon induction that mimics key aspects of multiple sclerosis pathology observed in humans (but over a much shorter time frame relative to the decades over which the human disease develops).

Choice of life stage:

Experiments will typically involve the use of adult mice. A more immature life stage of mice cannot be utilised for experiments where multiple sclerosis-like disease is induced as the induction and development of disease requires the use of adult mice as their immune system and brain and spinal cord must be fully developed. In addition, the multiple sclerosis-associated genes/genetic variants to be assessed are associated with disease in adults, and studying their expression and function in nonadult life stages may lead to inaccurate results and conclusions.

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

An animal in the project may be used as follows (from birth onwards):

- Bone marrow transplantation (irradiation and single intravenous injection at age 8 weeks; circulatory system near-full recovery by 3-4 weeks after injection; if multiple sclerosis-like disease is induced then this intervention is optional)

- Multiple sclerosis-like disease induction (subcutaneous injection at two sites on one occasion and intraperitoneal injection at a single site, under general anaesthesia on day 0; a second intraperitoneal injection up to 48 hours later; at age 13 weeks)

- Humanely killed at age 17 weeks (after disease developed, peak paralysis lasting about 48 hours was reached 2 weeks after disease induction, and there was partial recovery thereafter)

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

The maximal adverse effects expected are for animals that undergo bone marrow transplantation followed by induction of multiple sclerosis-like disease (although bone marrow transplantation preceding disease induction is optional and will only be performed for a subset of animals). The likely adverse effects associated with these interventions are as follows:

<u>Bone marrow transplantation:</u>

- Mice will be exposed to gamma irradiation to deplete the immune system and then they will receive an intravenous cell transfer of bone marrow cells to recover the immune system no later than 24 hours post irradiation.

- Weight loss (no more than 15%) will peak at about a week after cell transfer, after which the mice will recover

Multiple sclerosis-like disease:

- Tail weakness beginning about a week after induction, followed by hindlimb weakness, and tail and hindlimb paralysis (peak disease) occurring at about 2 weeks after induction. Peak disease typically lasts about 2 days after which recovery occurs

- Weight loss (no more than 20%) that will peak at about 2 weeks after induction after which mice will recover

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 (only animal type utilised in this project):

Sub-threshold: 60%; Mild: 7%; Moderate: 11%; Severe: 22%

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

- Killed

A retrospective assessment of these predicted harms will be due by 09 June 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?

Multiple sclerosis is a complex disease that involves the attack of the brain and spinal cord by immune cells, and about 200 different genes have been implicated in the development of the disease.

Experiments to fully understanding how these genes may promote or protect against disease, and how they may be modified for therapeutic benefit cannot be performed

directly in humans. However, such experiments require the capacity to manipulate the genome and the circulatory system (that gives rise to immune cells) and to investigate the interaction between immune cells and the brain and spinal cord. Ex vivo and in vitro systems cannot fully recapitulate this complexity, and hence animal models are essential to achieve the project aim. Moreover, as the mouse genome and circulatory system can be readily manipulated in inbred strains, and multiple sclerosis-like disease can be induced and studied using these strains in much shorter time frames than the decades it takes for the disease to develop in humans, the mouse presents the ideal species for this project.

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

Non-animal alternatives that have been considered for use in this project include those listed below, noting that the 'ALTEX: Alternatives to Animal Experimentation' journal was searched for alternatives relevant to brain research.

1) Human cell lines or primary human cells, including peripheral blood-derived primary haematopoietic stem cells and immune cells, cerebrospinal fluid-derived immune cells, and cells derived from induced pluripotent stem cells.

2) More complex in vitro cell culture systems, such as brain organoids, ‘brain-on-a-chip’ platforms

3) Post-mortem brain and spinal cord tissue from multiple sclerosis patients and controls

Why were they not suitable?

We are using some of the non-animal alternatives listed above, however, whilst these are useful for addressing parts of our overall project aim and can generate insights before embarking on animal work and also for refining the animal work, they do not represent true alternative options.

We routinely study multiple sclerosis-associated genes using human cell lines and primary cells derived from human peripheral blood to investigate how these genes affect cell type and function. Such investigations provide insights at the cellular level but do not extend to how the cells affect whole organs (specifically the brain and spinal cord). Moreover, although the cells can be activated, for example through the administration of stimulants, to try to mimic the activation that likely occurs during disease, such stimulations do not recapitulate the true complexity of the different stimulants and microenvironmental cues that cells would be exposed to within the whole organism, and which are not as yet fully characterised. Thus, the use of human cells and cell lines is a useful tool that will be employed in conjunction with this project, but which cannot replace the use of animal models.

‘Brain-on-a-chip’ (and organoid) platforms represent a more sophisticated culture system that more closely mimics certain structures present in the whole organ, such as the blood-brain-barrier. We will consider employing such chips as an alternative if the genes being

studied specifically affect the specific structures mimicked by these chips. However, our current understanding of multiple sclerosisassociated genes and of the development of disease pathology suggests that these platforms are likely to be too limited to recapitulate the complexity of the interaction between the peripheral immune system and the brain and spinal cord that is observed in patients.

We utilise post-mortem patient and control brain and spinal cord tissue to characterise markers of brain and spinal cord damage and repair and to try to infer the preceding mechanisms that lead up the observed damage. However, this tissue cannot be used to actively study these mechanisms and perturb the genetically-determined pathways that promote damage; instead in order to address this question we need to use an in vivo system.

A retrospective assessment of replacement will be due by 09 June 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 overall number of animals estimated for use in this project (a maximum of 9000) is based on the numbers of animals likely to be used for any single line of genetically altered animals across the three main protocols of this project, given previous experience with analogous experiments and use of experimental design tools.

For breeding and maintenance (first project protocol) we estimate that the maximum number of mice used will be 6000. We have used The Jackson Laboratory Breeding Colony Size Planning calculator tool (https://www.jax.org/jax-mice-and-services/customer-support/technical-support/breeding-andhusbandry-support/colony-planning).

For our experiments we estimate that the maximum number of mice used for each of our two experimental protocols is 1000 and 2000, respectively. These numbers have been estimated using the NC3R’s Experimental Design Assistant (www.nc3rs.org.uk/experimental-design-assistant-eda).

