Home Office

Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted July - December 2022 that require a retrospective assessment

Contents

1. Development of Novel Nanomedicines for Therapeutic, Diagnostic, and Regenerative Medicine Applications

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

nanomedicines, drug delivery, gene therapy, theranostics

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

This project aims to develop novel nanomedicines, packaging drugs in nano-sized drug carriers (i.e. nanoparticles), and explore the therapeutic/diagnostic potential of for their applications in cancer, neurological disorders, immunotherapy, cardiovascular diseases, and regenerative medicine.

Nanomedicines represents the next era in drug innovation as they can aid in drug delivery in a controlled and targeted manner, improving drug performance, reducing side effects by keeping the drugs out of tissues where toxicity may result, and concentrating them in the areas of interest to do the greatest good.

A retrospective assessment of these aims will be due by 12 January 2028

The PPL holder will be required to disclose:

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

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

Why is it important to undertake this work?

In this project, we particularly focus on the development of new therapeutics for diseases such as cancer, cardiovascular diseases, and central nervous system disorders which are debilitating and rapidly increasing in our community. Seeking new therapeutics, particularly by improving the delivery using nanoparticulate formulation, is of extreme importance. This is because they offer a more targeted delivery approach to the diseased site, reduced side effects and more desirable therapeutic outcome.

The work we propose will allow us to assess the drug distribution and safety profiles, identify, characterise the developed new treatments/diagnostic tools in reliable disease models in animals, and gain understanding of disease progression in response to treatments, enabling further clinical translation.

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

This project will advance our knowledge of nanomedicines to facilitate and maximise their applications in disease treatment, diagnosis and prevention. Findings from this project will be made available to other scientists through publication in peer-reviewed journals and presentation at scientific conferences and meetings. Research findings from this project will provide data for further applications to translational funding grants, bringing the research closer to clinical application.

Proposed research to be done under this project has the potential to generate intellectual properties with regards to novel formulation design, standardised manufacturing methods, and treatment regimens innovation etc. We will place legally binding agreements, aimed at capturing all relevant intellectual properties. A commercialisation strategy for the project will be developed using this experience by discussions, at the appropriate time, with contacts in the commercial world. Our previous work has led to patent filing, application and future applications to be submitted. Thus, we anticipate that we will develop novel and clinically applicable nanomedicines for therapy/diagnostic purpose within 5-10 years.

Who or what will benefit from these outputs, and how?
The beneficiaries from the above-mentioned outputs will be varied and include the academic, clinical and pharma sectors, and patients. The outcomes we generate from our

programme of work will provide new knowledge of therapeutic/diagnostic intervention utilising revolutionised controlled and targeted nanomedicines, providing further opportunities to drive academic excellence. Material, bioengineering and pharmaceutic science will be bridged together to explore how nanomedicine strategies can influence the drug delivery efficiency (e.g. by layering different materials to package drugs) and/or realise therapeutic/diagnostic synergy (e.g. by multicomponent formulation), and what key factors are important to achieve specific targeting (e.g. by surface engineering a targeting moiety). In the short-term, the research findings will be valuable to other peer academic researchers working in the multidisciplinary fields such as drug development science, bioengineering, biomedical imaging, oncology, neuroscience, cardiovascular disease, regenerative medicine.

Our work programme is focused on several unmet clinical needs. Using well-established disease models that mimic disease conditions in humans will allow us not only to explore the disease mechanisms in relation to treatment response and to develop novel treatments to improve therapeutic outcomes, but also allow clinical colleagues insight into the potential causes and management of disease for clinical translation in the next stage.

There are also potential advantages for the pharma industry, who remain highly interested in developing therapeutic agents for the targeting diseases proposed in this project that are debilitating. Developing effective nanotherapeutics may be costly and timely and will impact economically on health budgets/spending in the short-term. But the increasing medical necessity/relevance of nanomedicine will improve treatments and the success could impact on return to the health of patients and thus benefit economy in the long-term.

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

Publications in leading refereed scientific journals and presentation of research findings to major international nanotechnology/immunology/medical conferences will be the main routes to reach the scientific community. Unsuccessful approaches will be reported in publication, conference proceedings or preprint repository platforms (e.g. bioRxiv). We will contact the university's media office to make the discoveries as widely available as possible, such as publishing online as School/Faculty/College News, to outreach the general public. We will attend international scientific conferences to present and share the research findings. We will seek to establish new collaboration while networking with other peer scientists, to create new technologies and knowledge to complement each partner’s research strength.

We acknowledge that research to find out new treatment modalities is of significant patient and public interest. It is important to keep the scientific community, patient population and caregivers regularly updated through blog posts, open access publications and press releases, and engage with them to seek their views. We regularly invite schoolchildren to laboratories, on summer placements for example, to gain experience and raise public awareness. We will establish an online platform to engage with patients and caregivers to disseminate and discuss different elements of our research throughout this project.

Species and numbers of animals expected to be used

- Mice: 16100
- Rats: 2550

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 experimental procedures will be done in mice and will use rats in some experiments. Rodents are the ideal experimental hosts not only due to their size and short life cycles but because their genetic, biological and behavioural characteristics closely resemble those of humans, and many symptoms of human conditions can be replicated in rodents to a high degree of similarity. We use adult rodents in most of the protocols, as they can tolerate a large degree of therapeutic interventions. In the protocol to evaluate the treatment effects in genetically modified neurodegenerative mice, juvenile, adult, and aged mice will be used to study neurodegeneration and brain repair across these life stages. Juveniles can be aged and with age express more of the relevant phenotypes.

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

The purpose of the project is to develop novel nanomedicines, packaging drugs in nano-sized drug carriers, to improve the drug performance as potential new treatments or diagnostic tools for a range of diseases. Candidate nanomedicines which prove to be safe and effective in cells will be investigated for their behavioural improvements and therapeutic efficacy in animals. Selected nanomedicines will be administered in non-diseased animals initially to assess the distribution and toxicity profiles. Only the most promising nanomedicines will be further assessed in diseased animals which are used to model therapy in the clinic.

For drug tissue biodistribution and safety assessments, animals will typically be administered with studied drugs, their developed nanomedicines, or empty nanocarriers, by a variety of routes, usually by injection via a tail vein. Animals will undergo non-invasive whole body imaging assessments as used in human patients under general anaesthesia and imaging will be repeated over the study period.
Animals will also have blood samples taken either from the tail vein or by sampling from a heart chamber under anaesthesia (in which case the animal does not regain consciousness before humane termination). The duration of the experiment is usually up to 7 days after administration for drug tissue distribution studies and up to 12 months for drug safety assessments.

For evaluating the anti-cancer effects of the developed nanomedicines, an animal will be challenged with a tumour. Murine cancer cell lines can be transplanted into rodents sharing the same genetic background without rejection. Human cancer cells will be transplanted into immunocompromised animals to establish the tumours. For superficial tumour models, cancer cells will generally be implanted under the skin or sometimes surgically injected into mammary fat pads of female animals (e.g. for breast cancer model). For metastatic/internal tumour models, cancer cells will be injected into the blood stream to study cancer spread (metastasis) or intraperitoneally or surgically implanted into the relevant organ of tumour origin. Whenever possible, the anticancer effects of the nanomedicines will first be evaluated in a superficial tumour model prior to testing in a metastatic model. However, in some cases (e.g. targeting lung metastasis), it may be necessary to evaluate the nanomedicines in the metastatic model without prior evaluation in a superficial cancer model. Tumour growth will be monitored regularly by either use of callipers for superficial tumours, or by imaging methods for internal/metastatic tumours. Animals will be treated with nanomedicines or appropriate controls by a variety of routes

as identified previously in non-diseased animals and may receive repeated administrations. Animals will be studied for up to 6 months after a period of therapeutic agent treatment for tumour growth. Animals will have blood samples taken as described previously.

For developing nanomedicines for targeting/treating liver fibrosis, mice will be introduced with liver damaging chemicals to express liver fibrosis. It is a process when the liver is exposed to repeated injury; scars can form and persist. In this project, mice will be induced to express early-stage reversible liver fibrosis by pharmacological interventions through intraperitoneal administration for up to 4 weeks. Prior, during or after the development of the chronic liver fibrosis, animals will be administered single or multiple doses of the nanomedicines or appropriate controls by a variety of routes as identified previously in non-diseased animals. Throughout the course of the study, peripheral blood may be drawn periodically to assess levels of enzyme markers of liver damage and biomarkers of immune response to assess the effectiveness of liver regeneration. Animals will be studied for up to 6 months after a period of therapeutic agent treatment for liver fibrosis.

For developing nanomedicines for targeting/treating neurodegenerative diseases, genetic and pharmacological models of degenerative diseases will be used. For the genetic neurodegenerative

model, the phenotypic signs will be mild and animals will be kept until maximum of 24 months of age. For the latter lesion-induced model, animals will undergo a brief surgical procedure which involves injecting neurotoxins into the brain to cause brain damage (neurodegeneration) and the animals will be aged out typically to around 6 months old. In this model, animals will experience moderate levels of severity as involving surgery and injection of substances into the brain. Animals will undergo mild behavioural tests to look at how well their brain works. These will include tests of memory, and tests for mobility. Animals will be treated with developed nanomedicines by a variety of routes as identified previously in non-diseased animals. Animals may undergo imaging one time under anaesthesia with recovery (for cross-sectional comparisons e.g. model vs. wild type) or multiple times longitudinally to measure a given feature or abnormality overtime. Throughout the course of the study, peripheral blood may be drawn periodically, and cerebrospinal fluid may be sampled under surgical anaesthesia to assess levels of nanomedicines and/or biomarkers of disease progression.

One of the aims of this project is to develop nanomedicines for targeting/treating myocardial infarction, a condition with a blockage in blood flow, causing heart tissue damage. An animal will typically be induced to express myocardial infarction by surgical interventions (e.g. tie of a coronary artery to produce a myocardial infarct). The muscle layers and skin are closed after surgery and the animals are to recover for a period of up to 14 weeks after the myocardial infarction operation. The animal will be treated with developed nanomedicines for modulation of myocardial infarction by a variety of routes as identified previously in non-diseased animals. The animal may undergo non-invasive whole body imaging assessments as used in human patients under general anaesthesia and imaging will be repeated over the study period.

At the end of any protocol animals will be humanely killed. Occasionally, animals may be administered organ preservative whilst under non-recovery anaesthesia to allow us to undertake investigations on slices of selected organs observed under a microscope.

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

The majority of the animals are not expected to show signs of adverse effects that impact significantly on their general well-being such as normal behaviour to move, eat or drink. In the protocols to assess treatment efficacy in diseased models, due to the onset of the diseases and the side effect of therapy, some animals may experience some discomfort and express abnormal behaviour that does not prevent eating, drinking and other normal activities. These animals will receive supportive treatment and will be humanely killed at the earliest opportunity when study objective has been reached or if the animals begin to deteriorate, whichever comes first. There is a risk that we may observe toxicity with treatments. To limit this, we will use the doses that have previously been shown to be well tolerated using the route of least severity and the minimum number of administrations to produce the anticipated therapeutic effect. If the monitoring described above indicates toxicity, we will reduce the dose and/or stop treatment.

Below are the specific adverse effects associated with disease development for each diseased animal.

- Cancer

Cancer growth within an animal, as in a human, can cause detrimental effects. The tumours can take between 2 weeks up to 12 weeks to form and the potential adverse effects of tumour growth at a later

stage are associated weight loss, hunched posture, or inactivity. Tumour growth will be monitored regularly, and we monitor weight and specific behaviours in the animals that would indicate pain or distress. For some procedures that involve surgery under general anaesthesia, such as implanting cancer cells into the relevant organ of tumour origin (e.g. brain cancer; breast cancer) or removing a primary tumour in order for a secondary tumour to grow, we will administer pain killers and monitor the mice closely during recovery from anaesthesia. In the unlikely event that we observe effects of the tumour on the behaviour of the animals, or significant weight loss (>15%), or if the tumours ulcerate, the animals will be humanely killed.

- Liver fibrosis

Mice will receive liver damaging chemicals over up to 4 weeks to cause liver injury, an early-stage reversible liver fibrosis. Mice will be re-inspected one hour after induction of chemicals for abnormalities and every day thereafter. Mice that receive the treatment schedule limited in this protocol usually do not develop noticeable complications. All animals in control and liver fibrosis groups will be closely monitored for signs of illness or discomfort, and animals reaching moderate severity limit will be humanely killed.

- Genetic neurodegenerative disease

Genetically modified mice will be bred as part of the project, checked shortly after birth for the presence of a human gene which causes disease. Some will be kept as breeding stock to generate new experimental animals. Some animals will be taken forward for experimental work which will be aged out typically to around 2 years old as we study diseases of the ageing brain. The genetically modified mice bred under this project are expected to have only mild phenotype. This means that the animals will look, behave and appear normal, and any behavioural or phenotypic signs will be mild, such that can be picked up only by behavioural testing or prolonged observation/video recording and

scoring (eg. cognitive inflexibility, decreased working memory or slight reduction or imbalance in locomotion). Any animals that show unexpected moderate phenotype such as obviously reduced locomotion, seizures, signs of stress or prolonged sickness behaviour will be killed immediately. A proportion of the animals will be allowed to grow old and develop symptoms reminiscent of a neurodegenerative disease of old age, such as motor deficits and memory loss. For aged animals (greater than 12 months) we expect some to develop age-related conditions, much like humans do. These conditions include fur loss or overgrown teeth and in some cases arthritis or sore patches on the skin which will be monitored closely, and animals will be used for experiments at the earliest stage possible. If these effects are only mild, and cause minimal distress, the animal may be maintained for several months; however, if any of these issues are thought to cause significant pain, discomfort or distress, the animal will be culled. Animals will be treated with developed nanomedicines injected into the body or injected directly into the brain/spinal cord using surgery, to attempt to cure the disease.
Animals will be very closely monitored following the onset of any symptoms and the disease will only be allowed to progress to a limited degree. Animals that receive substance administration under surgery will be monitored closely including weighing, clinical examination and body condition in consultation with the NVS, and any adverse effect due to surgical procedures or treatments. Animals will undergo non-invasive, mild behaviour tests which cause only minor and transient discomfort induced by the test itself or drug injection. Animals are observed throughout the tasks and monitored on return to the home cage to ensure there is normal home cage behaviour. Limits are placed on the duration and frequency of testing to reduce the harms to the animals.

- Lesion-induced neurodegeneration

In this model, animals will experience surgical procedure to induce neurodegeneration and may present a transient lack of motivation that is associated with the neurodegenerative process. Animals should be supplemented with nutritional hydrogel or diet gel inside the cage until they recover. This is also to prevent possible postoperative weight loss. Animals whose disease progression will be monitored closely, will be kept until they develop the disease symptoms which are required for a number of scientific objectives to be met. These signs can include weight loss or gain and changes in movement and behaviour which are expected to mostly resolve within 48 h such that after they will be detected only upon behavioural testing. The animals may show slight gait abnormality or tendency to veer to one side, but this is not likely to adversely affect their general welfare. We have a well-defined scoring system in place to recognise when these signs occur and allow clear recognition of when the humane endpoint is reached. Animals will be treated with developed nanomedicines injected into the body or injected directly into the brain under surgery, to attempt to cure the disease. Animals will be very closely monitored following the onset of any symptoms and the disease will only be allowed to progress to a limited degree. Animals that receive substance administration under surgery will be monitored closely including weighing, clinical examination and body condition in consultation with the NVS, and any adverse effect due to surgical procedures or treatments. Behavioural testing will be used to assess the disease phenotype. In most cases behavioural tests that will be applied to mice are mild, non-invasive and are not expected to cause any lasting distress or harm.

- Myocardial infarction

Inducing myocardial infarction has clear adverse effects on the animal. The procedures involve surgical opening of the chest and the induction of a myocardial injury by a sudden

closure of a coronary artery, which result in pain and other complications such as heart failure, formation of a blood clot inside a blood vessel and death. There is a risk of sudden death associated with cardiac surgery. The majority of sudden cardiac death will be during or immediately following surgery while the animals are being monitored for recovery, with a small proportion suffering sudden death due to cardiac insufficiency which cannot be predicted by clinical signs as with human patients, though increased monitoring used to mitigate. These animals will be closely monitored for signs of discomfort or distress, relating to cardiac insufficiency such as increased rate of respiration, and increased heart rate, breathlessness, inactivity and decreased feeding. Any animal showing these signs will be humanely killed if these symptoms do not approve within the time frame carefully set.

Most of the protocols are designated with moderate levels of severity because of the needs for animals to undergo prolonged anaesthesia for imaging or surgery procedures. Only the protocol using the myocardial infarction model, when the heart receive reduced supply of oxygenated blood, is designated the severity limit as severe in this project due to the involvement of heart surgery and the nature of the disease (high mortality rate). To alleviate the risk of adverse effects, all recipient animals will be carefully monitored to assess their health status.

Animals will be killed humanely at the end of the experiments.

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)?

Breeding of colony (mice) – mild.

Experimental protocols: Most studies (80%, mice and rats) will be classed as moderate severity. Over 15% of animals (mice and rats) should be subject to mild severity. Among the animals that undergo myocardial infarction operation (<5%, mice and rats), majority of the animals (3/4) will experience moderate severity and the ones that cannot survive due to operative complications (i.e. sudden death, 1/4) will experience 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 12 January 2028

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 the aims of this project because they have genetic and biological characteristics closely related to humans, making them a good model for the evaluation of the performance of the developed nanomedicines. The biodistribution and toxicity profiles are complex outcomes which depend on the interaction of the developed nanomedicines with blood, the immune system, and multiple biological barriers. The data will provide valuable information such as how long they stay in the blood? how much they accumulate in different parts of the body? could they reach the target tissues with sufficient amount to be effective? will the administration of nanomedicines induce immune responses that are not favourable?, which are crucial for further therapeutic investigation in disease models.

In this project, we have chosen the rodent models of diseases such as cancers, neurological disorders, liver fibrosis, and cardiovascular diseases. These models are well-established and properly designed in this project to mimic the human disease condition. Investigations using these models will allow cellular mechanisms of diseases to be studied in detail as well as the effectiveness of novel diagnostics and therapies.

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

We will make every effort to evaluate the therapeutic potential of developed nanomedicines in alternative in vitro systems using cultured cells prior to or in parallel to this project. In addition, we will

be using cell lines or cells isolated from human tissues to develop organoid disease models such as cancer to complement this research.

Why were they not suitable?

It is an unfortunate truth that testing drug’s therapeutic efficacy using cultured cells does not shed much light on the drug performance in the body due to the lack of the complex nature of biological environment. Many biological barriers (e.g. blood to tissues and tissues to tissues) and animals' physical conditions (e.g. immune system), for instance, have great influence on the transport/delivery of drugs. While making every effort to evaluate the therapeutic potential of developed nanomedicines in cultured cells, it is still necessary to assess the drug distribution and safety profiles, identify, characterise the developed new treatments/diagnostic tools in reliable disease models in animals, and gain understanding of disease progression in response to treatments, enabling further clinical translation.

A retrospective assessment of replacement will be due by 12 January 2028

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 are estimated from similar studies performed under the previous two project licences over the past 10 years or from collaborators/colleagues' experience for new protocols. The numbers of animals for each experiment are calculated based on effect size, known from previous studies or the literature, in order to make statistically sound conclusions. Where effects size cannot be pre-determined, pilot studies will be performed to establish preliminary data that can be used for further statistical calculation to determine an appropriate sample size for the definitive experiment.

Breeding of genetically modified mice can involve relatively large numbers of animals. The transgenic mouse breeding strategy aims to generate as few 'unusable' mice as possible. The research requires the use of both wild type and heterozygous mice and hence no mice are wasted as a result of our breeding strategy.

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

Experimental design is given priority and we have used our previous experience (results have undergone statistical analyses and published in peer-reviewed journals), published data from others, and the NC3Rs' Experimental Design Assistant' software. Appropriate positive and negative control treatments are included where necessary. Wherever possible, attempts will be made to reduce the number of animals used in order to address specific scientific questions, while making maximal progress in achieving the goals of the project.

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

Several imaging technologies are employed in this project to obtain comprehensive information in animals that can be translated into the clinic to treat humans. The distribution of the nanomedicine in the body can be captured and semi-quantified at different time points after administration using the same animal. Imaging approaches thus hugely reduce the number of animals needed to study the dynamic changes of nanomedicines in animals.

All procedures are carried out by well- trained personnel following standardised protocols to maximise the success of each experimental step, particularly for the surgical procedures that require rigorous training. We will use the experimental and the analysis methods that are designed to maximise the information obtained from the minimum resource. Continuous data analysis on results as they are obtained will allow us to continually refine the number of animals that are needed in order to achieve a statistically significant result. Unsuccessful drug candidates will be filtered out earlier in the research and development stage which will lead to a reduction in the net number of animals used.

Our breeding strategy is that animals are only bred as required to supply animals for experimental requirements and produce meaningful and useful results in order to answer the experimental questions.

A retrospective assessment of reduction will be due by 12 January 2028
The PPL holder will be required to disclose:

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

Refinement

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

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

Rodents (mice and rats) are animals to be used in this project for many reasons. Firstly, they have a well-characterised genome that is sufficiently similar to humans that give data applicable to human clinical studies. Secondly, it is possible to generate rodents with specific genetic modifications to study. Furthermore, there is considerable experience in the wider scientific community regarding the use of rodent models for drug development and delivery studies as the responses to a variety of traditional drug delivery are well characterised.

For the development of lesion-induced neurodegeneration model such as Parkinson's disease model, rats are the most commonly used animals compared to mice and are a reference point to help compare results with previously published data.