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

To reduce the number or animals being used in this project, we have carefully considered how to best design our experiments, to ensure that experiments are statistically well-powered and that bias is avoided, through e.g. randomisation and blinding. This means that the number of mice used per experimental group is not unnecessarily large but is appropriate for the hypothesis being tested given the potential effect size, and avoidance of bias reduces the likelihood of inconclusive results that might otherwise lead to the need for experiment repetition and thus increased numbers of mice being used.

To help optimise the experimental design we have employed the NC3R's Experimental Design Assistant (www.nc3rs.org.uk/experimental-design-assistant-eda), and taken into consideration the PERPARE guidelines (https://norecopa.no/prepare).

All experiments will be conducted in compliance with the ARRIVE guidelines (www.nc3rs.org.uk/arriveguidelines), to ensure maximal quality and reliability of research that is published as a result of this project, hence facilitating others to scrutinise, evaluate and reproduce our findings.

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

Best practices for good colony management will be employed to ensure that minimal animal numbers are used. A database that provides breeding data such as litter sizes, and that therefore helps to create good breeding programmes will be employed.

Induction of multiple sclerosis-like disease

Commercially pre-prepared immunisation kits which give a consistently high frequency of disease induction and a highly reproducible disease course will be employed to minimise the overall numbers of animals used.

Pilot studies

For experiments where the appropriate sample size cannot be formally pre-determined, for example when testing new reagents, techniques or technologies, pilot studies will be performed. This will help to minimise animal numbers used by ensuring that wasteful larger scale experiments with unforeseen complications leading to biases or inconclusive results are not performed.

Maximising information obtained from minimal animal numbers

We have optimised experimental protocols to ensure that maximal cell numbers can be obtained from any single animal, and that assays for cellular profiling use a minimal number of cells with maximal information output, for example by using methodologies that enable assaying of multiple markers or genes simultaneously, such as next generation

sequencing technologies. Such optimisations ensure that minimal numbers of animals are required for experiments.

A retrospective assessment of reduction will be due by 09 June 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.

Extensive research over decades has established the mouse as the most suitable animal model for multiple sclerosis-like disease, as the disease induced in mice mimics clinical and histopathological features of the human disease, thereby enabling the study of complex cellular interactions and signalling pathways that drive disease development and progression. Several drugs and treatment strategies currently used to treat multiple sclerosis patients were initially developed in the mouse model of the disease, further demonstrating the suitability of the species for the proposed project.

Given the research that has been amassed over time using the mouse model, key procedures used in this project, including induction and development of multiple sclerosis-like disease, cell transfer, and genetic modification have been most optimally refined in mice as compared to other species. For all these procedures we have also carefully considered how to refine them further with respect to the experimental methods and techniques employed, post-procedure care, housing, and monitoring to ensure that the mice undergo the least possible pain, suffering, distress or lasting harm.

The choice of mouse strain is based on its susceptibility to induction of multiple sclerosis-like disease, the ability to generate genetic modifications, and the capacity to perform cell transfers. The strain that meets these criteria is highly susceptible to induction of multiple sclerosis-like disease, and the disease course typically observed in this strain is the least severe single-phase form of the disease, compared to the relapsing-remitting or chronic progressive disease observed in other strains. The induction protocol of multiple sclerosis-like disease in this strain is well established and relies on the administration of a mixture of substances that stimulate immune cells to respond to components of the brain and spinal

cord, and the administration of a substance that renders the brain and spinal cord permissive to the entry of such responding immune cells. Once inside the brain and spinal cord these cells bring about damage of nerve cells that leads to the development of the multiple sclerosis-like disease.

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

Species that are less sentient than mice cannot be used for this project. This is because to study multiple sclerosis-associated genetic variants, the species utilised must:

1) show substantial conservation of genes relative to the human genome

2) show highly analogous diversity of immune cell types and similarity in the structure and cellular composition of the central nervous system (brain/spinal cord)

3) have the capacity to develop a disease upon induction that mimics key aspects of multiple sclerosis pathology and clinical manifestations observed in humans (e.g. paralysis).

Mice are the least sentient species that meets these criteria.

Mice that have been terminally anaesthetised cannot be used for all aspects of this project as after induction, symptoms of multiple sclerosis-like disease take approximately 7-10 days to begin to manifest, and for experiments involving cell transfer, haematopoietic system reconstitution and the impact of cell transfer cannot be ascertained within the short time frame of terminal anaesthesia.

A more immature life stage of mice also cannot be utilised for experiments where multiple sclerosis-like disease is induced: the induction and development of disease requires the use of adult mice as their immune system and brain and spinal cord must be fully developed. In addition, the multiple sclerosisassociated genes/genetic variants to be assessed are associated with disease in adults, and studying their expression and function in non-adult life stages may lead to inaccurate results and conclusions.

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

Induction of multiple sclerosis-like disease

We will use the subcutaneous route to introduce the reagents that induce disease as it is the least invasive route and we will use short-term general anaesthesia. Typically, we use the flank of the mouse as this gives the best results in terms of disease induction. Animals are always closely monitored to ensure full recovery from any general anaesthesia used.

Multiple sclerosis-like disease course

We will use the least harmful, single-phase disease model to reduce the need for a higher degree of suffering. Animals will be regularly monitored and provided with added housing enrichment to minimise welfare costs.

Irradiation for immune system depletion

For experiments where the immune system needs to be depleted by irradiation, the required dose will be divided into two exposures rather than a single large dose as this has been shown to reduce adverse effects. Animals will be regularly monitored and provided with added housing enrichment to minimise welfare costs.

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

In order to ensure that experiments are conducted in the most refined way, the published best practice guidance that I will follow includes that available from:

- www.nc3rs.org.uk https://norecopa.no
- https://www.lasa.co.uk

I will also liaise with the Named Information Officer if seeking additional best practice guidance materials.

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

To stay informed about advances in the 3Rs I will attend internal 3Rs meetings and will regularly check the www.nc3rs.org.uk and https://science.rspca.org.uk websites. To help implement these advances effectively the NC3Rs regional manager may be contacted for further support.

A retrospective assessment of refinement will be due by 09 June 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?

47. Production of blood products for scientific use

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

Horse, Sheep, Blood, Microbiology

Animal types	Life stages
Horses	adult
Sheep	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?

The aim of the project is to provide a regular source of fresh sterile donor blood products from horses and sheep. Clinical and veterinary laboratories use the animal blood as a nutritious supplement for the manufacture of of culture media for the identification of microorganisms.

A retrospective assessment of these aims will be due by 06 June 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?

Microbiology/Pathology laboratories have established methods for the detection of pathogenic organisms by recognition of the growth characteristics and colony morphologies of the different organisms on culture media containing defibrinated blood. These classical microbiology methods are well established and although new media types are more readily available culture media products containing horse and sheep blood are essential for the diagnosis in microbiology/pathology laboratories.

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

The primary output of the project will be a consistent supply of good quality defibrinated horse blood products for use in pathology / microbiology testing.

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

Microbiology/Pathology laboratories have established methods for the detection of pathogenic organisms by recognition of the growth characteristics and colony morphologies of the different organisms on culture media containing defibrinated blood. These classical microbiology methods are well established and although new media types are more readily available, culture media products containing horse and sheep blood are essential for ongoing diagnosis in microbiology/pathology laboratories. The products supplied over the duration of the project will help ensure ongoing diagnosis within pathology / microbiology laboratories.

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

The shelf life of the blood products are maximised to ensure wastage is kept to an absolute minimum, and the bleeding processes are refined to ensure optimum volumes, with minimal impact to animals.

Species and numbers of animals expected to be used

- Sheep: 2000
- Horses: 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.

Classic microbiology methods rely on the availability of Horse and sheep blood. Similar to activities in Human Blood Donor clinics, Blood sampling from a superficial blood vessel is performed at regular intervals on healthy mature animals.

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

Healthy mature animals enter the bleeding programme where they are re-used throughout the term of the licence. Defined quantities of blood are removed from each animal at regular intervals, by qualified staff.

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

The adverse effects will be mild. Procedures have been designed to limit the suffering to donor animals. This includes refinement of the bleeding equipment and facilities to ensure animals are less stressed, and ensuring the bleeding process is efficient and ensures as little physical damage to donor animals as possible.

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 impact of the procedures are mild. Procedures are established to keep infection and wounds to an absolute minimum for each animal type.

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

- Kept alive
- Rehomed

A retrospective assessment of these predicted harms will be due by 06 June 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?

Classical clinical Microbiology relies on the supply of horse and sheep blood as they are major materials used for the manufacture of microbiological culture media. The blood is used in the media to help determine the haemolytic reactions in pathogenic bacterial species. This is an essential tool in the diagnosis of pathogenic bacteria and there is no effective alternative at present.

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

There are still no non-animal alternatives available. Classic microbiology methods still rely on the availability of defibrinated horse and sheep blood.

Why were they not suitable?

Classic microbiology methods still rely on the availability of defibrinated horse and sheep blood to determine the haemolytic reactions of pathogenic bacteria in culture media products.

A retrospective assessment of replacement will be due by 06 June 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?

These numbers are based on previous data and expected changes in demand.

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

The overall design of the project is unchanged, although refinements have been made over the past 30 years to ensure maximum efficiencies, with as few animals as possible.

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

The bleeding programme has been refined over more than 30 years to ensure only the minimum numbers of animals are used. The programme ensures the correct amount of blood is taken each time and that waste is minimal. All blood volumes are confirmed gravimetrically. Equipment is calibrated.

Shelf lives of blood have been established. The programme is managed to ensure fully trained staff adhere to their procedures keeping costs and animal numbers to a minimum.

A retrospective assessment of reduction will be due by 06 June 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.

With over 30 years experience, and data, we can see that the impact to the animals is mild, with little distress or lasting harm. The facilities and equipment have been refined to ensure the bleeding process is fast and efficient, and our livestock generally live long and healthy lives as part of the programme. Horses are bled in social groups alongside horses they are comfortable with. They are fed while in stalls during bleeding and the stalls are positioned to allow the horses to see outside. Sheep are bled in groups of 4. They are tethered securely to prevent movement and harm. The bleeding is fast so that that sheep are only held for the minimum amount of time before being released back to the flock.

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

Experience has shown that the most suitable animals are healthy, mature animals of the sizes defined in the programme. These animals are only mildly affected and generally live long and healthy lives.

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

Since the initial design and implementation of the programme, refinements have been made in all areas to minimise harms. These include changes to buildings, new more advanced equipment, improved monitoring systems, changes in feeding and care routines, and improvements in animal selection. Our Farm management team will continue to embrace new ideas, and advances to ensure animal harm is kept to a minimum.

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

The practices that are in place have been established over 30 years in co-operation with Home Office Veterinary expertise and ASPA guidance.

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

Our QA and Technical teams regularly review regulatory and ASPA guidance and communicate changes across the wider business as part of our Quality Management System procedures. Training needs are also reviewed and updated accordingly to ensure staff are aware of and able to implement advances and changes effectively.

A retrospective assessment of refinement will be due by 06 June 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?

48. Rodent toxicity, tumorigenicity and safety studies with chemicals

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

Rodent, Industrial Chemicals, Agrochemicals, Biocides, Food Additives

Animal types	Life stages
Mice	adult, aged, embryo, juvenile
Rats	adult, aged, embryo, juvenile
Hamster	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?

This licence authorises the conduct of studies in small laboratory animal species (rats, mice and hamsters) with the aim of evaluating the toxicity and tumorigenicity (ability to cause cancer) of non- pharmaceuticals (agrochemicals, biocides, food additives

/foodstuffs, ingredients of house-hold chemicals (where legislation allows) and industrial chemicals). This is to aid in the development of new chemicals, and to provide mandatory information to regulatory authorities to allow marketing approval (i.e. to show that they are safe when they come into contact with humans).

A retrospective assessment of these aims will be due by 08 June 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?

Governments require (and the public expects) that substances we are exposed to are safe or that their potential hazards are well understood and documented.