In the cancer models, immunocompromised mice are sometimes required when human cancer models are to be used. Tumour resection may be carried out to better model the human standard of care as surgical removal of cancerous tissues in many solid tumours is often accompanied by other conventional treatments such as immune, chemo or radio therapy. This will also allow administration of chemo/immune/radio therapeutic agents either prior to, during or post tumour resection, to model human adjuvant or neoadjuvant therapy. Tumour rechallenge following successful anti-cancer treatment or tumour resection may be performed to model cancer relapse, remission, or metastasis.
These procedures together represent a refinement method to better mirror the human therapeutic regime and also allow us to assess anti-tumour memory response, a key outcome in immunotherapy in particular.

Due to the likelihood that animals may undergo repeated anaesthesia (e.g. for imaging) and surgical procedures, the overall severity is moderate. In the case of genetically modified neurodegenerative disease model, there are occasions when it is necessary to allow animals to age in order to sufficiently model the human diseases. By leaving the animals to age (typically up to 24 months) the phenotype reaches a point where we can assess the full extent of neuronal degeneration. However, these are very few animals that might experience adverse effects of ageing. The myocardial infarction model is of severe severity as inducing myocardial infarction has clear adverse effects on the animal. It involves a surgical procedure to open up the chest and the induction of a myocardial injury, which results in pain and other complications including sudden death. Experience

with this model indicates that the expected harm can be controlled and minimised with resultant pathophysiology that can be used with human relatable data. Great care will be taken to reduce any distress or suffering in pursuit of scientific objective.

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

Rodents are the least sentient mammal species that is of proven relevance to accurately mimic both the anatomy and complex cell biology of the human counterparts. Less sentient animals (than rats or mice) will not be able to perform the behaviours we are studying, and thus such animals cannot be used.

In this project, adult animals will be used in most cases. Juvenile mice may be used to study the neurodegenerative changes in early brain development. Rat neonates will be used to obtain brain tissues.

Adult animals are chosen in many disease models such as cancer and neurodegenerative disease because these diseases cause morbidity mainly at advanced stages in humans. We cannot therefore use animals at a more immature stage of life if we are to study the aetiology of the disease. In lesion- induced neurodegeneration model, it is also required to use adult rodents in which the loss of the nerve cells and appearance of relevant symptoms mimic what is seen in patients. In cancer and liver fibrosis models, it is important to use adult animals where they have functioning immune systems (at least 6-8 weeks of age). By this age immune system responses have matured to a sufficient extent (that can fight a broad range of potential attacks against the body), and have immunological memory (the developed ability to quickly recognise something that the body has previously been exposed to, such as an allergen).

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

Animals will be given suitable bedding, shelter, chew-blocks, and nesting-material in appropriately- sized cages. Animal suffering will be minimised by appropriate environmental care, the use of general anaesthesia and analgesics in surgical procedures based on Home Office practices and veterinary advice, and implementation of clearly described early humane endpoints which are described clearly in the protocols. Several imaging techniques will be carried out to monitor the disease progression qualitatively. Diseased animals will be monitored by daily physically examination during the expected critical periods. Individuals performing any regulated procedures, particularly for surgical techniques, will be trained and supervised.

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

When designing experiments and sharing our results, we will follow the recommendations of the ARRIVE guidelines (Animal Research: Reporting of In Vivo Experiments), the PREPARE guidelines ((Planning Research and Experimental Procedures on Animals: Recommendations for Excellence), and the Experimental Design Assistant provided by the NC3Rs.
We will follow the Animals (Scientific Procedures) Act 1986 (ASPA) and the Home Office's Code of Practice for the Housing and Care of Animals Bred, Supplied or Used for Scientific Purposes. We will adhere to the guidelines published by Workman et al (Guidelines for the

welfare and use of animals in cancer research. Br J Cancer (2010) 102:1555-1577) for experimental design, best practice, and humane endpoints for cancer research in animals.
How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will regularly undergo reviews of our experimental and surgical procedures with our NACWO and NVS, and consult AWERB to ensure the work carried out under this project is conducted in the most refined way. We will also attend seminars on best practices in animal research that are held at the establishment or such events published on the National Centre for the Replacement, Refinement & Reduction of Animals in Research (NC3R) website for nearby (UK / Europe) opportunities. We will follow events, updated documents, and blog/news posts that are highlighted on the website (and in email newsletters) published by the NC3Rs.

A retrospective assessment of refinement will be due by 12 January 2028

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. Breathing in Health and Disease

Project duration

5 years 0 months

Project purpose

- Basic research

Key words

Respiratory rhythm generating circuit, Sleep apnoea, Cardiovascular disease, Dementia, Neurodegeneration

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

Retrospective assessment

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

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Contains severe procedures

Objectives and benefits

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

What's the aim of this project?

We aim to understand how complex breathing patterns can be generated under a whole host of pathological conditions, and the consequences when the system fails.

A retrospective assessment of these aims will be due by 19 February 2028

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?

Breathing is our first act upon birth, and the last action we complete before death. The first to last breath taken, is in fact, how we define someone's life. We don't love our children from their 'first blink', we don't love our partners until our 'dying thought', soldiers do not fight until their last 'heart beat', and nobody at a surprise encounter has ever said 'as I live and walk'. However we risk taking breathing for granted.

The breathing pattern is generated by specialised nuclei that control inspiration, post-inspiration, and expiration. Whilst much is known about the inspiratory oscillator, less is known about it's expiratory counterpart, and almost nothing is known about the controller of postinspiration. Unravelling the relationship between these nuclei would allow us a deeper understanding of the control of breathing. It would also act as a model oscillator for understanding other motor patterns that require multiple phases

When breathing goes wrong, put simply, you die. Often the loss of life in neurodegenerative disease occurs when the progression of cell death reaches the respiratory centres. Understanding how and why this happens may allow us to extend life in many disease such as Amyotrophic lateral sclerosis, Parkinson's disease, dementia with Lewis bodies, and Rett's syndrome. Furthermore progressive loss of the respiratory rhythm generating circuit is a natural part of aging, and any attempts at extending life will require preservation of the microcircuit for breathing.

Furthermore, failure of breathing often leads to significant repercussions in other bodily systems. Sleep-disordered breathing is linked to many other illnesses and disorders, such as heart failure, stroke, liver disease, atherosclerosis, metabolic syndrome, diabetes, dementia, and cancer. We wish to understand the influence of breathing on these disease types, particularly in the cardiovascular and neural systems as these are often the most costly both to the individual and to society as a whole.

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

We expect to create a deeper understanding of respiratory rhythm generation, and how dysregulated breathing can act as comorbidity for many diseases.

We expect to publish these findings in peer reviewed journals.

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

The point of death in many neurodegenerative diseases is the point that they reach the respiratory centres. Without understanding how the respiratory couple to produce a breathing rhythm, little can be done to help these patients. The current gold standard drug, riluzole, which is marketed as a life extending intervention for amyotrophic lateral sclerosis (ALS) only extends life by 2 weeks. By better understanding the respiratory centres we may be able to help these patients, as well as patients with debilitating breathing patterns, such as Rett syndrome suffers. Finally, loss of respiratory centre neurons is a natural part of the aging process and any hope of increasing our longevity in a healthy manner will have to include prolonging the life of these neurons.

Neurodegenerative and cardiovascular disorders are the 2 leading causes of death in the population. With an ever aging populations and an increased longevity of patients these numbers will steadily increase. There is a strong correlation between these 2 disorders and inflammation. During sleep apnoea (greater than 2 missed breaths during sleep) patients experience episodes of hypoxic (decreased oxygen) and hypercapnic (increased carbon dioxide), which activates inflammatory pathways. Cognitive performance is also linked to sleep-deprivation, and sleep apnoea leads to frequent usually incomplete, arousals, act to terminate the apnoea. As the arousals are incomplete, patients with sleep apnoea are usually unaware of their sleep disturbances, and also the associated cognitive decline. As sleep apnoea occurs in ~50% of men and ~25% of women in the world and often goes undiagnosed, this is a pandemic problem that most people are unaware of.

Here I wish to use my mode to probe the effect of sleep disordered breathing on cognition and neurodegenerative diseases. To do this I will use behavioural tests to test different forms of brain function these will include: behavioural assays of spatial memory such as the Morris water maze, the radial arm maze, and the Barnes test, but will also include tests of the behavioural response to anxiogenic situations and non-spatial, non-anxiogenic, tests that are essential controls for the cognitive behaviour tests. We wish to establish the effects on the cardiovascular system by taking physiological measurements of cardiovascular output and assessing cardiovascular tissue for remodelling.

Benefits:

Patients with sleep apnoea suffer from fatigue and the neurological deficits associated with it, which is why people with undiagnosed sleep-disordered breathing are up to 5 times more likely to be involved in a motor vehicle collision than the normal population. Not only do patients with sleep-disordered breathing have a whole host of complications but treating them is also difficult, the potential for drugs,

particularly analgesia and anaesthesia, to causes them to go into respiratory distress is high, and the increase incidence of liver disease means these patients often have difficulty to metabolise drugs. The decline in cognition due to sleep deprivation may also be compounded by inflammatory signals, which are elevated in sleep apnoea patients. Not only have neurodegenerative disorders been linked with neuroinflammation, treatment of obstructive sleep apnoea slows the decline in cognition in Alzheimer’s patients. This

project will explore fundamental mechanisms of protein dysfunction in dementia, such as Alzheimer's disease and vascular dementia, disease progression, and the development of inflammatory responses in sleep apnoea.

Patients with Sleep apnoea have a higher risk of heart failure (4-fold increase) and stroke (3 fold- increase), and treatment of sleep apnoea alleviates this risk. Given that sleep apnoea leads to heart failure, which in turns leads to sleep apnoea creates a vicious circle where one disease exacerbates the other. This interaction is so strong that the 2 year survival rate for patients with severe sleep apnoea following cardiac arrest is only 15%.

By identifying the relationship between sleep-disordered breathing, and neurological and cardiovascular pathologies we hope to provide information that will help to stop the perpetual downward spiral in the health of these patients. We hope that this will lead to improved patient outcomes and the quality of life for patients, and their families and carers. By the conclusion of the project, we should have identified markers of disease progression for cardiovascular and neurological risk in our sleep apnoea model. In the future we hope that the molecular markers we identify here, and in subsequent projects, could be used clinically to improve diagnosis of people with sleep apnoea, and to tailor their care to try and alleviate or even prevent secondary illnesses caused by sleep apnoea.

There are seven potential benefits arising from this project. 1) Increased understanding of the neural circuit for breathing that may be applied to understanding the terminal phase of neurodegenerative disease. 2) A model oscillator system that could help us to understand other natural rhythms that contain multiple phases. 3) Increase in the understanding of the fundamental scientific principles underlying sleep apnoea, inflammation, cognition, and neurodegenerative and cardiovascular diseases. 4) A blood panel profile that has the potential to be used to assess; a) whether patients have sleep apnoea, and b) whether patients with sleep apnoea are at risk of developing cognitive deficits, neurodegeneration, or cardiovascular disease. This will be of great use to clinicians, and will lead to improved patient outcomes and quality of life not just for patients, but also their families and carers 5) Identification of potential therapeutics and/or therapeutic targets, and to develop relationships with pharmaceutical companies to gain access to drugs through material transfer programmes. 6) Raised awareness of sleep apnoea within the general public, to increase the number of people being tested for sleep apnoea and reduce the number of undiagnosed individuals. 7) New policies for health providers that take into account the risks from sleep apnoea and make them more aware of the benefits to the general public for increased screening, and the benefits to the tax payer as early detection may lead to a reduction in the cost of these patients to the NHS.

Benefit milestones

Immediate Benefit: Publicize the success of the grant through the internal website, local media, and public engagement events.

Intermediate term benefit: Disseminate our findings, to academic communities, clinicians, and pharmaceutical companies, and begin to build contacts and collaborations. Train the next generation of scientists.

Long term benefit: Create a cardiovascular and blood panel profile to determine at risk patients. Find new therapeutics to stop sleep apnoea from inducing other diseases. Increase awareness of this issue with the general public and policy makers to improve the diagnosis of sleep apnoea. Create a model of sleep apnoea that will become the tour de force for studying this disease.

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

Dissemination of information

This benefit is attainable in the short term and will come about by effectively disseminating the results of this study, and therefore informing the scientific community about this new model of sleep apnoea will be through publication of scientific manuscripts. I will also use the Physiological Society (PhySoc),and Society for Neuroscience (SfN), meetings to present my findings to scientific researchers. This will allow me to achieve impact with academics and to begin the process to make this model the primary model used for studying sleep apnoea. Our success in the academic world will be measured through publications, citations, and collaborations.

Development of diagnostic tools

This is a much longer term benefit, but I will set in motion the elements required for long term realisation of this benefit. I shall prepare the ground by consulting with the Tech transfer office to gain a better understanding of the IP issues around developing biomarker panel. I shall start to build relationships with clinicians, who are likely to be receptive to my ideas and with whom I would like to collaborate with in the future. Collaborations such as these will be essential to obtain proof of principle data in clinical studies that will be essential to raise funding for major trials over much larger patient cohorts. I will consult with industry experts (e.g. Sarissa Biomedical) to gain understanding of what is required to develop a biomarker panel. Success will be measured by development of the multidisciplinary team needed to develop a biomarker based approach and successful generation of follow on funding to pursue this goal.

Testing pharmaceutical interventions

To realize this benefit, I will have to develop industrial collaborations. I have begun this process by contacting Pfizer to obtain potential therapeutics through their Compound Transfer Programme. The goal would be to work with pharmaceutical companies to test new therapeutics, such as the orally bioavailable P2Y1 antagonists we will test in this proposal, to see if they can have an impact in reducing mortality and morbidity in our model of sleep apnoea and in patients. I also work with a team developing novel adenosinergic compound that targets specific adenosine receptors and may be a

therapeutic target Testing compounds for pharmaceutical companies may lead to inclusion in clinical trials, which would help us to further realise benefit 1. Our success here will be measured through industrial partnerships and through procurement of therapeutics from industrial contacts.

Increased public awareness

To raise awareness, I will get help from our press office. The university also has an ongoing commitment to public engagement. The findings of this project would also be presented to local audiences at the university's science nights and other public engagement events. We will engage with charities such as the British Heart Foundation and the British Lung Foundation to disseminate our information more widely to patient groups, and policy makers. Public Patient Engagement is a key part of the design of modern clinical trials and thus starting this engagement with patient groups will lay groundwork for developing diagnostic tools. I shall raise awareness of sleep-disordered breathing, to help increase the rates of diagnosis to well above the 10% we currently see clinically. Our measures of success here would be increased awareness of this issue by the general public, hopefully leading to increased diagnosis and treatment of this disorder. As milestones are reached, e.g., gaining funding, publishing papers etc, will be advertised on the University webpage, as well as announced through the University's press office.

Governmental policy change

My ultimate goal is to influence policy makers. This is a very long term goal that will come about validating our findings with patient data, discovering new therapeutics to alleviate sleep apnoea and its comorbidities, and raising awareness of this disease with the general public, charities, and at risk groups. By making regulatory changes we want to see improved screening of the general populace and improved treatment for patients.

Training

Post-Doctoral researchers and PhD students will receive science communication training, under the academic staff development programme at the University. They will also present their findings at public engagement events. Whilst I will be managing and at the forefront of realising these benefits, my post- doctoral researchers and PhD students will be expected to participate in writing papers, and to attend and present our findings at major national and international conference

Species and numbers of animals expected to be used

- Rats: 500
- Mice: 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.

Our experiments look at human diseases from mid to late stages of life. This diseases span the nervous, respiratory and cardiovascular systems. Therefore we require model organisms that have a central nervous and cardiovascular system, and most importantly inspiratory led breathing and a diaphragm. As such our model must be intact, so that it has complete systems, and mammalian in origin, as these are the only animals with a diaphragm. We use rodents, rats and mice, as these are the lowest animals on the phylogenetic scale, that fulfill these criteria.

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

The animals undergo a recovery surgery where we inject areas of the brain so that we may manipulate the way these areas of the brain work. We may also implant devices for telemetric measuring or drug delivery. The animals are allowed to recover. The animals then under a study that may last between between 1 day and 4 months.

Experiments that last 1 day are generally performed under terminal anaesthesia where breathing and cardiac output are recorded and different brain regions are manipulated to see how these output change.

For experiments that last up to 4 months the animals undergo non-invasive recording for 9 weeks to look at their respiratory and cardiovascular health. Then the animals undergo several tests of cognitive behaviour lasting ~3 weeks. Rodents then undergo a terminal procedure under general anaesthesia and tissue is collected for analysis. Rodents may have a second disease, and/or may receive a medical treatment.

Some animals will have dementia and or an underlying cardiovascular condition, which norammly develop in mid-life.

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

Immediately following surgery there is a risk of pain and infection, but these are managed with drugs. The animals may experience altered breathing, but this will likely go unnoticed. Some animals will see a reduction in their ability to perform some intelligence/cognitive tests, and deficits in short and long term memory.

Dementia models: Animals with dementia can display increased aggression, anxiety, depression, and seizures. The presence of these adverse effects in controls could affect our data. Therefore we only expect to see these if they are induced by sleep apnoea. Even then, we will minimise these conditions with handling and enrichment protocols. Animals with seizures are removed from the study.

Cardiovascular phenotypes: Animals with cardiovascular phenotypes have hypertension and cardiomegaly. This puts them at specific risk of drug overdose, fatigue, loss of appetite, and weight loss. We minimise these conditions by titrating drugs up in pilot experiments to get the dosage correct before larger cohorts are used. Animals are monitored regularly to look for weight loss, and more palatable higher calorific foods can be introduced. Animals are not forced to exert themselves and so limiting any chance of fatigue.

Inflammatory models: Animals with systemic inflammation may suffer from fatigue, loss of appetite, and weight loss. Animals are monitored regularly to look for weight loss, and more palatable higher calorific foods can be introduced. Animals are not forced to exert themselves and so limiting any chance of fatigue.

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 our previous returns due to the recovery surgery most animals will fall into the moderate category (~90%), though some with multiple diseases will fall into the severe category (~10%).

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

- Killed

A retrospective assessment of these predicted harms will be due by 19 February 2028

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 wish to study the effects of sleep apnoea on neuronal structure, function and cognitive ability across the life span, in health and disease. This study assesses how entire physiological systems interact over a period of time to lead to, or exacerbate, disease states. Unfortunately for this initial study there is no scope for replacement, as we cannot replicate the cardiovascular, respiratory, or complete neurological systems ex vivo, and it is unethical to perform these studies on humans, thus there are no suitable alternatives.

Where possible we will complement our in vivo work with studies conducted with in vitro or ex vivo brain tissue to maximise the data yield from each animal and to provide detailed cellular and molecular insight. If necessary we will screen drugs in cell lines before we inject them into animals. We continuously read the literature associated with our projects, and should any alternative to whole animal work that we deem appropriate for use on this project, we will endeavour to replace our in vivo work with said alternative work model.

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

Unfortunately there are none for testing the neurological and physiological systems. However, any pharmaceutical intervention will be modelled and tested on cell lines, reduced preparations, and terminally anaesthetised animals before being used in awake behaving rodents.

Why were they not suitable?

You can not replicate these systems using any alternative strategy

A retrospective assessment of replacement will be due by 19 February 2028

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 these numbers based on previous animal numbers used on other project licences held by the applicant

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

The experiments are designed so that following the instrumentation/transfection of the brain etc, all experimental parameters can be assessed chronically using non-invasive methods, e.g., plethysmography, telemetry, behavioural tests, inscopix microscope in behaving rodents. Therefore, a single animal can be used for multiple experimental time points, significantly reducing the number of animals used. In addition, at the end of the study, animals may be used in a non-recovery or an ex vivo experiment, to identify the

mechanisms that link these pathological disorders. By using a single animal for both the behaving and non- recovery experiments we halve the number of animal used, moreover we can assure that pathological conditions have occurred before the non-recovery experiment takes place, thus all terminal experiments will provide meaningful data: again optimising the use of the animal numbers employed in the project. We will also make all tissue, not used by the primary investigator in the respective studies, available to other investigators, so as to reduce the need to repeat procedures for different studies.

If necessary we will use the NC3R's research design tool and where appropriate we will publish in journals which support 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?

We plan to subject experimental animals to a number of behavioural tests. This will allow us to correlate the effects of sleep apnoea with cognitive function and disease progression in models of disease with a range of spatial and non-spatial cognitive functions providing valuable intra-animal comparisons whilst reducing the overall numbers of animals used in this study.

Furthermore, intelligent choices of model animals can also contribute to reduction. For example the effects of breathing disorders on cardiovascular health and cognition can both be assessed in spontaneously hypertensive rats. By utilising models that can be used for more than 1 objective, we are able to further reduce the number of animals used.

When dissecting the respiratory network, we can transduce multiple neuronal sub-types in the same animal. Not only will this reduce the number of animals need to test all the subtypes in the network, it will also allow us to understand how these subpopulation interact is if activate/supress them in tandem.

A retrospective assessment of reduction will be due by 19 February 2028

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 experiments will be performed on rodents, normally rats (though we may use mice if there is specific genetic advantages). We will use the following models.

Recovery surgery on whole animals for neural transduction and device implantation. There is no alternative to this, but we have an extremely effective analgesia regime that provides excellent pain relief without causes aversion or dysphoria.

Non-invasive recordings on whole animals, including Plethysmography (breathing), tail cuff blood pressure (cardiovascular output), behavioural equipment such as mazes and object recognition. These cause no harm to the animals, who in my experience often see these events as enrichment. Animals must be sentient to perform mazes. Cardiovascular and respiratory networks are significantly altered

Non-recovery terminal procedures to test the respiratory and cardiovascular circuits. As non-recovery procedures they cause no pain or suffering to the animal

Ex-vivo tissue will be collected from animals used in chronic non-invasive recordings. This will be used for; electrophysiology, immunocytochemistry, immunohistochemistry, and immunological assays. These cause no harm to the animal.

Stroke prone spontaneously hypertensive rats (SP-SHR): These rats are an excellent model of haemorrhagic stroke, and the subsequent dementia that ensues. All models of haemorrhagic stroke display similar levels of pain, suffering, distress, or lasting harm. However, as SP-SHRs are well characterised and so greater detail can be extracted from the results.