The data generated from the studies performed under this project will be used to inform decision- making processes on substances under development and, where appropriate, to satisfy governmental regulatory requirements necessary to gain marketing authorisation or product registration.

This safety assessment is of immense importance along with other non-rodent and non-animal studies in demonstrating to governments and the public the safety of these substances or highlighting their known hazards and safe handling.

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

This project licence authorises the conduct of in vivo safety studies in laboratory small animal species to evaluate candidate molecules and novel and currently-registered substances in terms of systemic toxicity, toxicokinetics, local tolerance and/or the potential to cause or influence development of tumours.

The overall benefit of this project is that it generates high quality data that is acceptable to regulatory authorities and enables internal decision making within our clients' organisations. This project will also ensure that chemicals and pesticides that the general population are exposed to are safe.

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

Manufacturers of chemicals will benefit, as the data generated will allow them to progress their substances under development and, where appropriate, to satisfy governmental regulatory requirements necessary to gain marketing authorisation.

The studies ensure that non-pharmaceuticals such as food additives, agrochemicals and industrial chemicals that the human population are exposed to during their lives are safe or that their hazards are known as that they can be handled safely.

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

The work will be shared with chemical manufacturers who will use it to determine their future strategy, or for submission in documents required by regulatory authorities. Whilst we have no direct control over what happens to the data after we have shared it, we trust from information given to us that it is used for, or to support, regulatory purposes (e.g. to show that a certain chemical is safe for human exposure).

Species and numbers of animals expected to be used

- Mice: 35000
- Rats: 50000
- Hamster: 2000

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.

Most of our experiments will be carried out on conventional adult mice and rats as these are the smallest relevant species that we can use that have body systems that are comparable to humans. In some specialist cases we may use the hamster because what we are trying to find out is better performed in that particular species rather than in the rat or mouse.

The only other time we would use a species other than a mouse or rat is to continue work that has been previously done in that species. For instance if previous work, and results gained, had been carried out in a hamster, it would make no scientific sense to start the next stage of a programme of work in a rat or a mouse.

In some protocols we may use genetically altered animals. These animals may be more sensitive to developing tumours (which means fewer animals and a shorter study can be conducted). The use of genetically modified animals is low compared to conventional animals.

Occasionally we need to dose pregnant rodents with test substances, to provide animals for other toxicity studies that have been exposed to these substances before birth. These studies are rare and for specific regulatory purposes.

All life stages used are mandated by the regulatory test that the individual study is run under.

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

Typically, on this project, animals are dosed over a period of time with test substances, and usually sampled (e.g. blood or urine) before having tissues taken after they have been humanely killed for extensive toxicology analysis. Studies would range from a single dose, to those which last a matter of days (much less than a month) although some can last for 1, 3 or 6 months, and sometimes up to 2 years (to specifically examine whether a test substance can induce cancer). Study durations are dependent on the specific regulatory test being performed. Some animals are left dose free for a few weeks after dosing is complete to see if any effects of the test substances can be reversed.

Dosing of animals is commonly done orally using a flexible tube. For agrochemicals, the test substance is most commonly added to the food to mimic human exposure. Other common routes include inhalation (when animals are dosed in specially designed tubes) or dermal to mimic potential human exposure. Less common routes are by injection using a syringe and needle, maybe directly into a vein or under the skin.

Blood samples are usually taken from easily accessible veins, for example, in the neck or the tail of rats or mice. We are limited to how much blood we can take at once or, cumulatively, over a month. If we need a large blood sample, we would do this when the animal is anaesthetised and we would not let them recover consciousness.

Where possible, we try and take as many of the tissues and samples we need after the animals have been humanely killed after all dosing had been completed.

Rarely, some animals we use are genetically altered, if required, to make toxicity testing more relevant (and often shorter).

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

When dosing an animal by injection or taking blood, the amount of pain an animal feels is similar to what a patient would feel having an injection done by a doctor. If we have to repeatedly inject animals using a needle and syringe, we would choose different sites to do this where possible. If we can take blood samples when an animal is deeply unconscious then we do. If we need to take repeated blood samples or need to dose repeatedly then we try and use different sites. Of course everyone who performs these procedures are trained to a high standard.

Routinely we need to take a urine sample for analysis, so we would then put an animal into a special cage which is smaller than their normal cage. The animal can still move around. Virtually every animal will get used to their new cage within about 15 minutes and are fine.

Dosing with chemicals may cause adverse effects in some studies. Experience shows that the majority (~65%) of animals are not expected to show any clinical signs of suffering (either no clinical signs or normal background signs expected of the rodent strain). A small percentage (~15%) may show transient subtle to mild clinical signs. Moderate signs of adverse effects may be seen in some animals (~20%), usually in the higher dose groups. Lethality and/or severe effects are not study objectives in any of the protocols within this licence, but for preliminary studies that may be the first animal studies with limited data available, a very small percentage of animals may inadvertently show severe findings before they are immediately and humanely killed.

We do observe our animals at least twice a day, and the people who do this know the signs when an animal is ill. If an animal is ill, we would check it more frequently, and get more senior staff involved in its care for advice, including vets.

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)?

On the last project, about 85% of animals displayed mild severity, and around 10% of animals were classified as having displayed moderate severity. This is because these studies can last between a few days and weeks to up to a year, and although the individual procedures are usually mild in nature on their own, the cumulative effects make them moderate overall.

It's impossible to predict the proportion of severities expected on a service licence like this, as this will be dependent on what study types we are asked to perform.

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

Killed
Rehomed
Kept alive

A retrospective assessment of these predicted harms will be due by 08 June 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?

There is currently no regulatory and scientifically acceptable alternative to the use of rodents in these studies. These studies are run to satisfy the regulatory requirements of governments around the world to ensure chemicals are safe for humans. These tests are very specific as to what they require in terms of testing in animals to ensure this.

We maintain a constant awareness of regulatory guidance and ensure that where non-invasive methods exist which fulfil the regulatory requirement they are used in preference to animal studies.

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

There are no other non-animal alternatives for the work being undertaken on this project. The regulations we are following will not allow safety decisions to be made on non-animal systems alone.