Spontaneously hypertensive rats: These rats are an excellent model of non-haemorrhagic cerebral small vessel disease and the subsequent dementia that ensues. Haemorrhagic stroke comprises only a small proportion of the stroke population. SHRs are a well characterised model of the vast majority of stroke patients.

Hyperhomocysteinemia: These rodents are an excellent model of atherosclerosis and the subsequent dementia that ensues. Atherosclerosis is a precursor to almost all forms of vascular dementia. This model therefore not only recapitulates the early stage of most vascular dementia, but does so with little side effects beyond early stage dementia.

Genetic models of Alzheimer's disease: There are many types of genetic mutation that lead to Alzheimer's' disease. Whilst many models recapitulate the different facets of Alzheimer's, none by themselves can replicate all of the different phenotypes. We use the models that recapitulate the most phenotypes or those they contain the most frequent mutations.

Left descending coronary artery occlusion: This perfectly replicates myocardial infarction and death of the left ventricle myocytes. There are currently no better models.

Vascular banding: This represents low blood flow through major arteries, such as that caused by emboli or atherosclerosis. The banding can be placed on any large artery to replicate numerous vascular diseases

Vascular stiffening: this can replicate emboli in small vessels. It is similar to vascular banding, but can be performed on small vessels for which banding is unsuitable

Isoproterenol injections: Replicates advanced heart failure in humans. It is non-invasive and so removes all surgical complications

Zymosan injections: Replicates a low grade chronic inflammatory response, more representative of mild to lower moderate sleep apnoea.

LPS injections: Replicates a high grade chronic inflammatory response, more representative of upper moderate to severe sleep apnoea.

Peripheral nerve denervation: Peripheral nerves may be sectioned to replicate therapeutic treatments.

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

We require mammals to model the mammalian respiratory circuit. Given we are looking at respiratory control and diseases of the aging the rodents must be adults, and usually aged to mid life.

We are using reduced or less sentient preparations wherever it is appropriate.

Rats are preferred as: they a closer to humans in phylogeny, and so are more translationally relevant; are larger allowing for more accurate injections; and, are more intelligent and less anxious, providing better data for cognitive testing.

Mice are used when specific genetically altered lines prove advantageous to answering a specific research question

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

Any animals bought in are body scored and weighed regularly from when they arrive until they undergo surgery to make certain that they are fit enough to begin the experimental protocol. The animals are then weighed, body scored and thoroughly assessed for 2 weeks or until they reach a stable weight for 3 days with a minimum of 7 days of assessment following any surgery. The animals are then body scored and weighed weekly during the experimental period to ensure they are not displaying signs of pain, distress, suffering of lasting harm, and that they are able to continue the experimental protocol.

We have an extremely effective analgesia regime that provides excellent pain relief without causes aversion or dysphoria.

All animals are acclimated to the experimental equipment to reduce stress.

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

Our animal welfare practices are often ahead of the standard. Our protocols and practices have been introduced to multiple UK universities as well as some in the US. We endeavour to continue to be at the leading edge of animal welfare in scientific studies.

We will use ARRIVE guidelines where appropriate, and review new information from the LASA website.

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

The widespread use of these animals in cellular, molecular and behavioural neuroscience ensures that species-specific tests can be conducted, and that standard and widely accepted behavioural and in vitro analyses can be performed rendering results comparable across labs. These tests have been developed over decades by researchers who specialist in behaviour. As pain, distress, anxiety, depression, etc, are all confounding factors that can render the results of these test uninterpretable, they have been developed to minimise all possible deleterious effects on the subject. We will consult with the literature and specialists in the field to ensure we are using the most up to date techniques and experimental designs. To further improve our results and the welfare of our animals, appropriate acclimation to the behavioural tests, including frequent handling of test subjects will be utilised to minimise animal distress or anxiety. For anxiogenic tests, the minimum period of time capable of inducing distinct morphological and functional changes in neurons will be used.

The process of refinement will be ongoing and assessed continually as new data arrive. During my PhD we began with an observation in vivo, and once we had homed in on a region we looked at this phenomenon in more detail, and discovered the cell types and a vital protein involved in the behaviour we were assessing. We then looked at the sensory and release mechanism in more detail by using electrophysiological and dye-loading techniques in cell lines, where we identified the salient sensory signal; furthermore we found that the entire sensory and release mechanisms were located on the same protein. We then came full circle and tested our hypothesis in vivo. If we are able, we would like to follow this kind of approach, were we use more reduced preparations or replacement strategies to inform our in vivo work, reducing animal numbers whilst improving our understanding of the subject.

Furthermore, the terminal experiments performed here will be used to refine our approaches in the chronic studies.

We also are in regular contact with out NIO, NACWO, and NVS, with whom we discuss our procedures and the latest techniques.

A retrospective assessment of refinement will be due by 19 February 2028

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. Provision of Blood and Tissues

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

Primate, Blood, Tissue

Animal types	Life stages
Rhesus macaques	adult, juvenile
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?

To obtain blood samples from healthy animals housed in their home groups within a breeding colony. Data obtained will be used to inform future or on-going studies by refining tests and defining the normal range in multiple clinical read-outs before any separate investigative study occurs.

A retrospective assessment of these aims will be due by 10 February 2028

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?

Expected benefits of this project licence are to enable the provision of non-human primate (NHP) blood on a regular basis and other tissues as they become available to support a wide range of research programmes and to provide materials either for existing diagnostic tests or to support the development of novel laboratory tests.

This information is vital to inform the conduct of scientific studies involving NHPs.

Tissues and blood from macaques that are of high health status are a rare resource and their similarity to the equivalent human tissue gives added relevance to any tests or research that uses them. In many instances equivalent human tissues cannot be obtained or are rarely available. This work will help to refine our understanding of the natural diversity of numerous parameters within healthy populations, and any natural age-related changes which can be detected. Such research is aimed at reducing human suffering by understanding infectious and non-infectious disease pathogenesis or producing life-saving vaccines, interventions or therapeutics.

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

Expected benefits of this project licence are to enable the provision of non-human primate (NHP) blood on a regular basis and other tissues, as they become available, to support a wide range of research programmes and to provide materials either for existing diagnostic tests or to support the development of novel in vitro tests. This may include a profile on natural degenerative processes in aging to help understand and mitigate disease susceptibility. This material and information is vital to inform the conduct of scientific studies involving NHPs.

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

Tissues and blood from macaques that are of high health status are a rare resource and their similarity to the equivalent human tissue gives added relevance to any tests or research that uses them. In many instances equivalent human tissues cannot be obtained or are rarely available. Such research is aimed at reducing human suffering by understanding disease pathogenesis or producing life-saving vaccines or therapeutics.

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

The research programs supported with the blood and tissue samples provided from this licence involve worldwide collaborations. It is expected that research findings will be published to further the knowledge in the relevant fields.

Species and numbers of animals expected to be used

- Rhesus macaques: 150
- Cynomolgus macaques: 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 samples generated under this licence will provide blood, tissues and organs with a close similarity to human tissues, that can be used in a wide range of laboratory experiments or tests. Samples taken will be from animals (Juvenile (post-weened) or adult) that are an integral part of a UK breeding colony, with no additional animals being bred specifically to provide them; thus the same number of animals required to form the breeding and issue stock will additionally provide material that will replace the use of other animals for that specific purpose. An example of this is the intention to provide material for cell culture, tissue and organ culture, thus allowing the generation of authenticated primary or immortalised cell lines for distribution for use in medical science and healthcare laboratories.

There are no alternatives to using NHPs for the provision of this tissue. It is anticipated that by being able to provide high quality tissue to appropriate research teams; we can create a resource that replaces the requirement for additional animals (e.g. control groups) to be used.

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

Small volumes of blood will be taken and always less than 10% of the circulating blood volume. These procedures will be conducted by experienced licence holders. Experience shows that no adverse effects are expected and the level of severity will be mild. However, animals will be monitored after sampling to ensure that there are no adverse effects.

Tissue samples may be taken from macaques which have been terminally anaesthetised and then drained of blood. Tissue requests will be collated and pooled until a suitable animal is identified to be killed for colony management purposes e.g. reached the end of its breeding life, sustained injuries that require the humane killing of the animal on welfare grounds. At no point will an animal be killed purely for the harvest of requested tissue.

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

Transient stress due to induction of sedation/anaesthesia may occur during procedures. This will be minimised by use of rapid-acting sedatives or anaesthetics appropriate for the procedure and species based on experience and veterinary advice and by providing a suitable protective environment and level of observation until fully recovered.

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)?

100 Rhesus macaque - Mild - Protocol 1

100 Cynomolgus Macaques - Mild - Protocol 1 50 Rhesus macaque - Non-recovery - Protocol 2

50 Cynomolgus Macaques - Non-recovery - Protocol 2

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 10 February 2028

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 are no non-animal sources, or alternatives, for the blood, tissues and organs with a close similarity to human tissues, that will be provided in this project.

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

There are no alternatives to using NHPs for the provision of this tissue. It is anticipated that by being able to provide high quality tissue to appropriate research teams; we can create a resource that replaces the requirement for additional animals (e.g. control groups) to be used and assist in the development of micro-physiological systems to reduce the requirement for animal studies.

Why were they not suitable?

Not applicable - There are no non-animal alternatives

A retrospective assessment of replacement will be due by 10 February 2028

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 is based upon a reduced total to that in the previous licence application and based upon historic and future expected requirements.

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

Samples will only be taken from animals after completion and approval of a procedures request form that will require a full written justification for the work. Requests for tissue and blood will be coordinated/ managed to ensure the minimum number of procedures are conducted whilst maximising the use of the material i.e. requests will be consolidated through established project management structures. In this sense, a single sample from an animal is likely to fulfil several discrete requests.

Every effort is made to match breeding output with experimental demand for animals and numbers of animals produced will not be increased to solely match a specific demand for blood or tissue. It is envisaged that the use of samples from this colony will reduce the need for projects to acquire additional animals to provide control or naïve samples within their studies and the requirement to house them under more restricted experimental conditions.

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

Each request for tissues will be considered on a case by case basis. An application for blood or other tissues will be made by completion of a “Request form” in which the applicant will be asked to provide the following information:

The “primary purpose” for the procedure

Specific justification of why the use of NHP material is required

Why the use of animals is required and confirmation that all possible alternatives been considered

The completed form will be sent for review by the appropriate Named Animal Care and Welfare Officer, the Unit manager, and subsequently approved by the PPL Holder. Request forms will be retained and archived (irrespective of approval status) and will be available for review by the Home Office Inspectorate.

A retrospective assessment of reduction will be due by 10 February 2028

The PPL holder will be required to disclose:

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

Refinement

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

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

To avoid any undue stress, animals will be sedated before blood is collected from a vein near the skin surface. The volume of blood taken on any single occasion will not exceed 10% of total circulating blood volume based on body weight following well-established methods. Where the same animal is used to provide repeat samples, these will be taken at no less than 4 week intervals up to a maximum of three collections per year, and a total of 6 in-life. The animal must also not, on any occasion, have displayed any adverse effect (at, near, or after a bleed procedure). The classification of these in-life procedures is therefore considered to be “Mild” and the terminal procedure of blood removal followed by humane killing is considered as “non-recovery”

Taking samples from animals whilst they are still within their colony environment may be considered as a refinement as it either removes the need to transfer animals to the unfamiliar environment of experimental facilities or minimises the amount of time that animals are held in these more restrictive environments or removed from their social grouping. Animals that have had samples taken under this licence will be subsequently issued only to project licences where re-use is permitted and following documented assurance by the Named Veterinary Surgeon that they are fit for purpose.

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

These breeding colonies exist to provide experimental macaques for health-related research. Macaques are in many cases the best model for the evaluation of vaccine or therapeutic efficacy as their physiology and immunology is very similar to man. This similarity allows the application of tests identical to those used in human clinical trials. All animals present in the colony are there either as part of a breeding group or to provide experimental animals to UK Home Office approved projects. The work conducted under this licence aims to maximise and enhance information gained from these projects or from those where human tissues are unavailable, using animals from within the macaque breeding colony to provide control blood and tissues.

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

The ability to screen animals for existing antibodies to specific disease agents or to identify specific genotypes that pre-dispose to a particular outcome of infection will aid the allocation of animals to appropriate studies and will enhance study design. Other health checks may be conducted during sedation (eg. retinal scanning and hearing checks as quantitative indicators of aging)

Due to the limited availability of disease-free macaque tissues, the provision of samples will enhance the statistical validity of a wide range of research projects and will give added information on immune responses and histopathological interpretation of disease.

To avoid any undue stress, animals will be sedated before blood is collected from a superficial vessel with the use of rapid-acting sedatives/anaesthetics based on current veterinary advice. All animals will be placed in a suitable protective environment and monitored until fully recovered. The procedures will be performed by the animal's normal keepers.

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

Blood draw and anaesthetic techniques are constantly reviewed against industry best-practice and under guidance from a veterinary surgeon.

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

Active participation in committees and workshops, and ensure an up to date knowledge of published work associated with the use and care of non-human primates. Regular discussions and interactions with those in similar areas of work.

A retrospective assessment of refinement will be due by 10 February 2028

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. Companion Animal Vaccine Development

Project duration

2 years 0 months

Project purpose

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

Key words

Companion Animal Vaccine Development

Animal types	Life stages
Beagles	juvenile, adult, pregnant
Cats	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 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 main objective of this project license is to enable the development of new and improved vaccines for cats and dogs.

A retrospective assessment of these aims will be due by 14 January 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 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?

Companion animal ownership is thought to confer significant psychological as well as physical health benefits. Estimates put cat and dog ownership in the UK at approximately 8.5 million dogs and 7.4 million cats, the equivalent of 24% and 17% of households respectively. Vaccination is perhaps the single most important measure that can be taken to ensure these animals remain healthy; therefore, we are continually working to improve our companion animal vaccines and where possible, develop new ones.

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

The main output of the work carried out under this licence will be the licensing of new cat and dog vaccines, supported by publications. The main vaccines under development will be a new feline core combination vaccine (feline herpesvirus, feline calicivirus and feline panleucopaenia virus) with new feline leukaemia virus and Chlamydia felis components, and a new canine core combination vaccine (canine distemper virus, canine hepatitis virus, canine parvovirus and canine parainfluenza virus) with an improved canine parvovirus component.

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

The key benefit of this project will be the development of up-to-date companion animal vaccines that are safe and work well. Vaccination to prevent disease is preferable to relying on treatment once the animal becomes sick. Without vaccination, contracting disease can be debilitating or, at worst, fatal. This project will make sure that existing and newly developed companion animal vaccines protect the animal from currently circulating strains of disease without negatively affecting its overall wellbeing.

Ultimately, there are several key beneficiaries once a new vaccine is licensed and in use in veterinary practice:

• Cats and dogs: safe, up to date and efficacious vaccines benefit the health and welfare of the target animal.

• Pet owners: the psychological benefit of owning a cat or dog and the ‘humanisation of pets’ driven by the millennial generation have led to a generation of cat and dog owners who are more sensitive to animal welfare, social media reports of disease outbreaks and safety or efficacy issues in their vaccinated pets. It is therefore important that the portfolio is updated and increased with novel vaccines against new or emerging pathogens or new

combinations of existing vaccines to meet the high expectation of quality and convenience to improve the health of their animals.

- Other animals that can come in contact with cats or dogs and who are also susceptible to infection with the same pathogens will also benefit due to the reduced spread of the infectious agents, for example canine distemper virus affecting ferrets living in the same household.

- Conservationists: conservationists and zoo veterinarians often use cat and dog vaccines off label to protect endangered animals in conservation areas and exotic animals in zoos.

- Humans: dogs are increasingly viewed as an integral part of the family and have moved from ‘the barn to the bed’. This presents a much higher opportunity for zoonotic transmission and spread of infection, for example Bordetella bronchiseptica.

- The company: the interest of the company is to develop and market new and improved vaccines.

- In the short term the experimental animals are negative stakeholders/ beneficiaries since they are exposed to vaccination, challenge, sampling procedures and euthanasia.

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

After a new or improved vaccine is authorised by Regulatory Authorities, the company always aims to market new and improved canine and feline vaccines onto the global market for international benefit, and to publish the vaccines’ related information in journals. Global, regional, and local marketing conducts series of activities such as preparing product leaflets, liaising with small animal vets, presenting, and setting up stands at various conferences to launch and promote the vaccine.

Species and numbers of animals expected to be used

- Cats: 100
- Beagles: 225

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.

Cats and dogs (juveniles, adults and pregnant bitches) are vaccine target animals. Therefore, these types of animals have to be used to test any newly developed and improved vaccines for safety and efficacy.

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

The regulated procedures required by this licence are expected to be mild to moderate in severity; and limited to sedation (limited to some procedures such as intraperitoneal inoculation), inoculation, blood sampling, swabbing, and monitoring of temperature. The majority of these procedures are similar to those that a cat or dog would experience during an annual vaccination and/or veterinary check-up. The areas in which a moderate degree of suffering may occur are 1) some local reactions may last and be painful when touched for a few days during vaccine safety investigation; and 2) the onset of clinical disease following challenge with a pathogenic organism in efficacy studies.

Both feline and canine core vaccines will be given twice at 2 to 4 weeks apart for the first course of vaccination, the vaccinated animals will receive one vaccination in the second year. After that a booster vaccination will be given between one and 3 years later depending on vaccine components.

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

The regulated procedures required by this licence are expected to be mild to moderate in severity. To test vaccine safety procedures are limited to vaccination, blood sampling, swabbing and monitoring of temperature. Puppies are weaned as necessary to test safety in young dogs. The regulated procedures are similar to those that a cat or dog would experience during an annual vaccination and/or veterinary check-up. These procedures only cause minor and short-lived discomfort. However, some vaccines, especially with adjuvants, may cause some tenderness and/or minor swelling at the site of vaccination which may last a few days. These adverse effects are moderate in severity and may last up to 5 days.

To test vaccine efficacy, procedures are limited to sedation (in some procedures), vaccination, challenge, blood sampling, swabbing and monitoring of temperature. As can be seen in safety tests some vaccinated animals may experience some tenderness and/or minor swelling at the site of vaccination which may last a few days. In order to demonstrate to the regulatory authorities that a vaccine works, a number of vaccinated and non-vaccinated cats and dogs need to be given the relevant disease. Although vaccinated animals should be protected from disease and would only experience mild adverse effects, the non-vaccinated animals will succumb to the illness. Depending on the disease, they may develop clinical signs such as a high temperature, lack of appetite, depression, a runny nose, diarrhoea or vomiting. If an animal gets sick, we will nurse and look after it carefully.

Once the scientific objective has been obtained any sick animal may be treated to ease any suffering experienced or humanely euthanized to prevent unnecessary suffering. The moderate severity may last up to 5 days. Whenever possible, the cats and dogs will be re-homed at the end of the study 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)?

Overall moderate severity is requested for this licence. For both safety and efficacy, most procedures are expected to be mild, and some moderate. Approximately 90% animals in the safety protocol are expected to experience mild severity and the remaining approximately 10% are expected to reach moderate (as a result of local reactions). Approximately 75% animals in the efficacy protocol are expected to experience mild severity and the remaining approximately 25% are expected to reach moderate (as a result of local reactions and clinical signs caused by a challenge pathogen given).

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 14 January 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?

The aim of this licence is to develop vaccines for cats and dogs. During vaccine development a lot of work is done in the laboratory prior to testing the vaccine in the animal for which it is intended. We need to test the vaccine in the animal in order to ensure that it works and is safe.

Vaccines work by mimicking a microbe and teaching the immune system to recognise and destroy it. This protects the animal from disease if it encounters the genuine microbe in the future. The way in which a cat’s or dog’s immune system reacts to a vaccine or an invading microbe can only be confirmed by using the whole animal.

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

Vaccine virus or bacterium propagation and titrations are performed in cultured immortal cat, dog and other cells or bacterial culture media. In addition, sera from vaccinated animals are assayed for antibodies using in vitro cell based methods. Whilst serological parameters do not always correlate well with observed levels of protection, there is a good correlation for some, canine parvovirus for example. Therefore, wherever possible in vitro correlates will be applied. Virus titration in vitro will establish the compatibility of inactivated adjuvanted Bordetella with the live viral components.

Why were they not suitable?

Vaccines work by mimicking a microbe and teaching the immune system to recognise and destroy it. This protects the animal from disease if it encounters the genuine microbe in the future. The way in which a cat's or dog's immune system reacts to a vaccine or an invading microbe can only be confirmed by using the whole animal. Vaccine candidates need to be tested in target animals in order to ensure that they work and are safe.

A retrospective assessment of replacement will be due by 14 January 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 estimation is based on the number of studies done in the past per year, future planned and potential studies, and capacity of animal facilities.

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

The minimum numbers of animals required in safety and efficacy studies are governed by specific European Pharmacopoeia and European Medicines Agency guidelines. The number of animals used in each study will be guided by the legal minimum number required.

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 numbers of animals required in safety and efficacy studies are governed by specific European Pharmacopoeia and European Medicines Agency guidelines. The number of animals used in each study will be guided by the legal minimum number required.

A retrospective assessment of reduction will be due by 14 January 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.

As studies in the animal are the only sure way of showing that a vaccine is safe and works well, it is not possible to use an alternative animal (e.g. mice, rats) or computer model. The use of cats and dogs is therefore the most refined choice to fulfil the objective of this licence.

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

The ultimate test of safety for any vaccine is to administer the material to the target animal, which is the animal species for which the vaccine is intended. For a live vaccine both adverse reactions to it and immune responses induced by it are complex multifactorial events. As such there are no physico- chemical, cell line or organ explant assays that can reproduce what happens in the target species and therefore, animals have to be used. So, the efficacy of a vaccine can only be unambiguously shown in the animal for which it is intended.

Specifically the live attenuated viral agents will only infect their host species and therefore the correct degree of attenuation and hence safety can only be gauged by administration to that species. Likewise the efficacy can only be tested by administration to an animal in which the organism can replicate.

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

The severity of the regulated procedures required is expected to be mild in the majority of animals used in this licence, with a very low degree of pain, distress or suffering anticipated. Since adverse reactions to vaccination are highly undesirable (from both the animals' and owners' point of view), studies are largely conducted using vaccine formulations that have already been shown to be acceptable.

When the level of antibody present can tell us whether the animal will be protected from disease, this will be used in preference to challenge tests. However, in a small number of non-vaccinated animals, disease will be experienced. In these cases, at appropriate times, under the care of the attending veterinarian, animals will be monitored more frequently to check for humane end points to minimise any adverse effects. In addition, whenever possible pain killers and anti-inflammatories will be used to alleviate pain, and scientific end points will be used to end the study before HEP's are reached. Such additional checks and remedial actions will be documented in local records.

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

It is our policy to follow relevant guidelines (eg LASA, NC3R's) and best practices. As an example, we always use single use needles to the extent that we will often use one needle to withdraw an inoculum from its container and a then a fresh needle to inject it into an animal. Our team of NIO, NVS, NACWO's and experienced technicians gather varied new information to contribute to continuing improvements in our housing, enrichment, animal handling and early recognition and alleviation (where possible) of adverse effects. They are in regular contact with our Project Managers and Study Directors and are involved at the planning stage so that new advances and ideas can be incorporated into the protocol of every fresh study.

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

Our whole team is constantly looking for new advances in the 3R's. As with all establishments, we have a Named Information Officer to help gather and disperse relevant information. Our NVS regularly attends meetings on such topics as new advances in Animal Welfare Science, Ethical Reviews and general advances and practical tips for Veterinary Surgeons and Animal Nurses to improve the welfare of animals under their care. Our NACWO's and team constantly seek new ways of providing environmental enrichment (we have several times been used as examples of 'best practice' by HOI's for other establishments to follow).

A retrospective assessment of refinement will be due by 14 January 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?

5. Astrocyte Lactate Signalling in Health And Disease

Project duration

5 years 0 months

Project purpose

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

Key words

neuropsychiatric disorders, depression, anxiety, astrocyte, lactate signalling

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

Retrospective assessment

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 understand the role of astrocyte lactate signalling in regulating brain functions and behaviour in health and disease and to discover molecular and cellular mechanisms through which lactate works.

A retrospective assessment of these aims will be due by 06 March 2028

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?

Neuroscience research has mainly focused on studying the role of neurons in regulating brain functions in health and disease. However, non-neuronal cells called glia constitute about 50% of all brain cells. Astrocytes are the most abundant brain glia cells and their content in some brain areas can reach up to 20% of all the cells in the central nervous system.

This project will investigate a new pathway through which astrocytes contribute to brain functioning in health and disease. This project has a particular focus on the role astrocytes play in regulating behavioural responses to environmental stimuli. This is a process that goes awry in a number of neuropsychiatric disorders such as depression and anxiety.

Depression and anxiety are the most common mental health illnesses affecting 1 in 4 people in the UK. Depression and anxiety are considered to have similar causes and often occur together. Common symptoms of depression include feeling numb, pulling away from people and enjoyable activities, insomnia, low energy and poor concentration. Symptoms of anxiety partially over-cross with symptoms of depression and can additionally include racing thoughts, feeling irritable and angry, or feeling panic.

Depression is a leading cause of disability worldwide and significantly increases one's risk for suicide, tragically accounting for more than 6000 deaths per year in the UK. Various acute and chronic stressful events are the main risk factors for developing depression and anxiety. For example, recent changes to every day life due to global pandemic led to increased reported stress levels in the UK population. The prevalence of depression in the UK has almost doubled during the ongoing COVID-19 pandemic from 9.7% reported in March 2020 to 19.2% reported in June 2020. Such an increase in prevalence of depression during the pandemic has likely occurred due to a number of environmental factors such as a change to place of work, inability to socialise and increased worry given the severity of pandemic.

Other less prolonged and extreme environmental factors including shift work and moving house are also considered to be major risk factors for depression and anxiety.

First line treatments for depression and anxiety are different classes of antidepressant/anxiolytic medications - yet their effectiveness and the extend of adverse side effects varies from patient to patient. Recent research shows that antidepressants improve mood and attention only in about one third of all patients diagnosed with

depression. Our understanding of depression is very much incomplete and if we are to develop new antidepressants, particularly for treatment-resistant patients, we need to improve understanding of the biological mechanisms underlying responses to environmental stimuli thoroughly and extensively.

During this project I propose to investigate in detail the role of astrocyte lactate signalling in adjusting responses to environmental stimuli and the therapeutic effects of antidepressants. The results will pave the way for the development of new antidepressant and anxiolytic treatments with the aim to reduce the burden to relatives, carers and society and more importantly alleviate everyday suffering in patients.

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

The data generated as part of this project will provide fundamental insights into the role of astrocyte lactate signalling in neuropsychiatric disorders and will illuminate contribution of lactate in therapeutic effects of antidepressants. If successful, the study will discover specific signalling pathways, e.g. receptors, through which lactate elicits its actions on behaviour - which could be explored further as potential targets in depression.

Given the novelty of the approach and largely unknown astrocyte mechanisms in neuropsychiatric disorders, I foresee that the proposed work will result in 5-6 high impact publications and up to 3 scientific reviews. I will also present the results of my work annually at national and international conferences. I will create learning materials in collaboration with the mental health charity, clinicians and with support from people living with depression. I will also communicate the outcomes of my work to interested public through participation in public engagement events.

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

A number of stakeholders will benefit from the outputs of the current study including academics, patients living with depression and wider public, clinicians and industry partners.

The outcomes of this work will first of all benefit researchers interested in biological mechanisms of neuropsychiatric disorders and glia biology. Although such neuropsychiatric disorders as depression are complex disease, with many causes, a large proportion of research focuses on underlying neuronal mechanisms. Such a "neurocentric" view on neuropsychiatric disorders and mechanisms of antidepressant action affects clinical practise and research funding decisions. The findings of the proposed work will help to shift this view and contribute to the growing body of evidence implicating non-neuronal mechanisms in psychiatric disorders.

Patients living with depression and the wider public will benefit from the outcomes of this work. Specifically, during the project I will regularly meet with the focus group that consists mainly of patients who have experienced or are still living with depression. This will ensure a two-way dialogue rather than one-way communication of my results to patients – they

would be able to comment on how relevant the results of my work to their experience. It will also allow me to better understand the patients' needs so I can tailor my research towards those in the future. I will also create flyers and leaflets to illustrate in an accessible manner to lay audience the various existing theories of depression and the mechanisms of action of antidepressants currently used in clinical practice.

If the studies outlined below are successful, and we indeed arrive at new lactate targets involved in regulating stress response, then it will be made available to industry partners specialising on developing treatments against brain disorders. If further testing of these targets is successful, they will be taken further to clinical testing.

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

In due course the findings of this study will be published in peer-reviewed journals, and presented at national and international conferences and invited seminars. We will also ensure rapid communication of results through engagement with the social media. During the project an established network of collaborators with combined skills and expertise in place will also be essential in maximising the outputs of the work.

Species and numbers of animals expected to be used

- Mice: We estimate that up to 5,000 mice will be bred during the 5 year project, with up to 3,800 mice used in procedures.
- Rats: We estimate that 300 rats will be used in procedures during this project.

Predicted harms

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

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

We will use juvenile and adult mice to evaluate behavioural responses and modify lactate signalling, pharmacologically or genetically. Mouse brain anatomy and physiology is very similar to humans. Mice have been extensively used in neuropsychiatric research - and there are established behavioural procedures to evaluate behavioural responses to environmental stimuli in mice. We will use a number of transgenic lines during the project - and such transgenes are only generated in mice. Other organisms with simpler organisation of the nervous system (e.g. worms and flies) are not suitable for modelling of complex behaviour responses similar to humans and relevant to this project.

To address objectives of this project related to signalling pathways, we will use primary cell and slice cultures from neonatal rats and mice. We will initially work only with mouse cultures, however, at a later stage of the project we will use rat slice cultures to ensure that discovered signalling pathways in mice are conserved in another mammal (rat) - and similar mechanisms are more likely then to hold true for humans. It is not possible to

isolate primary cultures from adult animals since the survival of cultures will be massively compromised. Neonatal animals are widely used in neuroscience to prepare primary cell and slices cultures - and there are established protocols available to maximise the survival of these cultures.

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

Typically genetically altered animals will be bred, born and matured on this project.

About 60% of all animals (genetically altered and wildtype) undergoing experimental procedures will be infused with specific substances to modify brain lactate signalling or a control substance via one of the four routes (orally, s.c., i.p. or brain stereotaxic injections). Then either (1) brain cell signalling or (2) mouse behaviour will be evaluated at a baseline or as a response to acute or chronic environmental change – up to 50% of all animals, or (3) they will undergo surgery to implant cannula/stimulating devices to measure contribution of lactate signalling to brain metabolism as a response to acute or chronic environmental change – up to 10% of all animals.

About 30% of all animals (genetically altered and wildtype) will undergo behavioural testing and/or treatment with pharmacological agent (orally, s.c., i.p.).

The rest of experimental animals (genetically altered and wildtype) on this project (up to about 10%) will not undergo any manipulation but will be killed humanly during the first two weeks of life to prepare cell and slice cultures for in vitro studies that will be complementary and essential to in vivo studies proposed as part of this project.

To change cage environment we will modify lighting conditions, bedding, cage position, and objects in the cage or space available to animals. As part of the behavioural testing we will assess locomotion, aversive and social behaviour, motivation and cognitive functions. We will use behavioural tests that do not require use of any harmful or highly aversive stimuli but are rather based on a natural aversion of rodents to open illuminated areas or positive food reward. Only one task, novelty suppressed feeding, will require transient food restriction to motivate rodents to perform a task, in which food is provided as a reward for correct performance on the task. Food restriction will be only transient and following the test animals will be allowed to eat ad libitum. The test will be performed up to 5 times with at least a week in-between testing.

To deliver genetic constructs and pharmacological agents to a specific brain area animals will undergo stereotaxic brain surgery. If delivery directly to a specific brain area is not required, pharmacological agents will be administered through the least invasive route to answer the experimental question.

Where possible oral (e.g. via food or water) or subcutaneous administration will be favoured. LASA guidelines will be followed with respect to administration of substances.

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

Breeding:

Majority, up to 90% of the genetically altered mice will not suffer any adverse effects, since (1) majority of mice with genetic modification we proposed to use as part of this project will not have any harmful phenotype, (2) they may be bred and then killed for tissue extraction without undergoing any procedures.

Only knockout mice from one transgenic line, serotonin-free mice (less than 10% of all animals) will experience adverse effects – these knockout mice will be used to address a specific objective of the project related to the serotonin-independent mechanisms and the severe phenotype is innate to these knockout mice rather than being induced as part of the current project. Serotonin-free mice are the best way to study serotonin-independent effects of lactate on emotional behaviour and will be used only when necessary to address this specific objective. Severe phenotype is typical only for knockout but not heterozygous mice, and only for early postnatal age of this strain. Specifically, up to 70% of serotonin-free mice die during the first few weeks of life from unknown cause. However, death happens acutely, without animals displaying any signs of suffering or obvious harmful phenotype such as seizures. All (100%) serotonin-free pups have reduced body weight compared to wild type pups.

However, their body weight catches up at the age of about three months. Once adult, serotonin-free mice have higher aggression compared to wild type animals – however, it can be managed by single- housing which leads only to mild adverse effects.

Experimental procedures:

Up to 60% of all experimental animals (genetically altered and wildtype) will undergo surgical procedures to infuse specific substances in their brains and/or implant recording devices. Only a small proportion of these animals (<10%) may experience some adverse effects associated with the surgical procedures, including bleeding, infection and post-operative pain. Any animals which do experience these adverse effects will be treated appropriately.

Animals which have undergone surgical implantation of cannulas or stimulating devices (up to 10% of animals), then may be attached to equipment to record brain metabolite or to change activity of brain cells. Tethering might cause low level of stress displayed as, for example, changes in locomotion.

However, it is very mild and transient and majority of animals will acclimatise to the procedure rapidly.

Up to 60% of all animals (genetically altered or wildtype) will undergo testing in various behavioural tasks designed to assess locomotion, aversive and social behaviour,

motivation and cognitive functions. These tasks will not cause any lasting stress to an animal, but some will be associated with a certain transient level of stress. Restricting access to food might be used only in one behavioural test to motivate rodents to perform a task, in which food is provided as a reward for correct performance on the task. This will result in a certain level of hunger, however, food restriction will be only short-term and transient.

For some experiments we may modify cage environment, acutely or chronically. This may be associated with a certain level of stress. We may change lighting conditions, bedding, cage position, and objects in the cage or space available to animals. We will ensure that animals are housed and bred appropriately and are monitored regularly by researchers and the technical team - in case stress levels exceed defined threshold, animals will be taken out of the study and humanely killed.

In some experiments, we will use light or specific chemicals to activate or silence brain cells in behaving animals, techniques called optogenetics and chemogenetics. This would allow us to test cellular pathways controlling behavioural responses to environmental change. We will also use pharmaceuticals, for example, currently used in humans' antidepressants, to stimulate lactate metabolism. Most of these drugs are safely used in animal research and in clinical practice for humans and are not expected to cause adverse effects - while some of them have known side-effects, for example, gastro-intestinal symptoms, that are not life threatening or painful.

Mice will be single house when undergoing a surgery or chronic change to cage environment or due to their aggressive phenotype (serotonin-free mice) – this will lead to a short, transient reduction in body weight.

At the end of each study the animals will be killed and their brains will be taken for experimental analysis.

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)?

Majority, up to 90% of the genetically altered mice will be bred under mild protocol and will not suffer any adverse effects. Only knockout mice from one transgenic line, serotonin-free mice (less than 10% of all animals) will be bred under severe protocol.

About 60% of all animals (genetically altered and wildtype) undergoing experimental procedures will experience moderate severity: up to 10% will be implanted with cannula/stimulating devices and up to 30% will undergo a surgery; followed by behavioural testing and/or acute/chronic change to cage environment.

About 30% of all animals (genetically altered and wildtype) undergoing experimental procedures will experience mild severity – they may be infused with pharmacological agents and/or undergo behavioural testing.

About 10% of experimental animals (genetically altered and wildtype) on this project will not undergo any manipulation but will be killed humanly during the first two weeks of life to prepare cell and slice cultures for in vitro studies.

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 06 March 2028

The PPL holder will be required to disclose:

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

Replacement

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

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

The use of animals is vital to the success of the project. We are not able to use any in vitro models to study complex behavioural responses - to model such behaviours we need a functional brain that shares common systems with humans. Therefore, we propose to use mouse as a model. Mouse brain anatomy and physiology is very similar to humans and they have established behaviour patterns that reflect emotional states in humans.

We will use rats only for in vitro studies to ensure that discovered signalling pathways in mice are conserved in another mammal (rat) - and similar mechanisms are more likely then to hold true for humans.

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

It is not possible to assess behavioural responses in vitro in cell and brain slice cultures and induced- pluripotent stem cells (iPSCs).

Why were they not suitable?

Currently, there is insufficient data available to build a mathematical model that can fully reflect lactate metabolism in astrocytes and within brain networks. This study will be instrumental in producing and making available for other researchers' data on lactate

metabolism in health and disease. Eventually results of this study will lay the foundation for mathematical models that I and other researchers would be able to use in the future.

iPSCs technology allows the study of processes within a particular cell type - however, it is not be possible to use this in vitro technique to understand the role of lactate metabolism in regulating emotional behaviour.

A retrospective assessment of replacement will be due by 06 March 2028

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?

To estimate the number of animals required to address each objective we used a mathematical model to predict the group size needed to achieve significant difference which in turn were based on our previous experience, published and unpublished data by us and other laboratories

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

After consulting a resource on experimental design/statistics of the National Centre for the Replacement, Refinement and Reduction of animal in research (NC3R), we took a number of steps during experimental design to reduce number of animals used in the project: we adjusted sample size calculation, chose appropriate controls and integrated blinding measures.

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

To reduce number of animals used on this project we will

(1) where possible assess responses before and after intervention, so each animal may serve as its own control

(2) ensure that our breeding programme is the most optimal - for example, where possible we will ensure that maximum number of offspring carry the desired protein by breeding mice carrying the desired transgene on both alleles.

(3) carefully control the conditions under which animals are maintained - this will help us to reduce variability between experiments, from one animal to another. For example, animals tested in the same cohort will be bred and housed under the same conditions

A retrospective assessment of reduction will be due by 06 March 2028

The PPL holder will be required to disclose:

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

Refinement

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

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

To manipulate lactate metabolism and signalling in the brain we will use a number of genetic and pharmacological approaches. In studies where exogenous administration of a drug/therapeutic agent is required, the least invasive route of administration will be used to answer the experimental question to be addressed. Where surgical procedures are required, to minimise animal suffering, the surgeries will be performed using aseptic technique to minimize the risk of infection. Prophylactic analgesia will be given to all animals undergoing surgery to minimize suffering associated with post-surgical pain. In order to minimise the harm of brain injections, we will only infuse a maximum volume of 5µl that does not lead to substantial volume-induced damage base on our experience. As with all post-surgical animals, they will be closely monitored and observed at least once a day for several days following the procedure to look for any signs of adverse effects from the surgery.

Since serotonin has been widely implicated in a number of neuropsychiatric disorders we will also use a rodent model that lacks brain serotonin. We propose to use this strain of mice for some experiments to understand serotonin-independent changes to behaviour (1) as a result of environmental change and (2) following antidepressant action. No other mouse model would allow us to discover serotonin- independent effects of lactate on emotional behaviour and mechanisms of antidepressants. To improve the welfare of this

mouse strain we have developed a specific breeding and maintenance protocol – for example, to reduce effects of deletion of serotonin producing enzyme on both alleles on weight gain and lethality, litter size may be reduced to the amount of the transgenic mice (complete knockouts) and equal amount of "wildtype" and heterozygous pups.

We will use behavioural paradigms to test animal behaviour - activity, aversive behaviour, anhedonia, motivation and cognitive functions. We will use behavioural tests that do not require the use of harmful highly aversive stimuli but are rather based on a natural aversion of rodents to open illuminated areas or positive food reward. Only in one paradigm (novelty supressed feeding) mice will be food-deprived up to 24 hours prior testing – however, hunger caused by food deprivation will only be transient since animals will have free access to food straight after the procedure. Thus, these behavioural procedures are the most refined.

Animals may be implanted with cannulas and stimulating devices via stereotaxic surgery using aseptic technique. To minimise adverse effects of surgeries animals will be closely monitored and observed regularly following the procedure. During experiments mice may be tethered or connected to stimulating devices – a refined and widely used way to measure brain metabolites and control brain cells activity.

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

Complex behavioural responses being evaluated as part of this project cannot be modelled with less sentient organisms, such as flies or worms or with non-animal alternatives such as mathematical models.

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

To minimise welfare costs (harms) for animals we will ensure:

- most appropriate housing and husbandry: to minimise stress of housing, mice will be provided with environmental enrichment e.g., bedding, shelters, and chew toys
- most optimal breeding strategy – for example, using littermate controls as recommended
- most optimal care for a genetically altered mouse line with a phenotype (serotonin-free mice) – for example, reducing litter size to minimize effects of postnatal growth retardation
- that we use least invasive routes for drug administration and when testing new drugs, we will carry out small pilot studies in a few animals, to see if the drugs cause any harm to the animals. We will only carry out the full study if the mice in this pilot study appear to tolerate the drug
- most appropriate care when performing invasive techniques: we will provide appropriate analgesia and anaesthesia before, during and after the procedure and by carefully monitoring all animals until the full recovery

- where possible we will use non-aversive tasks

- to avoid bias: where possible experimental drugs will be diluted and code-labelled by an independent researcher and the experimenter will be blind to drugs they are using. If possible, behaviour will be video recorded and scored "offline" by a researcher blind to the animal genotype/experimental group

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

We will adhere to the NC3Rs and LASA guidelines on administration of substances. When planning experiments, we will refer to the PREPARE guidelines for checklists. We will use Arrive 2.0 guideline when reporting of research involving experimental animals. We will use NC3Rs guidelines on GA breeding to minimise wastage.

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

We have signed up for the newsletters for the National Centre for the Replacement, Reduction and Refinement of the use of animals in research (NC3Rs). We will regularly consult their website and attend webinars, and read the most up-to-date review and original research paper on best 3R practices to keep our knowledge in this area up to date – and will ensure implementing the most refined practises into our research experiments.

A retrospective assessment of refinement will be due by 06 March 2028

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. Anti-Cancer Therapy Discovery and 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

Cancer, Therapy, Tumour model development, Drug metabolism and pharmacokinetics, Pharmacodynamics

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 aim is to develop and progress an anti-cancer therapeutic (either sourced ‘in-house’ or through academic or industrial collaboration) towards clinical trials over the course of the 5 years.

A retrospective assessment of these aims will be due by 25 May 2028

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?

Around 1 in 2 people in England develop cancer in their life-time, with cancer now causing around 1 in 4 deaths. This picture is similar throughout the 'developed' world. There is thus a clear need to develop new therapeutic agents and strategies for cancer, to continue the progress seen in the past 20 or so years due to the movement towards targeted therapies, with improved survival rates seen for most common solid cancers such as lung, bowel, and breast cancer.

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

New information on novel therapeutics and/or target-specific tumour models. The tumour type will depend on the mechanism and specificity of the therapeutic, but types of cancers investigated will include solid cancers such as breast, colorectal, lung, prostate, head and neck squamous, melanoma and ovarian cancer, as well as childhood cancers such as neuroblastoma.