In vitro and in silico methods (test tube or computer work not using animals) are used in combination with animal studies to inform study designs and assist in understanding of potential toxicity but cannot yet replace in vivo (animal) studies.

Why were they not suitable?

Although there are test tube tests that can model some parts of how chemicals get into our bodies, and how our body deals with them, and can identify undesirable effects, for example, there is no series of test tube tests that brings all these complex happenings together, like we see in animals and humans.That is why we need to test chemicals in animals, as they have similar physiology and processes as humans, and that testing gives us a good idea what may happen if they were ever exposed to humans.

A retrospective assessment of replacement will be due by 08 June 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 we have used are based on figures of previous usage from previous projects, or a projection thereof (based on estimated incidence) based on requests received from chemical manufacturers in the past. It is, however, impossible to accurately predict the number of studies that may be performed, in the circumstances.

The regulatory guidelines we follow for each study usually indicate the number of animals in a study; otherwise, the number used is the minimum to achieve the aims of the study.

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

Studies are designed to provide maximal data and statistical power (where appropriate) from the minimum number of animals considering that it is better to increase the number of animals used to achieve the objective than to use too few animals and risk having to repeat the study. For regulatory studies, guidelines require the number of groups and animals per group to be adequate to clearly demonstrate the presence or absence of an effect of the test substance; core study designs are based on international guidelines where these exist. Otherwise reference is made to standard study designs with input from the Department of Statistics, where appropriate, to identify the optimum number balancing the need to achieve study objectives while avoiding excessive animal use. These internal designs are reviewed and updated in line with changing external guidelines and internal refinements that either minimise numbers or reduce severity.

Whenever possible, common species of animals are used such that a large amount of control background data is available. This reduces the need for large control groups.

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 try and get as many outputs as we can from a single animal where possible, without adversely affecting its welfare. So if we need to get several different samples, for example, we will often do that in the same animal, rather than use separate ones, when possible.

Before our main studies, we use smaller groups of animals to get an idea of the doses we need to use for the main studies. These studies are important as it gives us confidence that the doses we are using are correct prior to testing them in bigger groups of animals required by global regulators.

A retrospective assessment of reduction will be due by 08 June 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.

Most of our models involve dosing animals with test substances, and sampling them, with many outputs taken after the animals have been humanely killed. This is generally the least invasive set of procedures that can be done to give meaningful outputs to make scientific decisions about further tests, or to determine the safety of a test substance.

Throughout our studies, our animals are checked at least twice a day. This allows us to see over a period of time, whether dosing each individual animal is causing any adverse clinical signs. if this is the case, we can take action: get veterinary advice, add food supplements and extra bedding if needing, and even reduce dose levels or stop dosing completely.

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

Rodents (rats, mice and hamster) will be used in all of the studies conducted under this licence. Rodents are considered to be of the lowest neurophysiological sensitivity (their brain function and physiology) that will allow us to achieve the study aims and are considered suitable for predicting what's likely to happen in humans.

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

Many of the procedures performed on our rodents like blood and urine sampling, cause only transient distress to the animals. Blood sampling procedures are similar to and about as painful as having a blood sample taken by a doctor or a nurse. Blood volumes are kept to a minimum. Confining animals in special cages to allow us to take urine samples is similarly of little distress to the animals.

For inhalation dosing, where animals are restrained in tubes, training of the animals occurs for increasing periods prior to treatment commencing to accustom the animals. Dosing and

sampling procedures 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 and will be the minimum consistent with the scientific objectives of our studies. In addition, suffering will be further minimised by implementing clearly defined humane endpoints.

In addition, care is taken to provide as much environmental enrichment as possible. This includes plastic shelters in their cages, wood blocks and balls to gnaw on and push around; mice are given swings, mice and hamsters are given extra bedding for warmth and food supplements are given as appropriate.

In some tests we may use animals that are genetically altered, for example, transgenic mice because of their susceptibility to tumours. These animals are specially bred and don't display any harmful clinical signs due to their conditions.

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

For blood sampling and dosing then the following guidelines/literature will be followed:

Diehl et al. A Good Practice Guide to the Administration of Substances and Removal of Blood, Including Routes and Volumes, Journal of Applied Toxicology: 21, 15-23 (2001).

Gad et al. Tolerable levels of nonclinical vehicles and formulations used in studies by multiple routes in multiple species with notes on methods to improve utility. International Journal of Toxicology: 1-84 (2016).

LASA/NC3Rs: Guidance on dose selection for regulatory general toxicology studies for pharmaceuticals.

Guidance on the Operation of the Animals (Scientific Procedures) Act 1986. UK Home Office 2014

Regulatory guidelines.

This is not an exhaustive list and principally focuses on UK and EU documents:

EU Feed Hygiene Regulation (183/2005); EU Regulation 882/2004 on official controls for feed and food law (and animal health and animal welfare); EU Biocides Regulation 528/2012

Regulation (EC) No. 1907/2006 – Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).

Regulation (EC) No 1334/2008 of the European Parliament and of the Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for

use in and on foods and amending Council Regulation (EEC) No 1601/91, Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC.

Regulation (EC) No 1107/2009 of the European Parliament and of the council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC

OECD Guidelines:

- OECD 407 – Repeated Dose 28-day Oral Toxicity Study in Rodents
- OECD 408 – Repeated Dose 90-Day Oral Toxicity Study in Rodents
- OECD 410 – Repeated Dose Dermal Toxicity: 21/28-day Study
- OECD 411 – Sub-chronic Dermal Toxicity: 90-day Study
- OECD 417 – Toxicokinetics
- OECD 424 – Neurotoxicity Study in Rodents
- OECD 451 – Carcinogenicity Studies
- OECD 452 – Chronic Toxicity Studies
- OECD 453 – Combined Chronic Toxicity/Carcinogenicity Studies
- Summary of Considerations in the Report from the OECD Expert Groups on Short Term and Long Term Toxicology

US EPA OPPTS Guidelines:

- 870.3050 - Repeated Dose 28-Day Oral Toxicity Study in Rodents 870.3100 - 90-Day Oral Toxicity in Rodents
- 870.3200 - 21/28-Day Dermal Toxicity 870.3250 - 90-Day Dermal Toxicity
- 870.3465 - 90-Day Inhalation Toxicity870.4100 - Chronic Toxicity 870.4200 – Carcinogenicity
- 870.4300 - Combined Chronic Toxicity/Carcinogenicity 870.6200 - Neurotoxicity Screening Battery
- 870.7800 – Immunotoxicity
- 870.8355 - Combined Chronic Toxicity/Carcinogenicity Testing of Respirable Fibrous Particles 870.8500 – Toxicokinetic Test

Microbial Pest control guidelines:

- 885.3050 - Acute Oral Toxicity/Pathogenicity 885.3150 - Acute Pulmonary Toxicity/Pathogenicity 885.3200 - Acute Injection Toxicity/Pathogenicity 885.3500 - Cell Culture
- 885.3600 - Subchronic Toxicity/Pathogenicity
- Japanese Ministry of Agriculture, Forestry and Fisheries, 30 Shouan No. 6278. 2019.
- US FDA Redbook 2000, Guideline IV.C.3a – Short-term toxicity studies in rodents (July 2007),

- US FDA Redbook 2000, Guideline IV.C.4a – Subchronic toxicity studies with rodents (November 2003), US FDA Redbook 2000, Guideline IV.C.5a – Chronic toxicity studies with rodents (July 2007).
- US FDA Redbook 2000, Guideline IV.C.6 – Carcinogenicity studies with rodents (Jan 2006).
- US FDA Redbook 2000, Guideline IV.C.8 – In-Utero exposure phase for addition to carcinogenicity studies or chronic toxicity studies with rodents (July 2007).
- US FDA Redbook 2000, Guideline IV.C.10 – Neurotoxicity studies (7 July 2000).

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

This will be achieved by regular discussions with our Named Information Officer, colleagues in Animals Technology, and by attending appropriate training courses and conferences, or getting feedback from such events.

A retrospective assessment of refinement will be due by 08 June 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?

49. Metabolism and Pharmacokinetic Studies of Chemicals

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

Metabolism, Pharmacokinetics, Industrial Chemicals, Agrochemicals, Food additives

Animal types	Life stages
Chickens	adult
Beagles	adult
Mice	neonate, juvenile, adult, pregnant
Rats	neonate, juvenile, adult, pregnant
Guinea pigs	adult
Rabbits	adult
Hamsters	adult
Goats	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?

The aim of this project is to perform metabolism and pharmacokinetic studies to support the development of non-pharmaceutical compounds (including food additives and flavourings, Industrial Chemicals and Agrochemicals). These tests, commonly referred to as ADME studies, are designed to investigate the Absorption of test substance related material (how substances get into the blood), Distribution around the body, (including into

blood and urine), pathways of Metabolism (how the body breaks substances down), and routes and rates of Excretion (how the body removes the substance), by harvesting samples (blood, plasma and other bodily fluids, tissues and excreta) at different times after administration.

These studies will be performed for regulatory purposes to satisfy Global Safety Authorities that these substances are safe for humans if ingested deliberately (e.g. food flavourings) or exposed to them accidentally (e.g. Industrial Chemicals and Agrochemicals).

A retrospective assessment of these aims will be due by 10 May 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?

The primary benefit of work carried out under this licence will be to allow regulatory authorities (who are totally independent from the commercial interests behind every marketing application) to come to decisions regarding the distribution of new chemicals to which humans are exposed to, based upon safety data generated in these studies relating to exposure (pharmacokinetics) and ADME (metabolism) characteristics of the test compound. This will provide data supporting the rate and extent of absorption; distribution into tissues; key enzymes involved in metabolism; and the rates and routes of elimination; to assist in scaling safety margins of exposure and toxicity into man.

New agricultural chemicals may allow improved control of pests of food crops or of disease-bearing organisms. Testing of other chemicals may be necessary for new products that are designed to improve the quality of life. In all cases, the introduction of a new chemical may permit the removal of an older, more dangerous alternative, establish a new therapeutic approach or make an effective chemical more widely available, e.g. by reducing manufacturing costs. Testing of substances added to foods will ensure they are safe for human use.

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

The project will provide high quality, peer reviewed data for regulatory authorities, that will allow governments to decide whether the materials tested are safe for the public to use (Chemicals).

For Industrial Chemicals and Plant Protection Products, and other chemicals were there has been a regulatory call for data, there is an expectation, both public and governmental, that materials to which people/animals/environment are exposed should be safe to use or that their potential hazards are well understood and documented. This is a regulatory requirement, and testing of such materials will be performed to satisfy the needs of global

regulatory authorities under EC and OECD guidelines (including REACH, the Registration, Evaluation, Authorisation and restriction of CHemicals).

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

The primary benefit of work carried out under this licence will be to allow regulatory authorities (who are totally independent from the commercial interests behind every marketing application) to come to informed decisions, based upon data generated in these studies, regarding the risks and/or benefits when humans are exposed to chemicals.
The public will benefit from this data as will Sponsors, by obtaining data which allows them to make decisions on the development of their chemicals and to support regulatory filings.

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

Where confidentiality permits, data, study design and best practice will be openly shared at conferences, workshops, webinars, blogs and publications.
As 3R's benefits are also realised under this project licence, these will be shared more widely with other establishments.

Species and numbers of animals expected to be used

- Goats: 20
- Mice: 1700
- Rats: 1650
- Guinea pigs: 50
- Rabbits: 100
- Beagles: 100
- Chickens: 200
- Beagles: 50

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 majority of animals used during the course of this licence will be rodents (mice and rats). Adult animals will be used in all protocols; additionally neonate and juvenile rats may be used in specific exploratory studies. Scientific opinion, including that of the regulatory agencies (who are independent of governments), indicates the use of one rodent and one non rodent species for many of the metabolism and pharmacokinetic studies that are required, to allow chemicals to be properly assessed for safety and for potential testing in humans.

We also use goats and chickens to test the safety of agrochemicals (to protect the food chain). Using these species means we can also assess whether these substances can be found in milk and eggs as well as in blood/excreta and urine.