Scientific publications reporting on novel therapeutics and/or target-specific tumour models

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

In the short-term the data provided will impact on decisions to progress novel therapies further in the drug development cascade. The data produced may be used to strengthen research grant applications or to attract commercial investment in further investigations, so benefitting those developing the therapeutics: both in-house, and external academic and industrial partners, and ensuring every chance of long-term success to progress the therapy into the clinic.

In the longer term, dissemination in scientific publications of information on the development of novel target-specific models will benefit the scientific research community in this area, as these models will then be accessible to other groups to take advantage of, and also extend the portfolio of models we can offer for drug development to external partners.

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

Our approach to maximizing outputs will very much depend on whether the discoverer of the therapy is looking to patent the therapy or not. Any dissemination would most likely be lead by the collaborator, as the information we obtain will benefit the progression of their therapeutics.

If they are looking to patent then we will discuss with them about the dissemination of any non- confidential information, which we will either use in scientific publications, grant applications, and for use on bioscience industry websites to promote the work as a potential service going forward, and also to include in teaching materials for undergraduate and postgraduate courses.

If they are not looking to patent, then we would look to disseminate all novel information (included unsuccessful approaches and negative data) by the same routes.

Species and numbers of animals expected to be used

- Mice: 6,950
- Rats: 325

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 mice are the most frequently used species for tumorigenicity and cancer therapy studies. Thus this is beneficial when carrying out such studies due to the depth of knowledge available plus standard protocols, methods and reagents which have all been optimised for use in this species.

Immunodeficient (‘nude’) mice will be used where primary cells, or immortalised, established cell lines from a different species (e.g. human, hamster) are being transplanted. In the case of models which have been developed in rats and it would not be possible to transfer to mice (e.g. syngeneic tumours, organ size in the case of surgical manipulations), or where larger sample volumes are required for analysis (e.g. plasma for pharmacokinetic analysis) then rats will be used.

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

Each protocol covers different facets of the project, with some of the experimental steps similar across the project. For all invasive procedures, anaesthesia (typically by inhalation) will be administered, with analgesia also administered for more invasive techniques. Animals will be killed by a Schedule 1 method, unless blood is collected at the end of a study, and then this will be carried out by carried puncture under terminal inhalation anaesthesia.

The tumour type used in a study will depend on the mechanism and specificity of the therapeutic, but types of cancers investigated will include solid cancers such as breast, colorectal, lung, prostate, head and neck squamous, melanoma and ovarian cancer, as well as childhood cancers such as neuroblastoma.

To determine the maximum tolerated dose (MTD) that can be administered, if the compound under test requires tumour-specific activation, then tumour cells will be inoculated sub-cutaneously (s.c.) and then the tumour grown to a volume of at least ~100mm3 as measured by callipers before treatment. The therapy under investigation, or it's solvent, will then be administered either as a single or multiple dose by one of the following routes: intraperitoneally (i.p.), intravenously (i.v.), s.c., orally (p.o.), intra-tumourally (i.tum)., intravesically or intra-tracheally. Animals will be then monitored for signs of deleterious effects and if these are evident, then the dose will be reduced for a subsequent run, or if they are not evident then the dose may be increased. At the end of the study the animals are killed.

Typical duration for a study at 4 dose levels will be 2 months (non-tumour-bearers) up to 4 months (tumour-bearers).

Where data of tumour pharmacokinetcs (PK) and/or pharmacokinetics (PD) are required, then tumour cells will be inoculated s.c. and the tumour grown to a volume of at least ~100mm3 before treatment. The therapy under investigation, or appropriate controls, will then be administered as single or multiple doses at either maximum tolerated dose (MTD) or a dose below this by one of the following routes (i.p., i.v., s.c., p.o., i.tum., intravesical, intra-tracheal or via an osmotic pump implanted i.p., s.c. or by

i.v. catheter). At predetermined time points following administration, typically animals are killed and tumour (if present), plus blood and organs/tissues of interest are harvested for PK/PD analysis. In some studies, non-invasive imaging or a metabolic cage may also be utilised, or probes to label tissue or vasculature administered prior to culling. Typical duration for a study would be a few hours (non- tumour-bearers), up to 2 months (tumour-bearers).

Where the s.c. tumourigenicity will be evaluated, tumour cells or fragments will be inoculated s.c., and the primary tumour grown to a volume of up to ~1200mm3 as measured by callipers. In some studies, non-invasive imaging may also be utilised, or probes to label tissue or vasculature administered prior to culling. Typical duration will be ~2 months.

Where we are looking at tumour deposits at specific sites in the body, e.g. lung or liver metastases, then tumour cells or tumour fragments will be introduced as required and animals will be inspected with appropriate frequency for the model for signs of deleterious effects, and before these reach a moderate severity the animal will be culled. In some studies, non-invasive imaging may also be utilised, or probes to label tissue or vasculature administered prior to culling. Typical duration will be

~2-4 months depending on the growth rate of a specific tumour model at a specific site.

Where the therapeutic effects will be studied in s.c. implanted tumours, once the tumour has grown to a volume of ~100mm3 then the therapy under investigation, or appropriate controls, will then be administered as single or multiple doses at either maximum tolerated dose (MTD) or a dose below this by one of the following routes (i.p., i.v., s.c., p.o., i.tum., intravesical, intra-tracheal or via an osmotic pump implanted i.p., s.c. or by i.v. catheter). Tumour growth will then be monitored to a volume of up to

~1200mm3 as measured by callipers. In some studies, non-invasive imaging may also be utilised, or probes to label tissue or vasculature administered prior to culling. Typical duration will be ~1-4 months depending on the growth rate of a specific tumour model.

Where we are looking at therapeutic effects on tumour deposits at specific sites in the body, e.g. lung or liver metastases, then once it is known that tumour has established at the site of interest (from previous studies) then the therapy under investigation, or appropriate controls, will then be administered as single or multiple doses at either maximum tolerated dose (MTD) or a dose below this by one of the following routes (i.p., i.v., s.c., p.o., i.tum., intravesical, intra-tracheal or via an osmotic pump implanted i.p., s.c. or by i.v. catheter). Animals will be inspected with appropriate frequency for the model for signs of deleterious effects, and before these reach a moderate severity the animal will be culled. In some studies, non-invasive imaging may also be utilised, or probes to label tissue or vasculature administered prior to culling. Typical duration will be ~2-6 months depending on the growth rate of a specific tumour model. This approach will also be used in a more involved evaluation of PK/PD where studies also involve evaluating tumour deposits at specific sites in the body, e.g. lung or liver metastases are carried out.

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

Where substances are injected into an animal, then one might expect to see minor discomfort in each case with inflammation at the injection site.

Where tumours are implanted s.c. or another site superficial site, e.g. the mammary fat pads, , potential harms would be the tumour becoming sore, inflamed, infected or ulcerated, and approaching a maximum combined volume per animal of 1200mm3. Any showing signs which would suggest they are likely to exceed moderate severity are to be killed by a Schedule 1 method. It would be expected that animals would experience these symptoms for no more than 72hours.

For evaluation of MTD, it is possible that the animals may experience unexpected immediate, i.e. within minutes, symptoms of acute toxicity. These signs may include abdominal contractions, unresponsiveness to stimuli, hunched appearance, tremors or convulsions. Should this occur, the dose will be considered toxic, the animals killed and the dose reduced for subsequent administration in other mice. In addition, when monitoring of weight loss, if a drop below 85% of the starting body weight is seen over 24

hours then this is considered severe , and any animals showing such signs are to be killed by Schedule 1 method.

Where mini-pumps are implanted s.c. or i.p., at the end of their delivery period, pumps swell and begin to leak a concentrated salt solution resulting in local irritation to tissues around the pump and they can also induce reverse osmosis resulting in dehydration of tissues or of the whole animal. Thus if the animal is to survive longer than the pump's active infusion time, the pump must be removed no later than one half-life after the completion of its infusion time under brief general inhalation anaesthesia with recovery.

Where mini-pumps are implanted i.v., visible damage may develop at the insertion site. If this persists for over 72 hours then these animals will be removed from the study and killed by a Schedule 1 method.

Where animals are maintained under anaesthesia for periods on longer than 5 minutes, then there may be an adverse systemic reaction to the anaesthetic.

Where animals may be housed in a metabolic cage for up to 24 hours, then this may be a potential cause of stress, which should dissipate when the animal is returned to the home cage.

Where tumours are implanted orthotopically or at specific sites, then depending on the site of tumour growth, then it may have an impact on the animal, e.g. for a tumour implanted in the tongue, this may restrict the ability of the animal to eat and this is usually signposted by sudden weight loss outside of regular bodyweight fluctuations, , I.e. a sudden drop in weight compared with the starting body weight of more than 10% in a 24 hour period, or for tumours established in the lungs then there may be an impact on lung function as evidenced by abnormal breathing or panting.

In addition to the specific harms detailed above, in general, animals are checked at least twice daily by competent animal care staff who will notify the Personal Licence (PIL) holder and/or Named Animal Care Welfare Officer (NACWO) of any indication of pain, suffering or distress including but not limited to abnormalities in behaviour and/or appearance, such as poor coat condition, piloerection, unusual posture (hunching), discharge or lack of grooming to eyes, ears, nose, ano-genital region, facial grimace, obvious signs of injury, abnormal movement, reduced activity, reluctance to socialise, reduced alertness, vocalisation, indications of reduced eating or drinking. An informed assessment of the level of severity will be made based on the above observations, the known phenotype, and the procedures undertaken and appropriate treatments/ actions such as antibiotics or analgesics will be considered (taking Named Veterinary Surgeon (NVS) advice where needed).

For all studies apart from evaluation of MTD, in cases where side effects which are expected to exceed the moderate severity limit are observed, the animal will be humanely killed by a competent person.

Indications that a moderate severity limit is likely to be exceeded include but are not limited to, significant (easily detectable by a competent person) or lasting (more than a few hours following procedures, or 48 hours in stock mice) changes as listed above.

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)?

For evaluation of MTD, it is expected that ~50% of animals will experience no more than mild severity,

~40% moderate severity and ~10% experiencing severe effects of the types described in the section above on expected impacts/adverse effects..

For studies where tumour models are established s.c., it is expected that the majority of animals (~99%) will experience no more than mild severity, with ~1% experiencing moderate severities of the types described in the section above on expected impacts/adverse effects.

For studies where tumours are implanted orthotopically or at specific sites, it is expected that ~90% of animals will experience no more than mild severity, with ~1% experiencing moderate severities of the types described in the section above on expected impacts/adverse effects.

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

- Killed

A retrospective assessment of these predicted harms will be due by 25 May 2028

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 majority of the work carried out during cancer drug discovery is done in silico or in vitro (including evaluation in 2-dimensional (cell monolayer) and 3-dimensional (multilayered cell culture, tumour spheroids and organoid cultures)), which can give useful

information on how/if a drug is going to interact with a specific target or if it shows good potency against a culture of cancer cells.

However this work does not take account of how the drug behaves in a complex system, i.e. is it available in a stable form for sufficient amount of time to interact effectively with the cancer cells and not be metabolised to an inactive or toxic form, or if a drug target is still accessible when it is subject to physiological control from external systemic factors.

Thus it is necessary to evaluate the agent in a living organism since the ultimate goal of the project is to develop agents which will eventually proceed to the clinic. Ethically it does not warrant proceeding directly from formulating a potential cancer treatment in an in vitro laboratory to evaluation in clinical trials in human patients, and throughout the world there are regulations controlling the screening of a treatment in vivo.

Therefore some in vivo work on experimental animals has to be carried out before progress to the clinic, although through adherence to the 3Rs philosophy and good experimental design, the numbers of animals used is kept to the absolute minimum with the minimal amount of suffering to obtain statistically significant results which will aid progress of an agent into the clinic.

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

Replacement strategies which are adopted include use of human tissue homogenates for preliminary stability and drug metabolism and pharmacokinetic (DMPK) studies prior to animal studies, monitoring of efficacy in 3-dimensional (multilayered, tumour spheroids and organoid) culture models, and where effects on tumour dissemination are predicted for novel agents then extensive in vitro evaluation in 2D adhesion and 2D/3D migration/invasion assays will take place before progressing in vivo.

Why were they not suitable?

These non-animal alternatives will be used extensively in the early development stages of novel therapeutics before they are advanced to any in vivo studies under this Project Licence (PPL). However for the reasons covered in the section above, at some stage these therapies will need to be evaluated in vivo.

A retrospective assessment of replacement will be due by 25 May 2028

The PPL holder will be required to disclose:

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

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to

design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

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

This is based on estimate of novel therapeutics that will be evaluated and target-specific models which will need to be developed for this purpose over the course of the project, with the numbers of animals for each particular study calculated by power calculations where necessary.

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

Through previous experience of carrying out the majority of techniques on this Project Licence (PPL), discussion with local statisticians, and for techniques where we do not have extensive experience, reference to the literature and use of the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) Experimental Design Assistant.

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

Where tumour cells have been engineered to emit a bioluminescent or fluorescent signal, then we will be able to monitor tumour growth using non-invasive imaging techniques to detect these signals over time in a small cohort of animals. This will be instead of having to set up a larger number of animals and then have to kill a sub-group of animals at different time points to track tumour growth.

As on some occasions cells or fragments to do not successfully grow when initially implanted s.c., the licence will allow for a second attempt at inoculation if tumour doesn't take first time so that the mice are used effectively.

Taking as much of the tissue as possible from an animal that isn't required for post-mortem analyses relating to the particular study (including having the capacity to take a large amount of blood at the end of all procedures by cardiac puncture) so that this can be used by other researchers.

To reduce control animals required, where possible we will set up animals for pharmacodynamic analyses in satellite groups along with efficacy experiments, so that the control animals can be shared by the 2 studies.

Where analytical methods are sufficiently sensitive to detect compound levels, repeat bleed sampling from the same small cohort of animals can be used for monitoring blood levels of compound instead of setting up a larger number of animals and then kill a sub-group of animals at different time points to obtain a blood sample.

A retrospective assessment of reduction will be due by 25 May 2028

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.

Adult mice are the most frequently used species for tumorigenicity and cancer therapy studies. Thus this is beneficial when carrying out such studies due to the depth of knowledge available plus standard protocols, methods and reagents which have all been optimised for use in this species.

Immunodeficient (‘nude’) mice will be used where primary cells, or immortalised, established cell lines from a different species (e.g. human, hamster) are being transplanted. In the case of models which have been developed in rats and it would not be possible to transfer to mice (e.g. syngeneic tumours, organ size in the case of surgical manipulations), or where larger sample volumes are required for analysis (e.g. plasma for pharmacokinetic analysis) then rats will be used.

If the cells lines that are being used have been engineered to express a fluorescent tag, e.g. Green Fluorescent Protein, or luciferase, then this gives the capability to perform non-invasive optical imaging (fluorescence or bioluminescence) and allows us to monitor internal tumour burden throughout the ongoing experiment. This minimises the risk of developing an unexpectedly high tumour burden, as we can follow tumour growth ‘live’.

Animal suffering will be minimised by adhering to the UK National Cancer Research Institute Guidelines for the Welfare of Animals. Specifically, we will always use aseptic technique and will commit to the LASA Guidelines as set out in 'Guiding Principles for Preparing for and Undertaking Aseptic Surgery' (2nd edition, April 2017), and for blood sampling, the ‘LASA Good Practice Guidelines for Collection of Blood Samples’ (1988).

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

As the majority of therapeutics will be used in adults, then it is important that these therapies are evaluated in an adult mammalian model, as these more closely model the

environment to support tumour growth, and the systemic pressures (e.g. metabolising enzymes, circulatory system) which are placed on a novel therapy when administered systemically. Given that often weeks or months are needed for tumours to grow and/or for the therapy to take effect, then it would not be possible to carry out these studies in terminally anaesthetised animals as this would not provide a reasonable timeframe to monitor these effects.

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

Analgesics and anaesthesia will be used , and we will follow advice relating to care and welfare given by the NVS and the NACWO.

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

Animal suffering will be minimised by adhering to the UK National Cancer Research Institute Guidelines for the Welfare of Animals (Workpan et al. (2010), Br J Cancer,102:1555-1577). Specifically, we will always use aseptic technique and will commit to the LASA Guidelines as set out in 'Guiding Principles for Preparing for and Undertaking Aseptic Surgery' (2nd edition, April 2017).

For blood sampling we will follow guidelines as set out in the 'Handbook of Laboratory Animal Management and Welfare, Third Edition' Editor(s): S Wolfensohn & M Lloyd (2003) & use as a a guideline for clotting time, Emeis et al. (2007), J Thromb Haemost, 5: 670-679.

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

Through scanning most recent literature, referring to the NC3Rs website and through information passed on by the local Named Information Officer (NIO).

A retrospective assessment of refinement will be due by 25 May 2028

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. Preclinical Tumour Models

Project duration

5 years 0 months

Project purpose

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

Key words

preclinical tumour models, patient derived tumour models, cancer therapy

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 overall aim of the project is to develop clinically relevant models of cancer to test novel therapies. The goal is to provide a platform that will aid in the process of discovery of drugs that target particular pathways and to facilitate a reiterative benchside to bedside approach to research that will increase patient benefit.

A retrospective assessment of these aims will be due by 04 February 2028

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?

There is currently a high efficacy failure rate of novel compounds developed to treat cancer patients. The generation of models that replicate more faithfully the disease in humans is a high priority.

Tumours grown from patient material have been shown to retain the genetic changes observed in patients and reliably predict clinical activity of novel compounds in a variety of tumours.

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

We expect to generate new patient derived tumour lines, which will serve as preclinical models to study the efficacy of novel anti-cancer therapies. The data generated in this project will be communicated to the research community as part of peer-reviewed publications and talks at international scientific conferences.

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

In the short term, the outputs of the work will benefit other clinical and research scientists in the cancer field. The availability and analysis of relevant preclinical tumour models will help advance the discovery of novel cancer treatments. In the long term, we expect our data to inform the design of clinical trials in cancer patients by our clinical colleagues.

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

Our close collaboration with clinicians ensures that our work has direct impact on patient benefit. The data generated in this project will be communicated to the research community as part of peer- reviewed publications and talks at international scientific conferences.

Species and numbers of animals expected to be used

- Mice: 11000

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.

Animal models are required to fully replicate the properties of the three dimensional tumour tissues growing within specific organs in cancer patients. These properties cannot be adequately recapitulated in vitro. Similarly, the effects of drugs need to be tested in vivo so that the effects of the natural microenvironment where the tumour resides and how the drugs access the tumour and how specific is the drug to its target can be assessed. Mice are the most effective choice of species for these experiments and the availability of strains with fewer immune cells allow for the grafting of human derived tissue with minimal rejection.

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

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

Prior to tumour cell injection, mice may be prepared in the following ways: (i) by changing the hormone environment to allow growth of tumour cells that are hormone dependent; (ii) in rare cases, mice will be subject to a bone marrow transplant to assess the contribution of specific bone marrow cells to the growth and aggressiveness of the tumour.

Tumour formation will be induced through the injection or implantation of tumour cells. In most cases, cells will be placed under the skin. Other sites such as the prostate, salivary gland, bone, intraperitoneal, may be chosen to further mimic the tumour site in patients and to increase growth.

In some cases, mice will be treated with drug therapies. Mice will receive the optimal drug dosing that have been assessed using (i) optimal doses and schedules derived from the literature, (ii) previous studies carried out at our establishment or (iii) using dose tolerability studies for novel compounds.

We will use non invasive imaging and taking biopsies for molecular analysis to follow the development of the tumour and minimise the numbers of animals required at different time points during the course of experimental regimes.

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

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

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

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

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

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

A small proportion (<2%) may experience a severe severity. This is because with new test agents we need to make sure they are safe by conducting toxicity studies, and at times there are unforeseen effects from these new test agents.

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 2028

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 generation of accurate preclinical models is essential for cancer drug discovery as there is currently a high failure rate of new agents. These models need to replicate the properties of the three dimensional tumour tissues growing within specific organs in cancer patients. These properties cannot be adequately recapitulated in vitro. Similarly, the effects of drugs need to be tested in vivo so that the effects of the natural microenvironment where the tumour resides and how the drugs access the tumour and how specific is the drug to its target can be assessed. It is increasingly clear that to reliably predict clinical activity of novel compounds in the clinic in vivo preclinical models must be used.

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

We are continually developing in vitro cell culture and three dimensional tissue assays, including organoid generation from patient derived tissue, and comparing them to the in vivo models in an effort to establish animal replacements.

Why were they not suitable?

Although in vitro models allow us to test novel hypothesis and treatment regimes, they are not suitable to address the contribution of the tumour microenvironment to tumour growth and response to treatment. In addition, they are not able to reproduce the process of tumour spread to different organs. 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 04 February 2028

The PPL holder will be required to disclose:

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

Reduction

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

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

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

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

We do the following to ensure that we are using optimum number of animals for each experimental protocol:

We consult colleagues with statistical expertise, including our clinical colleagues, to ensure that the optimum and minimum number of animals are used to obtain significant data. These will be most relevant for our therapeutic studies and will depend on the therapy to be studied and tumour diversity.

We base our work on data from previous studies using similar tumour models.

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

- We take a number of measures to ensure that the minimum number of animals is used including:
- Using non-invasive imaging to follow the development of the tumour in time and so that we do not have to kill mice at different time points during the course of the experiment.
- We ensure that the maximum amount of information is obtained and analysed per experiment to reduce the need for repeats.

Using optimum procedures to reduce the number of mice and to reduce experimental variability. For example, where possible, we use ultrasound guided tumour cell inoculation into organs to achieve higher precision.

A retrospective assessment of reduction will be due by 04 February 2028

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 widely used for preclinical models for cancer research. The generation of genetically altered strains that are immunodeficient makes it possible to model the growth of human tumours as xenografts, which have been shown to be effective predictors of clinical response to drugs. The animals are maintained in ventilated cages using sterile food and bedding and all procedures are carried out in laminar flow cabinets to avoid infections. Animal suffering will be minimised by keeping tumour burdens within acceptable limits. Therapeutic drugs will have been assessed for toxicity and therefore we expect high tolerability of the regimes. Where possible, imaging will be used for earlier study end points.

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

The similarities in genetic make up between human and mouse makes them appropriate in vivo models for growth and treatment of patient derived tumour tissue. This is particularly the case for the tumour microenvironment and organ physiology, which cannot be fully reproduced in less sentient animals.

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

Mice will be housed in cages with sterile bedding, food, and water. Trained competent personal with experience of using mice in cancer research and who are familiar with the effects of anti-cancer drugs on rodents will perform all procedures. Studies will be designed to use the minimum number of mice. The welfare of mice entering a study is closely monitored throughout each procedure. Anaesthesia and analgesia will be used to minimise stress and suffering during surgical procedures. Where possible, we will use ultrasound to guide tumour inoculation at specific sites as a refinement.The procedures chosen are always considered to be the least severe ones that would produce satisfactory results.

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

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

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

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

A retrospective assessment of refinement will be due by 04 February 2028

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. Preclinical Development of Interventions Against Respiratory Pathogens

Project duration

5 years 0 months

Project purpose

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

Key words

Pathogen, Respiratory, Emerging, Preclinical, Interventions

Animal types	Life stages
Mice	adult
Hamsters (Syrian) (Mesocricetus auratus)	adult
Ferrets	adult
Cotton Rat	adult
Rhesus macaques	adult
Cynomolgus macaques	adult

Retrospective assessment

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

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Uses non-human primates
- 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 and/or assess new vaccines, therapies and treatments (interventions), in animal models of infection, against several infectious respiratory diseases that are considered to be a public health threat, in the UK and globally. (e.g. SARS-CoV-2, influenza, adenovirus, RSV).

A retrospective assessment of these aims will be due by 19 January 2028

The PPL holder will be required to disclose:

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

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

Why is it important to undertake this work?

This project will allow for the use of established animal models as well as furthering their development. These models will enable studies into pathogenesis and transmission of viruses impacting on public health. They will also support the development and approval of vaccines and treatments.

This will benefit humans because respiratory pathogen outbreaks (e.g. COVID-19, flu) can harm or even kill large numbers of people.

This project will also increase knowledge and expertise in the field of respiratory pathogen research which is important to the future, rapid development of drugs and vaccines for emerging respiratory viruses, e.g. a novel Coronavirus or variant.

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

There are several benefits of this project in developing models for and evaluating the efficacy of treatments for respiratory pathogen.

Outputs will include:

- Model development and optimisation in a range of species; new information on the models will lead to publication and dissemination of refinement information to the wider respiratory pathogen and animal community
- Pre-clinical evaluation of novel vaccines and treatments against respiratory pathogens to assist in development and licencing

- Capability to assess vaccines and treatments against emerging respiratory pathogens, where human challenge trials are not possible, e.g. for potential respiratory pathogen threats

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

This project will contribute to the understanding and refinement of animal models of respiratory pathogens allowing long term contribution to the field. We anticipate that model development and optimisation in a range of species can be achieved within this project.

This project will enable us to select effective vaccines and medicines from a range of candidates. By filtering these candidates through our models of infection, we will reduce the number of candidates required to be tested in humans and advance translational research.

This project will provide the capability to assess new interventions against the most serious forms of infectious disease, where human challenge trials will not be possible due to the severity of the form of disease being investigated. Regulatory bodies will, however, accept preclinical data generated in animals in such circumstances so this project may assist in the licensing of new or improved interventions against serious forms of infectious disease.

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

Our work is highly collaborative, with the assessment of potential new vaccines and medicines being carried out with either commercial partners, through research programmes or as collaborative efforts with international public health and pandemic preparedness agencies. Knowledge and findings will be transferred to subsequent studies and disseminated through the scientific community through publications where appropriate to ensure that our findings are subject to the scrutiny of the scientific community. In addition to peer-reviewed publications, the work performed under this licence will be disseminated widely at international conferences which would provide opportunity for informal feedback and in-depth discussions to disseminate new knowledge. The data provided to collaborators and customers will direct the appropriate generation of products which will have direct benefits to human health.

Species and numbers of animals expected to be used

- Rhesus macaques: 60
- Cynomolgus macaques: 60
- Mice: 1100
- Hamsters (Syrian) (Mesocricetus auratus): 1600
- Ferrets: 1050
- Other rodents: 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.

Mice, cotton rats, hamsters and ferrets are all recognised animal models for the assessment of the respiratory pathogens mentioned within this project. Mice, cotton rats, hamsters, and ferrets will be used to evaluate the effectiveness of vaccines and medicines against respiratory pathogens. The scientific community working on these respiratory pathogens worldwide use the same animals as these are the most appropriate species in these disease investigations. Using the same animals allows for data that is generated from this project to be compared to other related work that has been published. This is an important and critical step in the development and assessment of various vaccines and medicines.

The use of two or more species is required by licencing authorities to provide reliable data, which fully assess new vaccines and interventions candidates. Non-human primates will only be used after assessment has been conducted successful in a small animal model and where the data is integral for respiratory pathogen assessment or the evaluation of vaccines and medicines.

Adult animals for each of these species are the most suitable for these experiments.

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

Typically, the following will occur during and experiment:

Following acclimatisation, animals will be sedated and undergo a baseline bleed to assess normal immune responses prior to intervention. Animals on this project may also be implanted with a biometric chip or other telemetric device to provide a unique identification code and/or to track body temperature or other data.

Animals may then be vaccinated, most commonly intramuscularly or intranasally. Alternatively, therapeutics may be administered, most commonly intranasally or orally.

If a vaccine is being assessed the duration of the experiment can range from one week to several months depending on the length of the vaccination phase.

A respiratory pathogen will be administered to animals, usually via the intranasal or aerosol route. Once administered the experiment is usually completed 14 days later, depending on the pathogen.

The animals will undergo regular health monitoring by trained and expert animal care staff at specific timed points. The frequency of these health monitoring checks may be increased as symptoms present in these animals. If clinical signs approach the severity

defined within the project, the animals will be humanely euthanised using an appropriate schedule 1 method.

Animals can be expected to be sedated and sampled (nasal washed/swabbed) daily in order to study the shedding of the pathogen administered. Animals may be sedated and bled over the duration of a study ensuring only a maximum of 10% of total blood volume is taken at any one time and no more than 15% is taken over a 30-day period.

At the end of all studies, animals will be euthanised by a schedule 1 method or terminal exsanguination under full anaesthesia. After being humanely euthanised, the animals will undergo necropsy where the maximum amount of relevant biological material will be collected for analysis.

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

Potential adverse effects to pathogen challenge, anaesthetics and administration of substances have been identified.

Expected effects from respiratory pathogens are commonly sneezing, nasal discharge, lethargy, laboured breathing, and loss of appetite. Animals may lose weight and they may have a temperature. Clinical signs are expected to be transient, however clinical signs of emerging pathogens may not yet be defined.

Any adverse effects associated with specific viral challenge will be identified by detailed behavioural monitoring. Together with this behavioural monitoring, objective data (animal weight and temperature) will be collected and analysed.

Any adverse effects associated with the anaesthesia will be monitored closely. Animals that have been anesthetised could become dehydrated and could develop hypothermia, however, this is highly unlikely due to refinements put in place.

Animals may experience mild discomfort when being handled during the administration of respiratory pathogens, vaccines or medicines.

Adverse effects associated with the administration of substances will be minimised by using highly trained staff competent in the delivery of substances via various routes, by using the smallest volumes and lowest rate administration of substances.

At the end of all studies, animals will be euthanised by a schedule 1 method or terminal exsanguination under full anaesthesia.

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)?

Humane clinical endpoints will be defined, therefore, unnecessary suffering is avoided. At the end of all studies, animals will be euthanised by a Schedule 1 method or by terminal exsanguination under full anaesthesia.

Mice, Cotton Rats, Hamsters and Ferrets

The expected severity from respiratory pathogen infection is expected to be to be severe in less than 20% of mice, cotton rats, hamsters and ferrets on this project.

Non-human Primates

The expected severity from respiratory pathogen infection in NHPs on this project will be moderate at most.

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

- Killed

A retrospective assessment of these predicted harms will be due by 19 January 2028

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 research focuses on assessing the effectiveness of treatments for infections caused by established, emerging and potentially dangerous respiratory pathogens. There is a requirement to develop new treatments and vaccines to ensure that they are optimised and to provide evidence for safety and efficacy against new or emerging health threats.

The ways in which the human airways protect themselves against respiratory pathogens and the ways in which the immune system works is complex and has not yet successfully, completely and reproducibly been modelled in computers or cell-based systems. We cannot show whether a vaccine or treatment can both reduce symptoms and prevent progression of disease in the body except by using animals.

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

Limited trials can be conducted in human volunteers. There are no suitable non-protected animal alternatives in which to model respiratory pathogens. If possible, vaccines or

medicines will be tested for simple effectiveness (i.e. an antibody stopping a virus from entering cells) in non-animal systems as a stop:go decision point prior to being assessed in animals on this project.

Why were they not suitable?

There are ethical and safety reasons that prevent us from studying what we aim to study during the project in humans.

A retrospective assessment of replacement will be due by 19 January 2028

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 numbers of animals used during the five-year period of this licence is based on the numbers of animals used on the predecessor of this licence and refinements made during its use.

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

Individuals associated with this project have a vast amount of knowledge and experience when designing studies, with the aim of using the minimum number of animals per group while still generating data that are useful. Statistical advice is available, and this advice will be used to minimise animal usage.

We always use statistical calculations to identify and use the minimum number of animals required on a study, whilst still providing robust scientific data which will be accepted and give statistical relevance and comply with any relevant regulatory requirements.

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 required for a study will be determined by how variable the infection model outcome is. We reduce our model variability by optimising our infection procedures using good microbiology and world class instrumentation.

Studies will be conducted in a step-wise manner so that the number of animals used will be minimised if the vaccine/treatment shows no likelihood of working; for example, if a new vaccine does not elicit an appropriate immune response, then it would not progress to a challenge efficacy study.

Where appropriate, pilot studies with a reduced number of animals or groups, will be performed. This is particularly important where challenge dose must be determined, or to assess vaccines ability to elicit an immune response or to establish a dosing regimen. Although statistical significance would not be determined, in the long term, this avoids a negative outcome involving a large number of animals.

Robust scientific quality control of the test materials and methods will ensure studies are carried out successfully the first time, minimising the need to repeat studies and subsequently reduce the number of animals used. All protocol and study designs would be reviewed by our AWERB and improvements and suggestions would be implemented where possible.

A retrospective assessment of reduction will be due by 19 January 2028

The PPL holder will be required to disclose:

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

Refinement

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

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

The majority of work carried out in this project will either use mice, cotton rats, hamsters or ferrets depending upon the respiratory pathogen. Non-human primates will only be used in particular circumstances where required.

To assist in the prompt recognition and subsequent intervention, critical periods have been identified (depending upon pathogen) and monitoring frequency increased. Our staff work in shift patterns, to ensure that animals considered to be in the critical phase of an experiment are regularly monitored. This high frequency, hands-on monitoring, has been shown to effectively minimise the welfare costs to animals and hence reduce the severity and quickly identify if an animal is heading towards a humane end point. Humane clinical endpoints have been clearly defined therefore unnecessary suffering is avoided.

In order to provide animals with maximum social interaction and environmental enrichment we will aim to group-house animals for the duration of studies where possible.

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

The majority of animals used within this project will either be mice, cotton rats, hamsters or ferrets depending on the respiratory pathogen being investigated. Aspects of this project rely on the ability to observe clinical signs in the infected animal, for example antiviral testing upon symptom onset, therefore using an appropriate species for the selected pathogen is essential. Non-human primates will only be used in exceptional circumstances, smaller animal models will always be used preferentially.

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

We strive to conduct the minimum number of experimentations as possible, to collect data to exemplify the interventions associated within this licence. We use rigorous monitoring processes to ensure that we minimise the welfare costs to the animals. Our staff work in shift patterns to ensure that animals considered to be in a critical phase of an experiment are regularly monitored. The frequency of which can be increased whenever necessary depending on the severity of the presenting symptoms. The health scoring system we have developed over many years provides a more holistic overview of the potential harms we are causing and when to intervene such that harms are minimised. The high frequency of hands on monitoring has been shown to effectively minimise the welfare costs to the animals.

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

We will follow a variety of published guidelines where appropriate, including: Local AWERB guidelines

ARRIVE guidelines of the NC3Rs

Guidance on Animal Testing and Research from the Home Office Good research practice guidelines from the Wellcome Trust LASA and RSPCA guidelines and

Handbook of Laboratory Animal Management and Welfare, Fourth Edition Editor(s): Sarah Wolfensohn, Maggie Lloyd. Published Online: 04 Jan 2013, Print ISBN: 9780470655498.

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

We will stay informed regarding advances in the 3Rs and keeping up to date with advances in the field of animal modelling of respiratory pathogens. This will be done by attendance at meetings, conferences and continued discussion with our peers.

Discussion around advances in the 3Rs that will benefit the animals used in the project will be encouraged. These changes will be presented internally to the AWERB committee. Any changes that will be beneficial to the animals while maintaining the scientific integrity of the protocols will be implemented. Changes will be made to this project, if required, to implement advances in the 3Rs effectively.

A retrospective assessment of refinement will be due by 19 January 2028

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. Repair and Regeneration of the Injured Heart

Project duration

5 years 0 months

Project purpose

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

Key words

Myocardial Infarction, Heart Failure, Cardiac Regulation, Immodulation

Animal types	Life stages
Mice	adult, embryo, neonate, juvenile, pregnant, aged
Zebra fish (Danio rerio)	adult, 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 overall aims of this project are to identify potential drug targets to harness and enhance resident molecular and cellular mechanisms that already exist in the heart; to facilitate optimal repair and tissue restoration following acute and chronic stages of heart injury and to improve outcomes and prevent heart failure.

A retrospective assessment of these aims will be due by 06 June 2028

The PPL holder will be required to disclose:

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

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

Why is it important to undertake this work?

Cardiovascular disease (CVD) is the single most common cause of mortality and morbidity worldwide http://www.who.int/cardiovascular_diseases/en/ of which ischaemic heart disease and myocardial infarction (heart attack) is a major contributor. A consequence of heart attack is irreversible loss of tissue, scarring and progression to heart failure. There are no drug treatments for 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 candidate pathways for drug development and treatment.

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

We will gain new information as to how the heart responds after a heart attack, in an attempt to repair the damage caused. We will also gain specific insights into the changes that occur within resident heart cells and incoming immune cell types which drive the process of scarring (fibrosis) that leads to heart failure. From this knowledge, we aim to identify new ways to repair the damaged heart, restore lost cardiovascular tissue and modulate the local injury environment to prevent heart failure. We will publish our findings in high impact journals and disseminate our studies via online web sites, social media and at conferences and symposia.

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

After a heart attack, a significant portion of the heart’s muscle is irreversibly damaged. With rapid intervention to restore blood flow survival rates after the initial “attack” are improving, however, there is an increase in the debilitating condition of heart failure in a growing number of patients. We are researching novel ways to promote heart regeneration targeting resident and infiltrating cell types after acute and chronic injury. Beyond this project (approximately 10-15 years), this information may be used to develop new drugs to repair the heart by a combination of enhancing new muscle and vessel formation and dampening the immune and scarring responses. This would provide treatment for the 900,000 heart failure patients in the UK and millions more worldwide, currently an unmet clinical need.

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, online and via preprint repositories such as BioRxiv.

Species and numbers of animals expected to be used

- Mice: Maximum 28,000
- Zebra fish (Danio rerio): Maximum 36,000

Predicted harms

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

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

We make use of both zebrafish and mice to study cellular and molecular responses to heart injury, using surgical approaches which mimic human ischaemic heart disease, heart attack and heart failure. The adult zebrafish can inherently regenerate its heart, as can the neonatal mouse, whereas the adult mouse undergoes fibrotic repair with ensuing heart failure over time mimicking human patients. Use of these different animals (zebrafish and mouse) at different stages (neonate versus adult) enables us to directly compare regenerative versus non-regenerative models in a single programme of work. This in- turn facilitates the identification of potential molecular targets that might promote more optimal tissue repair or regeneration. Moreover, directly comparing findings from zebrafish and mouse serves to identify key evolutionarily conserved factors which helps us to prioritise target pathways for application to non-regenerative adult mice and in the future to human patients.

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

A smaller number (approximately 20%) will undergo surgery to model human cardiovascular diseases:

In some zebrafish (maximum 20%), we will stimulate a heart attack by removal of ventricular tissue or damage through cryogenic injury.

In some mice (maximum 20%), we will simulate a heart attack by tying a suture around a major coronary artery as standard or via needle occlusion of the coronary artery with ultrasound guidance.

Up to 10% of mice will be fed a high fat diet with the infusion of the naturally occurring hormone Angiotensin II to model heart failure.

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 tissues postmortem, 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; intravital imaging) 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 in zebrafish requires the induction of cell death and scarring by surgical or cryogenic damage to the heart tissue . This is very well tolerated by the majority of fish (>90%) and they recover and resume swimming within minutes. The remining 10% may show signs of distress, due to the complexity and invasiveness of the procedure, which include alterations in behaviour such as reduced activity, lower frequency of swimming, abnormal swimming behaviour; rapid gill opening and closing indicative of altered respiration rates; bleeding from the gills or failure to respond to food.

Modelling a heart attack in mice (neonatal and adult) 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 continued use of 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 48 hours.

Modelling heart failure: as with humans, the majority of cases will be asymptomatic during the initial stages, however, beyond the 16-week protocol animals may develop clinical symptoms of heart failure, including increased heart rate and elevated blood pressure. This can lead to breathlessness, tissue oedema, fatigue, disorientation and lack of appetite.

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 - 75% mild; 20% severe.

Zebrafish - 75% mild, 15% moderate, 10% severe

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

- Killed

A retrospective assessment of these predicted harms will be due by 06 June 2028

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 and extracellular matrix of the heart, blood vessels and the immune system. Despite advances in cell based systems, organoids , tissue slice cultures, computer modelling and the benefits of using clinically relevant patient biopsies, none of these methods faithfully models events such as heart attack or heart failure. All of these approaches fail to accurately model the complex cell-cell and cell-tissue cross-talk in a 3D context. Hence, the use of animals is unavoidable if we are to answer important questions about causality and to 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, examples include embryonic stem cell derived epicardial cells and induced pluripotent stem cell derived macrophages (that are relevant to our project). Importantly, we can also obtain cardiovascular cells from patient biopsies (for eg. atrial appendage from patients undergoing coronary artery by-pass). We have used such cells to test the efficacy of small molecule drugs in a dish. Only once promising compounds with potential for therapeutic use in humans have been identified and reproducibly validated (by dose response and structure-activity relationship studies) would we test them in live animals.

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, blood vessels and immune cells

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 06 June 2028

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 26 years of experience in designing experiments with animals. Our previous studies and the published literature 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 statistical and experimental design courses, and have previously consulted statisticians on the quantitative aspects of animal experiment design.

All of my recently awarded funding applications required me to demonstrate how animal numbers were calculated (including the use of power calculations) and these have been externally 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 from 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 animal. For example, we may take the heart and blood from a single animal which, depending on the treatments given, can be used in multiple projects. We can use a single heart for next generation sequencing and proteomics to obtain information on literally thousands of genes/proteins; we can then validate expression changes of approximately 35 genes per sample or we can collect around 100 sections to localise and quantify gene/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 06 June 2028

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 congestive heart failure are serious, life-threatening conditions in human patients and can only be accurately modelled using severe protocols in animal models.

In zebrafish we induce heart injury using the established approaches of either ventricular resection or cryo-cauterisation of the ventricle; both require extrusion of the heart but are rapid procedures and we have refined the extent of resection or cryo-probe exposure to minimise adverse effects whilst ensuring sufficient injury to invoke the appropriate cellular responses.

In mice (neonatal and adult) to induce a heart attack, the step to insert an intubation tube into the trachea to ventilate 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. As a potential significant refinement, we plan to establish a protocol for minimally invasive technique to create a heart attack by occlusion of the left anterior descending artery via ultrasound- guided needle to block blood flow and induce a heart attack in mice to replace open heart surgery.

The surgical procedure to induce heart failure in adult mice (implanting a small pump under the skin for delivery of Agiotensin II) causes only mild discomfort for up to 24 hours. However, heart failure develops at a different rate in genetically altered mice and the possibility and timing of clinical symptoms is, therefore, difficult to predict. Our experiments are designed to end before major discomfort (breathlessness, fatigue, disorientation), however, in a small number of mice (10%), adverse clinical symptoms acute clinical signs can develop, as all animals will manifest some degree of expected adverse effects following surgery. 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 heart rate or blood pressure to detect the earliest signs of abnormal heart function.

In all surgical models, pain, suffering and distress is minimised by analgesia, daily monitoring and use of humane endpoints when necessary. This work is carried out by very experienced and who's competence reduces the loss of animals.

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

Lower model organisms that are used in biomedical research, such as worms (C. elegans) or fruit flies (Drosophila), either do not have a heart (worms) or a very rudimentary “dorsal vessel” (flies) which does not model the human heart and does not enable studies of complex responses to heart injury, including multiple cell-cell communications, inflammation and scarring, or heart regeneration.

Zebrafish are a genetically tractable model organism and of major interest for our studies because of their inherent ability to regenerate their hearts after injury. Moreover, they can do this is the absence of scarring or following the formation and resolution of scarring, depending on the nature of the insult (resection or cryoinjury, respectively). This makes them an invaluable model to identify intrinsic processes that modulate scarring and/or restore lost tissues which can then be applied to non- regenerative adult mice and eventually humans. Mice are essentially the only mammalian model amenable to genetics to assess individual loss or gain of gene function in the context of cardiac function and regeneration. Neonatal mice can regenerate their hearts during the first week of life making this so-called regenerative window of particular interest to identify molecular and cellular events which alter during this critical period and for comparison with adult zebrafish findings followed by extrapolation to adult mice. Ultimately, the cardiovascular diseases we wish to model occur in the adult human population in which there is limited regenerative capacity, thus adult mouse stages are very relevant. Studying heart function and the changes that occur both acutely and chronically require the animal to remain alive for several weeks or months following a simulated heart attack.