The most appropriate non-rodent species (dog) will be selected with reference to studies performed in rodents; in non-animal models evaluating cross-species metabolism and toxicity (similarities in effects between rodents and dogs); and pilot pharmacokinetic and

safety assessment studies. The species selected based upon this information is the one which is predicted to be most similar with human in terms of sensitivity, pharmacokinetics and metabolism.

The most widely used and best understood second species for metabolism studies is the dog. Dogs are only used where the purpose of the programme of work can only be achieved by their use. Under this project licence the use of dogs will be limited to the support of testing agrochemicals and plant protection products, and dogs will not be used in industrial chemical or food additive testing.

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

Typically, on this project, animals are dosed singly or over a period of time with a chemical, and sampled (e.g. blood or urine) before having tissues taken after they have been humanely killed. Most studies would last a matter of days (much less than a month) although some, occasionally may last for longer than that.

Most studies will involve animals being dosed with a chemical, and a series of sequential samples will be taken. These will usually be bodily fluids such as blood (generating plasma or serum) as well as urine/faeces and even expelled air. Dosing of animals is commonly done orally using a flexible tube, or by injection using a syringe and needle, maybe directly into a vein, or into a muscle into the arm or leg, or just under the skin. Blood samples are usually taken from easily accessible veins in the neck, leg or the tail. We are limited to how much blood we can take at once or over a month. If we need a large blood sample, we would do this when the animal is anaesthetised and we would not let them recover consciousness.

Some animals will have to be confined for periods in special cages (metabolism cages) to collect urine/faeces/air. They will usually have access to food and water, and adapt to these new surroundings well. To dose and bleed animals we often have to restrain them for their own safety for short periods, but this won't harm them.

In some studies, we also have to surgically prepare animals for testing. This is limited to rodents. These surgeries are needed to get specific samples (e.g. bile). We only surgically prepare animals when there is no other option to dose or collect a specific sample**.**

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

When dosing an animal by injection or taking blood, the amount of pain an animal feels is similar to what a patient would feel having an injection done by a doctor. If we have to repeatedly inject animals using a needle and syringe, we would choose different sites to do this where possible. If we can take blood samples when an animal is deeply unconscious then we do. If we need to take repeated blood samples or need to dose repeatedly then we try and use different sites. Of course everyone who performs these procedures are trained to a high standard.

Animals undergoing surgery receive the same sort of care as a patient would in hospital. We discuss their pain relief and use of antibiotics with a veterinary surgeon before we start. We administer drugs as necessary and give them plenty of time to recover from surgery before we use them in experiments.

We often need to take a urine/fecal sample for analysis, so we would then put an animal into a special metabolism cage which is smaller than their normal cage. The animal can still move around however, and we'd normally introduce an animal to this cage to acclimatise them to it. Virtually every animal will get used to their new cage within about 15 minutes and are fine.

Dosing with chemicals may cause adverse effects in some studies, although this is rare. We do observe our animals at least twice a day, and the people who do this know the signs when an animal is ill. If an animal is ill, we would check it more frequently, and get more senior staff involved in its care for advice, including vets. We also help sick animals out by giving more bedding, more heat and special food to make them more comfortable.

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)?

On the last project, about 52% of animals were classified as having displayed moderate severity. This is because legally, all surgical procedures carried out on an animal must be classified as moderate, and on occasions, there were prolonged periods of dosing and sampling required to get the information we needed (also moderate). The rest of the animals were classified as having displayed mild severity.

It's impossible to predict the proportion of severities expected on a service licence, as this will be dependent on what study types we are asked to perform.

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 10 May 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?

Metabolism is a very complicated process, and although there are metabolic tests that can be performed in-vitro (without the use of animals), there are no adequate models to replace the whole animal experimental model, as the complexity of the human body cannot be fully replicated in a test tube.

Similar international guidelines and directives are in place regarding agrochemicals and their ability to contaminate the food chain and industrial chemicals and their effect on the health and welfare of humans (especially if accidentally ingested).

Work condusted under this project will be for regulatory purposes, and this work is legally required by Global Regulatory Authorities. There are no accepted non animal tests that will allow the fulfilment of regulatory criteria alone, hence the use of animals is required.

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

There are some studies that can be carried out in-vitro that can be used to support ADME work including tests that assess metabolism and absorption of substances, and how well they bind to key proteins in the blood. Predictive software can also be used.

Why were they not suitable?

None of these tests can yet model the complex and integrated mechanisms governing the ADME of new chemicals fully and hence, animal testing is still a requirement.

A retrospective assessment of replacement will be due by 10 May 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 used on this project is estimated from those used under previous projects and after consideration of regulatory trends, and in review of future services being developed and offered for the selection of new chemicals for regulatory studies.
Studies will be designed under this licence such that the minimum number of animals will be used in order to obtain the maximum information, whilst the scientific objectives of each study are met, in accordance with regulatory requirements and agreed standard practices.

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

We have a wealth of experience and knowledge regarding regulatory requirements and the industry standards used, hence we are in an ideal situation to influence study designs when discussing study requirements, and will always consider ways of reducing animal numbers. We also have available professionally trained statisticians to help design studies, especially where the test compound is a biological and use of statistics can provide a guide as to the best number of animals required to determine if a novel new compound is

a statistically acceptable biosimilar to an original molecule. Standard study protocols are reviewed by the AWERB against known guidelines and the Company's ethical compliance policies.

In order to fulfil the requirements of the regulatory agencies, animal group sizes for metabolism studies are variable, depending on the chemical being used, the specific type of study being undertaken and the strain of animals being used. When dealing with agrochemicals for example, group sizes for rodents are usually 3-5. However, only a single goat will be used for each group and hen studies may consist of group sizes of 10 or more.

Furthermore, reduction in animal use continues in study designs where a number of candidate compounds (each in trace amounts) may be co-administered in a single (cassette) dose with consideration to avoid drug-drug interactions. This approach minimise the total number of animals required for pharmacokinetic studies. Notwithstanding the above, studies will be subject to ethical review and approval by the Project Licence Holder. Alternative blood sampling techniques (eg saphenous vein sampling) have allowed a reduction in the total number of animals required to generate the necessary data. Also, increased sensitivity of analytical techniques has allowed us to investigate a number of procedures that have allowed microsampling of blood in several species. These changes allow a more comprehensive profile to be generated from single animals (hence reducing the overall number of animals used), or by allowing a cross-over design to be used on smaller animals (typically rodents).