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

The Zebrafish has proved to be a powerful tool for studying human cardiac development and disease. As use of the Zebrafish has expanded in research, unfortunately the understanding of pain and therefore welfare management has been slow to catch up. In particular the type, dose, administration route and effect on scientific outcomes. A welcome progress in the field of Zebrafish analgesia is the outcomes of the FELASA working group and their report on 'Pain Management in Zebrafish' regarding effective analgesia that might be employed pre or post operatively, the recommended route of administration and the dosage. We will actively use this as a guide to analgesia in fish and refinements that we can introduce to our protocols and work closely with the VET and NACWO to identify and implement analgesic regimes.

For all surgical procedures in mice, 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 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

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

I attend the termly Departmental (HO animal licence) Animal Welfare Meetings, which includes presentations from our in-house biological services staff and affiliates and updates on the 3Rs. 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 to attend. We frequently discuss 3Rs at group meetings.

A retrospective assessment of refinement will be due by 06 June 2028

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. The Safety Evaluation of Chemicals in Dogs and Pigs

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

Non-Clinical, Safety, Dog, Pig, Toxicology

Animal types	Life stages
Beagles	embryo, neonate, juvenile, adult, pregnant, aged
Pigs	embryo, neonate, juvenile, adult, pregnant, aged
Minipigs	embryo, neonate, juvenile, adult, pregnant, aged

Retrospective assessment

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

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- 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 project aims to test non-pharmaceuticals (agrochemicals, biocides, food additives /foodstuffs, ingredients of house-hold chemicals (where legislation allows) and industrial chemicals) in large animal species (pig, mini-pig and dog).

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).

The dog will only be used when, for scientific reasons, the pig or minipig cannot be used to fulfil the aims of an individual study.

These studies are run to satisfy the requirements of UK/EU (and sometimes international regulatory authorities) who are independent of governments) which require the testing of pharmaceuticals in a non-rodent species. Study designs are based on OECD guidelines for chemical testing.

A retrospective assessment of these aims will be due by 20 January 2028

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?

Governments require (and the public expects) that substances we are exposed to are safe or that their potential hazards are well understood and documented. Before potential new chemicals are exposed to consumers, their safety must be evaluated. This is mandated by law to ensure public safety.

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.

New chemicals/agrochemicals have the potential to increase or protect food production while minimising safety risks to consumers and/or adverse effects on the environment.

Before potential new chemicals/agrochemicals are used in the environment, their safety must be evaluated. This is mandated by law to ensure public safety.

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 large animal species to evaluate existing chemicals and novel and currently-registered substances in terms of systemic toxicity and toxicokinetics. This licence only authorises the testing of non-pharmaceuticals.

The overall benefit of this project is that it generates high quality data that is acceptable to regulatory authorities and support these submissions, and enables internal decision making within our clients' organisations. This project will also ensure that chemicals that the general population are exposed to are safe.

Supporting studies, including preliminary studies and candidate selection, will enable appropriate dose selection and appropriately focussed observations and investigations in the definitive regulatory studies.

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

Our customers will benefit, as the data we generate will allow them to progress their substances under development and, where appropriate, to satisfy governmental regulatory requirements necessary to gain marketing authorisation.

The project aims to test non-pharmaceuticals (agrochemicals, biocides, food additives /foodstuffs, ingredients of house-hold chemicals (where legislation allows) and industrial chemicals) in large animal species (pig, mini-pig and dog).

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 so that they can be handled safely.

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

The work will be shared with customers 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).

Where appropriate, we collaborate with our customers to share data we have produced in the form of Scientific publications that are in the public domain.

We are able to advise our customers on which studies are required in their development programme and on suitable study designs, based on our experience and on knowledge

gained from previous post- registration feedback from customers and/or regulators, leading to focussed and effective studies.

It is difficult to predict how the benefits of any work done on this project will be seen in the future due to confidentiality issues. However this work will contribute to the safety of chemicals that the public and animals are exposed to.

Species and numbers of animals expected to be used

- Beagles: 2500
- Pigs: 280
- Minipigs: 2050

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 dog, pig or minipig are used in these studies, as they are well characterised species with a lot of background scientific data available over many years. They also satisfy the requirements of global regulatory authorities for safety evaluation in non-rodent species, which is required by law prior to allowing use that may result in exposure of humans. Testing is performed to OECD guidelines.

Most chemicals are tested in a rodent species prior to testing in a non-rodent species, as covered by this project.

It is a legal requirement in the UK that dogs (or cats or equidae) may only be used in a programme of work involving regulated procedures when the objectives of the work cannot be achieved by using another species. In this project, the dog will only be used when use of the pig or minipig would not achieve the aims of the experiment, or satisfy regulatory authorities. All requests for studies using dogs are assessed by means of an internal review process; the review panel, including scientists, project licence holders and responsible persons under ASPA, consider the information presented to reach a consensus decision, and will only approve the use of dogs where there is robust justification that the study could not be successfully performed using pigs or minipigs instead.

Most studies would be carried out in adult animals; juveniles would only be used for specific studies where necessary to evaluate potential risks of human juvenile exposure. Similarly, studies in pregnant/breeding animals will only be conducted where there is a need to assess safety of test items to which reproductively active humans or animals may be exposed.

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

Animals are dosed by the intended/likely route of human or animal exposure (for example oral administration, injection, infusion or inhalation), and observed regularly to monitor appearance, behaviour and clinical health.

Some animals may undergo a surgical procedure under general anaesthesia, eg placement of a deep vein catheter for intravenous infusion, or implantation of a monitoring device or minipump. Investigative procedures carried out in these studies are similar to diagnostic procedures that might be used medically to monitor progress of a human patient and include, for example, collection of blood and urine samples for laboratory investigations, or ECG monitoring to assess heart rate/function, or examination of the eyes using an instrument similar to those used by opticians. 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.

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 repeat-dose studies which can last up to 1, 3 or rarely 12 months. 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 capsule, or sometimes by incorporation in food Other common routes include inhalation (when animals are normally dosed via a face mask) 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 of dogs, pigs or mini-pigs. 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 to take as many of the tissues and samples we need after the animals have been humanely killed after all dosing had been completed.

If we need to take a urine sample for analysis, we would put an animal into a special collection cage which is smaller than their normal cage. The animal can still move around.

Other more unusual tests might include assessment of retinal function, assessment of neural function, taking small samples of tissue under general anaesthesia, collection/examination of body fluids such as tear fluid or semen, collection under general

anaesthesia and examination of lung washings or spinal fluid, body temperature by rectal thermometer. A minimal degree of restraint or confinement may be required for some procedures. Where appropriate, positive reinforcement training (using treat rewards) is used to encourage co-operation in (and minimise any stress of) handling/procedures.

Some animals may be used on procedure on more than one occasion (re-use); such re-use is limited and strict criteria are applied, eg veterinary examination indicates that it is appropriate to do so. Some animals (dogs only) may be re-homed via the establishment's rehoming scheme if it is in their best interests, but most animals are humanely killed at the end of the study to allow detailed examination of the organs.

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 degree of pain or discomfort an animal feels is similar to what a patient would feel having an injection done by a doctor.

Most animals confined temporarily in a urine collection cage will get used to their new cage within a short time and show no adverse effects; however, in a small number of cases, some animals may show a degree of stress or anxiety unless the confinement is discontinued.

Dosing with chemicals may cause adverse effects in some studies. Experience shows that the majority (~60%) of animals are not expected to show any clinical signs of suffering (either no clinical signs or normal background signs. A small percentage (~30%) may show transient subtle to mild clinical signs. Moderate signs of adverse effects may be seen in some animals (~10%), usually in the higher dose groups. Lethality and/or severe effects are not study objectives in any of the protocols within this licence.

We 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 consult vets and other senior animal care staff for advice and guidance in its care.

Most animals are expected to experience no, or only mild, adverse effects during the course of the study such as slight weight loss. A small percentage of animals may show more significant adverse effects, such as more marked weight loss, reduced activity, vomiting or tremors. No animals would be expected to die or to suffer prolonged adverse effects as a result of the procedures, and where necessary early humane end-points are applied, under veterinary guidance as necessary, to prevent this; such end-points might include interventions to discontinue dosing, or to provide supportive treatments, or if necessary to humanely kill the animal.

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 experienced mild severity, and around 10% of animals were classified as having experienced moderate severity. The moderate severities in the last project were either due to treatment-related signs of moderate severity (mostly in preliminary studies) or because a surgical procedure, e.g. cannulation, was involved.

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, however, a distribution between ‘mild’ and ‘moderate’ severities similar to those in the last project are anticipated..

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 Kept alive
- Rehomed
- Used in other projects

A retrospective assessment of these predicted harms will be due by 20 January 2028

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 animals in these studies. These studies are run to satisfy the legal and 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.

The vast majority of non-pharmaceutical testing takes place in rodents. However, in some instances, the use of a rodent to test non-pharmaceuticals is not suitable for, e.g. metabolic, physiological or other reasons. In these cases, the use of the dog, pig or mini-pig may be more suitable.

We maintain a constant awareness of regulatory guidance and ensure that where non-animal methods exist which fulfil the regulatory requirement they are used in preference to animal studies.

The regulatory requirements are mainly for UK/EU regulators, but occasionally other regulators in other countries like the US for example. If the requirements for these non-UK/EU tests are over and above the requirements for a UK/EU regulators, or the test required is more severe, then we consult the Home Office to ask for prospective authority to run such tests.

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

We maintain a constant awareness of regulatory guidance and ensure that where non-animal methods exist which fulfil the regulatory requirement they are used in preference to animal studies. Currently, however, 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 processes together interactively, as in animals and humans.

That is why we need to test chemicals in animals; they have similar physiology and chemical processes to those in humans, and such testing gives a good indication of effects in humans exposed to the chemicals under evaluation.

A retrospective assessment of replacement will be due by 20 January 2028

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?

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 to get as many outputs as we can from a single animal where possible, without adversely affecting its welfare. So if we need to take several different samples, for example, we will often do that in the same animal, rather than using 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 preliminary studies are important as they give us confidence that the doses we are using are correct prior to testing them in bigger groups of animals as required by global regulators.

A retrospective assessment of reduction will be due by 20 January 2028

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 dogs, pigs and minipigs We only use dogs when pigs or minipigs are unsuitable for scientific reasons.

The models we use are the least invasive procedures, for the least amount of time necessary to get the information we need. They are carried out using standard and recognised techniques by fully trained staff. We also have veterinary clinicians on hand for advice and on the occasions we have to anaesthetise the animals and for general advice on animal welfare.

If we have to repeatedly inject animals or withdraw blood using a needle and syringe, we would choose different sites to do this where possible to minimise local adverse effects.
Where appropriate we place temporary cannulas in blood vessels to reduce the number of needle punctures necessary. If we can take blood samples when an animal is deeply unconscious then we do so.

For all surgical procedures pain relief will always be provided. Surgical procedures will be carried out aseptically and to at least the Home Office minimum standards for aseptic surgery, and in accordance with the principles set out in the LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery (2017) (LASA is the Laboratory Animal Science Association). Basically any animals who undego surgery will get the same standard of care as a patient who needed surgery in hospital.

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

There is a scientific and regulatory requirement for safety/toxicity data in non-rodent species such as dogs or pigs to supplement rodent data which will enable a complete risk assessment to be made.

We use pigs in preference to dogs wherever possible (a legal requirement in the UK) dogs are only used where necessary to achieve the study objectives, ie when the pig is unsuitable (for example due to species-specific differences from humans, toxicological responses, or practical limitations due to anatomy or physiology).

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.

The dog is stated as the most commonly used non rodent species in the regulatory test guidelines covered by this licence.

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 animals are recovering from surgery, we give them extra heat and monitor them closely until they are fully recovered and showing normal behaviour. We then check them at least twice daily before they go on study.

During dosing and restraint, animals are constantly and closely watched for signs of distress. If equipment is used to enable us to achieve the scientific aims of the study (e.g. confinement in a metabolism cage for urine collection), then we would habituate animals to this equipment prior to dosing.

If we have to repeatedly inject animals or withdraw blood using a needle and syringe, we would choose different sites to do this where possible to minimise local adverse effects..Where appropriate we place temporary cannulas in blood vessels to reduce the number of needle punctures necessary. If we can take blood samples when an animal is deeply unconscious then we do so. 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.

For situations involving restraint or confinement procedures (e.g. in a metabolism cage) the animals are habituated to this equipment starting with short periods, then building up. Most animals habituate well to this equipment, but if they don't (rare) we remove them from the study.

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 any adverse events and provide supportive care and treatment as appropriate.

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, 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?

The conduct of Regulatory Toxicology and Safety Evaluation Studies. UK Home Office. 2005 Guidance on the Operation of the Animals (Scientific Procedures) Act 1986. UK Home Office 2014

OECD (1998), Repeat Dose 90-day Oral Toxicity Study in Non-Rodents, Test Guideline No. 409, OECD Guidelines for the Testing of Chemicals, OECD, Paris.

OECD (2018), Chronic Toxicity Studies, Test Guideline No. 452, OECD Guidelines for the Testing of Chemicals, OECD, Paris

OECD (2009), Draft Guidance Document on the Design and Conduct of Chronic Toxicity and Carcinogenicity Studies, Series on Testing and Assessment No. 116, available on the OECD public website for Test Guidelines at www.oecd.org/env/testguidelines.

Regulation (EC) No. 1907/2006 – Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).

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).

Forster R (ed.) (2010) The Rethink project – Minipigs as models for the toxicity testing of new medicines and chemicals: an impact assessment. Journal of Pharmacological and Toxicological Methods: Volume 62, No. 3.

Trennery P, Smith D (2002) Non-rodent selection in pharmaceutical toxicology. A ‘Points to Consider’ document developed by the ABPI in conjunction with the UK Home Office.

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 Animal 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 January 2028

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. Mapping Brain Networks in Experimental Epilepsy by Eeg and Mri

Project duration

2 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

imaging, epilepsy, EEG, epileptogenesis

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?

This project aims to develop, evaluate and optimise clinically relevant, translational biomarker of epilepsy that is created by combining brain imaging by MRI and brain activity recording by electroencephalography (EEG) in conjunction with computer modelling. We will conduct these studies in epileptic animals under controlled conditions where we can be sure to understand if our modelling ("MINM") is accurate, and to optimise it, before taking this methodology into clinical settings. The aim of methodology is to improve the detection

of the source of seizures in the brain, so that the brain tissue can be more accurately and precisely treated by either resection or optogenetic silencing.

A retrospective assessment of these aims will be due by 30 March 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 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?

Epilepsy is a severe brain disease that is sometimes very difficult to treat, even requiring a surgical removal of a part of the brain in an attempt to alleviate the patient's suffering. If not properly treated, epilepsy can result in severe incapacitation. In cases where surgery is needed, locating the exact area of the brain to be removed is extremely important, not only to ensure that patients are seizure free, but also to minimise post-operative impairment. At this point, it is not possible to accurately predict which area of the brain to remove, often resulting in failure or inadvertent incapatication of patients. By modelling epilepsy in animals, and optimising methods to detect the origin of seizures, we hope to be able to improve these methods of epilepsy treatment.

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

The present project will produce high quality data that will be published in peer-reviewed journal(s). The combination of data acquisition and computational modelling methodology developed during this project will be of translational value, helping to validate, refine and improve tools in development for clinical use.

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

The data acquisition protocol developed during this project has the potential to benefit neurosurgeons as well as patients undergoing pre-surgical assessment, by improving the quality of pre-operative evaluation and the precision of epileptogenic focus localisation, which in turn, will lead to a reduction of the invasiveness of the current techniques, with reduced morbidity and increased treatment success rates. Our computational model will provide a framework for validation, refinement and improvement of future tools aimed at determining the target area for surgical treatment, improving surgical outcome and reducing post-operative impairment.

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

This project is based on a multidisciplinary approach, involving collaborators from neurosciences, neuroimaging, mathematics and computer sciences. This approach will harness the strengths of the different disciplines, maximising outcome and fostering further multidisciplinary collaborations.

Findings will be published in peer-reviewed journals, in accordance with the ARRIVE guidelines. In addition, we will post news and blogs related to most interesting findings on our websites. We routinely organise meetings which includes people with epilepsy in the audience, and we will aim to present preliminary findings. We will also build on our track-record of presenting scientific findings in this field through theatre and other arts media.

Species and numbers of animals expected to be used

- Rats: 40 rats

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.

Models of epilepsy using adult rats have been used for decades and have contributed significantly, not only to the understanding of how epilepsy develops, but also in the discovery and testing of novel anti- epileptic drugs. There are clear and reproducible similarities between the brain changes observed in such animal models (such as lesion to a part of the brain called hippocampus) and those observed in patients.

In animals, there is strong evidence that an “epileptic network” gradually emerges in focal epilepsy, allowing seizure onset outside the primary “focus”. Sheybani et al (2018) used high density EEG to demonstrate dynamic development of epileptic networks in rodents injected with kainic acid in the

hippocampus. Electrical activity progressed to involve not only hippocampus but also frontal parts of the brain, and remote areas became independent from the original focus as epilepsy progressed. Early surgical intervention, removing the lesioned hippocampus, successfully treated the epilepsy, while the same intervention after a longer period of epilepsy did not result in control of seizures.

These observations are of significant translational value in the quest to develop computational models of epileptogenic network progression and focus identification.

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

Animals’ brains will first be imaged using simultaneous EEG and fMRI (under light anaesthesia) in order to establish a baseline reading of "normal" brain activity. One week after this, they will receive an injection of a pro-convulsive “epileptogentic” compound (e.g.

pilocarpine, kainic acid), either systemically directly into the specific area in the brain that is known to produce seizures. During seizure induction, animals may be pre-treated with drugs that decrease seizure threshold (e.g. lithium) or that decrease side effects of epileptogenic compound (e.g. scopolamine). Such injections will induce an initial sustained epileptic seizures lasting up to 2 hours. After this initial episode, animals will be treated with anti-epileptic drugs and the seizures will be stopped. This initial episode will cause animals to later (after 2-3 weeks) develop spontaneous seizures after which they will be imaged again at several time points (up to 3 times). After the final imaging session, animals will be killed (Schedule 1, perfusion fixation or decapitation under anaesthesia) in order to harvest brains for histological study.

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

Animals are expected to develop spontaneous seizures. These should have a frequency of 2-3 per week, be self-contained and should not have any significant impact in animals' wellbeing. A small percentage of animals (less than 5%) might develop more severe seizures, which might result in weight-loss, reduced mobility, reduced grooming and signs of dehydration (e.g. reduced skin elasticity). Whenever animal weight loss is higher than 15% of initial weight, or they show signs of deteriorating health, animals may be initially treated with appropriate supportive measures (e.g. modified diet, hydrolitic fluid injections, short treatment with AED). If animals do not show signs of recovery after 48 hours of supportive treatment, they 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)?

- Normal spontaneous seizures: mild to moderate severity, 95% of animals receiving epileptogenic treatment

- Severe seizures: severe, up to 5% of animals receiving epileptogenic treatment

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

- Killed

A retrospective assessment of these predicted harms will be due by 30 March 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?

Animals have to be used because it is not yet possible to model epileptogenesis without looking at a whole living brain. This is because our knowledge about structure and function of the central nervous system, as well as of the pathological events during development of epilepsy, are not yet sufficiently advanced. Indeed, one of the main aims of this work is to help advance knowledge about how epilepsy develops, which will eventually lead to improved non-animal modelling of the disease.

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

As stated above, there are no replacements, either in vitro or in silico, for the whole brain and, specifically, for how a normal brain becomes epileptic. Therefore, there are no known suitable alternatives.

Why were they not suitable?

The complexity of the central nervous system renders it impossible, with current knowledge and technology, to replicate and model with any level of certainty, the normal and pathological processes observed in brain.

A retrospective assessment of replacement will be due by 30 March 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?

In order to create the computational model which is one of the three objectives of this project, based on previous experience developing similar computational tools, between 10 and 15 animals per group (2 groups: control and epilepsy) will be needed in order to achieve the required statistical power.

Considering that up to 5% of animals may not develop initial frequent/severe seizures (during protocol 1), with an additional 10% that might not develop chronic spontaneous

seizures, we expect between 2 and 3 animals to be dropped out of the experimental group. An extra group of 10 to 15 animals, receiving intracerebral injections, may also be required if the systemic injection group does not yield suitable data for the computational model development.

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

The model of epilepsy selected has a high reproducibility (higher than 90% success rate in developing long-term spontaneous seizures); All animals will be recorded using high density EEG (32 channels) and simultaneous MRI, which will include a number of MRI protocols (e.g. resting state, structural, conductivity), ensuring that we maximise the amount of information extracted from every subject, hence reducing the need for extra groups/animals.

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 objective of this study is to create computational models of epileptogenesis and the epileptic networks, which will inherently lend themselves for reduction in the required number of animals used in future experiments.

A retrospective assessment of reduction will be due by 30 March 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.

The systemic injection of pilocarpine, in animals pre-treated with anti-muscarinic drug (e.g. scopolamine) and lithium, ensures the dose of the epileptogenic drug can be minimise, improving reproducibility and, at the same time, reducing its adverse effects. This model ensures a high level of reproducibility and relevance to the human condition.

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

The nature of this study is to use data acquired during the epileptogenic process, in initially normal adult brains, to create computational models for seizure focus prediction. Therefore, it is imperative that we use mature and freely-behaving animals, whose brains have enough complexity and analogy with human ones in order to ensure translational relevance.