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 where a new substance is being tested in animals for the first time, we would often test that in a small group of animals (usually 1-3) to give us confidence that the dose levels we chose are safe, and the substance affects the system its designed to, without making an animal ill. These are called pilot studies.

We will try and get as many outputs as we can from a single animal where possible, without adversely affecting its welfare. So if we need to get a blood sample, or if we need to find the levels of a substance in say urine, for example, we will often do that in the same animal, rather than use separate ones, when possible.

A retrospective assessment of reduction will be due by 10 May 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 ADME study designs used are regulatory driven and we have been submitting data and reports to all of the major regulators for more than 30 years. Scientific opinion, including that of the regulatory agencies, indicates the use of one rodent (e.g. mouse or rat) and one non-rodent species (dog, goat or hen) for many of the studies that are required. Rodents are generally used to investigate metabolism, (occasionally dogs), and goats and chickens are used to examine for residues.

In some studies, a low dose of radioactivity is used (incorporated into the test material) as a marker to allow us to track the distribution of drug in the animal. This is only used on specific short term studies (mainly in rodents), and the radioactivity levels are designed to be safe to both animals and humans, and to cause no harm on their own.

Other species (hamsters, rabbits, guinea pigs, goats and chickens) may be periodically used on this licence, where their use is specifically indicated (for metabolic or pharmacokinetic purposes, or that they are a continuation of previous work already carried out in that species).

In order to minimise animal suffering, all dose levels used under this licence will be expected to cause only mild to moderate effects on animals. Trained animal technicians observe all animals (after surgery and/or post-treatment) at least twice daily and at pre-set post-dosing intervals. Senior technicians and vets are available to assist the Personal Licence holder in documenting reactions to treatment and deciding when and how to intervene to prevent further suffering. In the case of surgically prepared animals, these will also be observed at least once a day during the post-surgical period.

Refinements for blood sampling include the development of a non-restraint dependent method of bleeding in rats (via a peripheral vein). The increased sensitivity of analytical techniques has led to micro sampling of blood (in several species) has reduced the volume of blood required to be sampled from animals and allowed better data to be generated.

We have an ongoing programme that investigates refinements involving husbandry and enrichment for all species.

Animal welfare is of utmost importance and Good Surgical Practice will be observed for any animal undergoing surgical procedures. Surgery will be conducted using aseptic techniques (to prevent infection) which meet at least the standards set out in the Home Office Minimum Standards for Aseptic Surgery. Before we start surgery, we agree with a Vet what pain killers or antibiotics the animals need both before and after the surgery. When recovering from surgery, we give the animals extra heat and monitor them closely until they start behaving normally again. We then check them at least twice daily before they go on study.

Dog metabolism cages have been modified to allow dual or triple housing (where appropriate), thereby introducing companion animals within the experimental space. Previously animals were housed singly, and by having companion animals it reduces stress for all of the animals

Periodically, investigations may be undertaken to develop additional refinements for animal welfare or sample collection. These will only be introduced following approval by the AWERB, and following conduct of a validation study if appropriate.

All protocols in this licence fall within the moderate severity limit.

However, the majority of animals will only experience mild to moderate severity.

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

Rodents will be used in most of the studies conducted under this licence. Rodents are considered to be the species with a similar enough brain/nervous system and physiology that will allow us to achieve the study aims and are considered suitable for the predicting what's likely to happen in humans.

For other species, there would need to be specific scientific justification provided for them to be used. This may be, for example, that other studies investigating a particular chemical has already been carried out in that species, or there is a particular metabolic pathway that is only available in that species, or say that is the target species for the actual test substance.

The use of dogs will be driven by the need to fulfil regulatory requirements (agrochemicals and plant protection products ONLY).

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

Many of the procedures used in this licence are standardised, well defined and already well refined over many years. We will continue to assess any future possible refinements over the duration of this licence.

Where animals do show adverse clinical signs after dosing or surgery, we will increase the frequency and length of observations, and provide supplementary interventions (like extra bedding/food/heat) where needed, until the signs resolve.

Similarly, if after discussing with a vet and senior technician, we decide an animal is not recovering from procedures, and there is no prospect of them doing so in the near future, we will humanely kill them to prevent further suffering.

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

For any surgical interventions, then the LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery (2017) will be followed.

For blood sampling and dosing then the following guidelines/literature will be followed:

First report of the BVA/FRAME/RSPCA/UFAW joint working group on refinement, Laboratory Animals, 27, 1-22 (1993).
Diehl et al (2001).A Good Practice Guide to the Administration of Substances and Removal of Blood, Including Routes and Volumes, Journal of Applied Toxicology, 21, 15-23.

Regulatory guidelines

COMMISSION REGULATION (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market.

The testing of plant protection agents or biocides will be performed to satisfy European and global regulations; US EPA OPPTS 860.1300, OECD 417, 503, EU 7030/VI/95 and 98/8/EC with subsequent amendments.

Industrial chemicals require testing under the procedures for the Registration, Evaluation, Authorisation and Restriction of Chemicals [REACH] within the EU (OJEU L 396, 30 December 2006).

The investigation of chemicals and substances that are added to foods intended for consumption by man or by animals is the subject of multiple European Directives, often with subsequent amendments. Examples include Council Directives 89/107/EEC on food additives intended for human consumption
(OJEC L40, 11 February 1989), 70/524/EEC concerning additives in feeding stuffs (OJEC L270, 14 December 1970), 88/388/EEC on flavourings for use in foodstuffs (OJEC L184, 15 July 1988), 89/398 on foods for particular nutritional uses (OJEC L186, 30 June 1989) and 2002/46/EC on food supplements (OJEC L183 12 July 2002).

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

This will be achieved by regular discussions with our Named Information Officer, colleagues in Animals Technology, and by attending appropriate training courses and conferences, or getting feedback from such events.

A retrospective assessment of refinement will be due by 10 May 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?