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

We have consulted with NVS and NACWO, and are endeavouring to follow refinement practices such as those described in:

Wolfensohn S, Hawkins P, Lilley E, et al (2013) Reducing suffering in animal models and procedures involving seizures, convulsions and epilepsy. Journal of Pharmacological and Toxicological Methods 67:9–15.

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

"Opportunities for improving animal welfare in rodent models of epilepsy and seizures" (National Centre for Replacement, Refinement and Reduction of Animals in Research – NC3Rs)

"Reducing suffering in animal models and procedures involving seizures, convulsions and epilepsy" (Wolfensohn S, Hawkins P, Lilley E, et al, 2013)

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

This project has been design with help of "Opportunities for improving animal welfare in rodent models of epilepsy and seizures", from the National Centre for Replacement, Refinement and Reduction of Animals in Research. Publications and guidelines from the NC3Rs will be continually revised throughout this project.

A retrospective assessment of refinement will be due by 30 March 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?

12. Study of Aggressive Paediatric Solid Cancer Evolutionary Processes and the Impact on Drug Responses Using Patient Derived Tumour Samples

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

childhood cancer, therapy resistance, cancer evolution, preclinical tools, translational research

Animal types	Life stages
Mice	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?

Paediatric cancers are thankfully rare, yet cancer remains the leading cause of disease-related death in children. Children who die from cancer often do so because their disease

has evolved to adapt to changing environmental pressures such as exposure to treatment, and has become drug resistant.

Indeed, despite the encouragingly high overall survival rates in paediatric oncology, children with tumours that come back after treatment are typically difficult to treat. We aim to further our understanding of how children's cancers evolve to become resistant to treatment, so we can help to overcome this major barrier to being able to cure more patients.

We aim to perform our studies using innovative preclinical tools that mirror the complex nature of cancer and have the potential to predict how the disease may evolve, and so how a child will respond to treatment. We will, for example, use a type of model called patient-derived tumour xenografts (PDXs), derived from samples of a patient's tumour which can be grown in the mouse. We hope that the knowledge these mouse experiments will give us, alongside existing understanding of genomics and evolution, will allow us to develop smarter, kinder, more personalised therapeutic strategies for children, and to increase their chances of cure or long-term survival.

A retrospective assessment of these aims will be due by 18 February 2028

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?

Cancer adapts to changes in the environment in a similar way to animals, plants, bacteria or viruses, following the principles of natural selection and evolution. This innate adaptability helps explain why cancer is so difficult to treat and underlies cancer's life-threatening processes such as relapse and treatment resistance. Here we aim to use refined preclinical tools that closely resemble the originating disease to study how and why some aggressive solid tumours in children are able to adapt to evade the effects of treatment, so that they can grow back and progress. Recently, it has become apparent that in order to treat cancer successfully it is necessary to understand how it evolves. Studies involving DNA sequencing and using enhanced mathematical and computational approaches have started to reveal the fundamental processes underlying how cancers adapt and evolve. However, the complex nature of cancer and gaps in our understanding of the effects of DNA changes during the course of the disease have made it challenging to translate scientific progress into advances in treatment. We also now know that cancer has additional ways of adapting to avoid the effects of treatment other than changes to the

DNA sequence (mutations), and we suspect that these non-genetic mechanisms may be particularly important in children's cancer, which is typically driven by a relatively small number of genetic mutations.

Evolutionary studies of children's cancers are currently in their infancy. We have until recently lacked adequate preclinical and clinical studies tailored to paediatric cancers – in part because cancer in children is thankfully a rare as well as a diverse disease. As a result, clinicians have had to make treatment choices in children based on studies carried out in adults – despite the fundamental differences between cancers in younger and older patients. In an effort to change this unfortunate reality, scientists have developed laboratory tools such as patient-derived tumour xenografts (PDXs) grown in mice from samples taken from children, as a mean to better understand the biology of the disease and use that knowledge to create improved treatments. These new mouse models faithfully reflect the molecular features of the original tumour, including cancer's ability to dynamically adapt and evolve. We hope that they will also predict how children with cancer will respond to drugs – so that they could be used to pick out treatments that are likely to be effective in clinical trials. Our proposed project aims to develop and use such refined mouse models to understand the molecular mechanisms underlying cancer evolution and drug resistance in children, and to improve our tools for predicting how children are likely to respond to drugs. Ultimately, we want to use this knowledge to create new, smarter, kinder, tailored treatment strategies for children

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

We aim to generate knowledge about the processes underlying cancer's adaptation and evolution in young patients that lead to treatment resistance. We aim to perform these studies mainly in samples of a patient's tumour which can be grown in the lab (named PDXs). We aim to complement bigger initiatives such as the major ITCCP4 study via collaborations and sharing of models and expertise. By reaching out to the wider scientific community we hope we can maximise the power of other studies by providing them with improved laboratory tools for cancer research and experience. We aim to publish our data in peer-reviewed journals and share our knowledge and observations via communications in meetings and workshops, talks to the scientific community and with our collaborators and supporters. We are in the process of establishing a new network of collaborators to promote, expand, share and support paediatric cancer evolutionary studies. We will also aim to communicate about our research with the wider public through science communication, engagement and fundraising activities.

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

We aim to model paediatric cancer using improved and refined laboratory tools. During this time we aim to share our models, knowledge and expertise with the scientific community via publications/communications and educational workshops and meetings.

Treatment resistance is the main barrier to our efforts to cure cancer. Our approach aims to use laboratory tools that mirror patients' tumours and innovative technological approaches to study and understand how and why some children's cancers adapt to treatment and progress. This approach is an important advance on previous approaches as it will use mouse models that are tailored to paediatric tumours with the potential of anticipating patients' response to treatment. The information gained from these studies will help us to explore how to overcome or steer cancer evolution to avoid the development of resistant disease. The predictive value of our studies in patients will be explored in collaboration with our clinical colleagues. To this end, we will aim to compare drug responses and evolutionary trajectories in the human and in the mouse by developing a co-clinical trial. This approach will help us test the predictive value in cancer patients of drug responses in PDXs. We further plan to open up therapeutic options in children with difficult-to-treat cancers by providing the information from the PDX generated from an individual's tumour along with other clinically relevant data to clinicians in order to integrate scientific study with clinical medicine.

By gaining a deeper understanding of the molecular mechanisms underpinning adaptation and evolution in children's cancers, we aim to anticipate the trajectory of an individual patient's disease in the clinic which will help guide doctors' treatment decisions and develop improved, refined and personalised therapeutic strategies for young patients. Our overall goal is to maximise therapeutic testing in paediatric cancer and avoid/re-direct cancer evolutionary trajectories that drive the generation of refractory disease by 1. Gaining a deeper understanding on the disease and its dynamics 2. Generating improved preclinical models and testing their responses to standards-of-care and novel clinically relevant therapeutic approaches 3. Investigate the use of such improved preclinical models to answer question of unmet clinical need such as the choice of sequential versus upfront combination therapy

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

We plan to further cultivate and expand our collaborative network.

We also plan to present our work in meetings and conferences, as well as in consortiums such as the ITCCP4 (Innovative Therapies for Children with Cancer) , a large-scale platform concerned with understanding childhood cancer and speeding up the development of new treatments and EuroPDX (an association of translational and clinical researchers involved in cancer research).

We will also be involved in workshops to share our experience.

We aim to publish our experiments and improved techniques to maximise the output of our research and hope to further expand into multidisciplinary, national and international collaborative efforts in both academic and industry settings, similarly to what we have achieved previously in the breast cancer field.

Species and numbers of animals expected to be used

- Mice: 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 will use mice (Mus musculus) genetically modified to lack an intact immune system. The choice of such mouse strains is essential to ensure human tissue can be engrafted without being rejected by the immune system. Each sample will be implanted in individual adult mice of typically three months of age to allow time for the engrafted tumour to grow and expand. From the ones that successfully engraft, we will further expand cells by serially transplanting tissue into more mi

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

Typically, an animal will undergo a surgical procedure to maintain and expand the children's cancer in the lab. No more than 1 procedure will be typically performed to expand the patient's tumour material in the lab for downstream experiments. Occasionally, and in order to mirror clinical practise, a second procedure to remove the tumour may be performed. Occasionally, mice will also be given anti-cancer treatments such as standard-of-care chemotherapy and/or radiotherapy to test the predictive value of PDXs and to maximise the clinical predictive power of preclinical studies. Tumour growth will be monitored typically by palpation and/or through the use of non-invasive imaging techniques.

Occasionally, small volumes of blood will be collected to monitor treatment toxicity.

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

Mice will experience some short-term post-operative discomfort – with the degree of impact related to the surgery performed and the site used for engraftment. It is also possible that mice could experience adverse effects as a result of anti-cancer therapeutic approaches, some of which may have a degree of toxicity.

However, all animals will be monitored daily for signs of ill health and assessed for clinical signs that necessitate intervention. We will follow the guidelines for the welfare and use of animals in cancer research to minimise the adverse effects and use appropriate humane endpoints when needed.

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 90-95% of animals are likely to experience moderate levels of severity. This is because they will undergo surgery to implant or inject tumour cell fragments or suspensions to generate PDXs, with occasionally repeated blood sampling and dosing of substances. The remaining 5-10% of animals are only likely to experience mild severity because they will not undergo the surgical preparation procedures and will be used as control mice. This project uses some immunocompromised mice which are susceptible to disease, but the chance of infections is negligible because the mice are held in "barrier" conditions which protect them from picking up any infections.

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

- Killed

A retrospective assessment of these predicted harms will be due by 18 February 2028

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 very complex disease, characterised by the interplay between cancer cells and surrounding non-cancerous cells, which means many aspects of cancer can only be adequately modelled using animals. Further, many anti-cancer drugs require metabolism in an organism in order to exert their effects. Historically, good laboratory models of childhood cancer have been missing, impeding the understanding of the biology of the disease. The lack of adequate laboratory tools has now been recognised as one of the main factors contributing to the very high failure rates of potential new drugs during clinical trials. Use of cell lines and mouse models that inadequately mirrored the characteristics of human disease has made it challenging to translate laboratory results into patient benefits. However, more recent studies have shown that growing human tumour samples, including those from children and young adults, in mice (known as PDXs), can give us improved preclinical tools that are more predictive of how patients will respond in the clinic. Our aim is to understand the mechanisms underlying tumour dynamics in patients to develop improved therapeutic strategies that avoid treatment resistance. To this end, we need to use improved mouse models of the disease (PDXs) to maximise the clinical predictive power of our results.

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

We have considered use in parallel of other innovative patient-derived approaches such as organoids

– which are miniature three-dimensional tumours grown in special dishes. Occasionally, and in order to complement our studies on the molecular mechanisms of treatment resistance, we may also use cell lines in experiments.

Why were they not suitable?

Cells grown in dishes are likely to have drifted in genotype or to have been selected for specific molecular features that allow them to adapt to in vitro culture. They are therefore normally not ideal models to assess the preclinical trajectory of cancers and predict what a patient's response may be in the clinic

A retrospective assessment of replacement will be due by 18 February 2028

The PPL holder will be required to disclose:

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

Reduction

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

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

A critical step in our approach is sample availability. However, due to the strong basic and translational applications we aim to implant any available sample that comes to our laboratory through our internal and external collaborations. Our animal estimations have therefore been based on past experience, literature, sample availability.

All experiments will be designed using the ARRIVE (Animal Research: Reporting of In vivo Experiments) guidelines. Prior to carrying out any studies we take into consideration our previous experience, data in the literature, we seek the advice of the BSU team and we consult with bioinformaticians to ensure that studies are appropriately powered. Before embarking upon any study with little prior information we would start with a pilot study. We use non-invasive imaging where possible to assess parameters such as tumour volume this allows us to use the minimum number of animals to provide statistically appropriate studies.

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

We will typically use a balanced design, in which all experimental groups have equal size, as this maximises sensitivity. We will however typically consult the NC3R's Experimental Design Assistant during the experimental design phase and further use ours and published data to reduce the number of animals used without compromising the goal of the experiment.

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 typically write a protocol for each experiment including: a statement of the objectives; a description of the experiment, covering such matters as the experimental treatments, the size of the experiment (number of groups, number of animals per group), and the experimental material; and an outline of the method of analysis of the results. We will typically make appropriate arrangements to reduce the variance and increase the reproducibility of the results by randomly assigning animals to experimental groups and blinding studies and data collection and analysis. Moreover, we intend to run a pilot study in a small group of animals to better identify sample sizes and reduce toxicity without affecting the efficacy of larger studies

A retrospective assessment of reduction will be due by 18 February 2028

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 will usually be immune compromised, such as the NSGs, to increase engraftment rates. Any new models we develop will be closely monitored for symptoms indicative of tumour burden. Suffering will be minimised by keeping tumour burdens within tolerable and acceptable limits and using non-invasive imaging wherever possible for internal tumours. Pain from any surgery will be minimised by the use of refined technique,

analgesia and anaesthesia as appropriate and as previously advised by Named Veterinary Surgeon.

For a brain tumour model involving a number of procedures – surgical, chemo- and potentially radiotherapy. Initially we will carry out a pilot study. Any animals on this protocol will be vigilantly monitored. For this we have designed an Ethogram task (Table 3) for observation and neurobehavioral evaluation and score/actions details, this may be modified according to observations. This involves a five-point severity score (SS). Animals will be scored from 0–5, with 5 indicating maximal impairment at which the animal will be killed by a Schedule 1 method.

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

Mice have been shown through long experience to be the best animal system to capture and model cancer's complexity. The use of mouse models is standard in preclinical cancer research.

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

We will engraft tumours and carry out any further procedures using aseptic technique and following the LASA (Laboratory Animal Science Association) guidelines. Our past experience has helped us to know when we should consider ending a procedure because of the old age of the mice. We normally limit mice used in experiments to 12 months of age because our usual choice of mouse strain starts to develop signs of ill health after that time, although this time will occasionally be extended for a couple of months if the animal is healthy. We have also become experienced in identifying signs of rapid tumour growth, which ultimately can impact on the health of the animal because, for example, of tumour ulcerations. We may perform experiments to disrupt some genes, for example using a gene editing technique called CRISPR-Cas9, or drugging approaches. When possible, we will disrupt gene function in such a way as to only affect tumour growth, reducing the likelihood of causing severe outcomes.

We will only use well-established reagents and protocols to induce expression or deletion of the candidate gene. Where a target gene shows the potential to drive the development of tumours, we will apply growth inhibitors (putative cancer drugs) but not tumour-promoting agents to avoid severe outcomes.

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

I will use the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines which were developed with input from the NC3Rs and the scientific community on a whole.

These have further been in-bedded into Protocols which collectively will assist me in ensuring that my studies both maximise information published and minimises any unnecessary replication.

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

We will continue to refer to the guidelines for the welfare and use of animals in cancer research to minimise the adverse effects and use appropriate humane endpoints when needed plus any updates from the NC3Rs and any relevant published papers.

A retrospective assessment of refinement will be due by 18 February 2028

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. How Resolving Inflammation Shapes Tissue Immunity

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

Inflammation, Resolution, Autoimmunity, Barrier function

Animal types	Life stages
Mice	adult, aged, juvenile, 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?

Acute inflammatory responses to infections are a natural sequence of events that, until now, was believed to lead the affected tissue back to the physiological and immunological state they experienced before inflammation occurred.

However, my research is revealing that this is not the case and that as inflammation resolves a novel set of immune events occur that help to shape timmue immunity.

Therefore, I wish to understand how the resolution of inflammation triggers this novel phase of tissue immunity.

I also wish to utilise this knowledge to better treat, predict or prevent chronic inflammatory and autoimmune diseases in humans.

A retrospective assessment of these aims will be due by 10 February 2028

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?

Currently, there are no treatments to halt or reverse the underlying pathology that drives chronic inflammatory diseases only interventions that treat their symptoms.

This research will elucidate novel immune pathways that "hone" the immune system to deal more efficiently with future immune challenges. Indeed, I hypothesise that these pro-resolution pathways prevent the development of autoimmunity Consequently, I rationalise that the breakdown of these protective pathways, as what happens in old age or in the context of inherent diseases, leave the host susceptible to the development of chronic inflammatory diseases.

Indeed, these pro-resolution pathways may become subverted in otherwise healthy people when they experience an unusually over-exuberant inflammatory response such as that experienced by some people to SARS-Cov-2

Therefore, understanding post-resolution phase of acute inflammation, the mechanisms by which it becomes dysregulated and the consequences of this in terms of chronic inflammation may lead to novel therapeutic strategies that directly modulate the underlying cause of autoimmune diseases.

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

New Information

The outcome of project will provide new insight into to the internal pathways that help inflammation switches off and how these novel processes establish the next immune-driven phase of equipping tissues to better respond to future infections, so called secondary infections.

This means that every time we get an infection, the affected tissue becomes educated to better respond to future pathogens.

This is new biology that promises to provide new insight into the aetiology of many chronic inflammatory diseases.

My hypothesis is that failure of inflammation to resolve will lead to tissue being more susceptible to over exuberant inflammatory responses in the future, development of chronic inflammatory diseases and autoimmunity.

These outcomes will inform on focused experiments that that maybe carried out using "the human arm" of my research programme where I have developed various models of translation and experimental medicine in healthy and diseased humans.

New models of human research

By translating my research into humans this will have the added benefit of reducing the use of animals in research and focusing our attention developing further models of inflammation in humans that can be shared within the academic community.

Publications

Publications are the cornerstone of how our research is communicated world-wide. These are peer- reviewed journals of international renown either publicly available or through subscription via their websites or Pubmed. In addition, large data sets such as that derived from of microarray datasets and RNA-seq data will be deposited and publicly available public repositories including TACITuS NCBI GEO or ArrayExpress.

Outreach/Media/Conferences

These are additional platforms by which our novel data and findings can be disseminated in an “easy to digest format” often pre-publication thereby offering critical feedback before formal publication.

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

The impact of this research will become apparent during this programme or research and in the years to follow.

In the short term, the beneficiaries will be the scientific community and pharmaceutical industry interested in inflammation, immunology, and drug development.

In addition, knowledge, and samples generated will be shared world-wide as well as training for postgraduate students, postdoc and clinician scientist training will benefit.

Translating this research into humans will have the added benefit of reducing the use of animals in research and focusing our attention developing further models of inflammation in humans that can be shared within the academic community.

Ultimately, data generated from this project will benefit the academic community and offer new opportunities for drug development and/or refinement of the use of existing drugs.

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

Presentation of the data at scientific conferences (mainly international, some national ones)

Publication of research papers describing our findings in scientific journals eg PLoS Pathogines, Nature Communications, mBio, Infection and Immunity, Frontiers Immunology etc.

Publication of reviews and editorials that highlight our work and place the data in context, including their clinical implications

Patents on new vaccine and therapeutic products developed through close partnership with university commercialisation departments (as we have already done for experimental data obtained using the last PPL for two vaccine candidates)

Interactions with biotech and pharma to obtain external support for development of potential vaccine and therapeutic candidates.

Continuing established collaborations in the UK, Europe, USA, Thailand, and Australia and developing when necessary new collaborations

Species and numbers of animals expected to be used

- Mice: 5000
- Rats: 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.

My research group is interested in developing and characterising models of inflammation in humans. However, we are still reliant on rodents to gain mechanistic insight into how our immune system works. Mice, in particular, have emerged as the rodent of choice due to

the ease with which their genome can be altered in order to label, delete and modify specific genes and cells. Indeed, such are the advances in this field that the scientific community have moved relatively quickly from global knockouts to conditional deletions where specific genes in specific cells can be targeted in a therapeutic manner. It is this level of precision that is required to definitively elucidate cell function and origin during homeostasis and in disease states. It is for this reason that I plan to use predominantly mice for my research.

In the field of inflammation research, we are finding many parallels between events occurring in murine resolving pneumonitis, for example, and resolving human skin blisters in terms of mononuclear cell profiles, phenotypes and long-term impact on tissues post inflammatory resolution. Members of my research group are constantly drawing comparisons between our mice data and data obtained from humans using similar stimuli. Our strategy is to use clinically relevant models and stimuli in rodents that best represent events that occur in man to generate meaningful data that helps understand basic processes, disease aetiology and ultimately treat diseases in humans.

Following from this, we will use both male and female mice in our studies as we find that male humans evoke a different type of innate immune response to females, namely more severe that takes longer to resolve.

We have identified a profound impact of increased aged on pro-resolution of inflammation processes following infection thereby necessitating to use of old mice in this programme of research.

Because of the ease with which the genome of mice can be altered in order to label, delete or modify specific genes and cells, genetically -altered mice will be used to provide definitive mechanistic insight into the role that a particular biochemical pathway play in driving or resolving inflammatory responses.

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

I will inject pathogens or chemicals into rodent body cavities (peritoneum/pleural cavity), skin and lungs to mimic inflammatory responses, events that are akin to when we experience a bee sting, for

instance. These injections will cause a resolving inflammation, which by its very nature is harmless, moderate and generally clinically silent. We will be examining how inflammation switches off naturally and the impact of pro-resolution of inflammation pathways on tissue immune function within days of infection. In addition, a core part of our investigation will be looking at how long these protective pathways remain operative within these tissues following resolution. Consequently, we will be examining tissues at several time points, including months following infection.

The nature of our investigations is to determine how well our immune system works and how we can make it work more efficiently and for longer. This will involve detailed analysis

of cell types and their phenotypes within affected tissues following schedule 1 termination and secondary challenge at selected time points with the same or different infectious stimuli. These types of mild/moderate procedures constitute most of our experimental approach.

Interestingly, we discovered that when tissues resolve their responses to infections, there is a period of unexplained immune suppression that provides a window for tumours to grow. Therefore, part of our research will be examining the role post-resolution biology plays in tumorigenesis.

We also apply genetic or pharmacological interventions where pro-resolution of inflammation pathways will be experimentally blocked in animals, resulting in inflammation failing to switch off. In which case, animals are closely monitored to avoid unnecessary pain resulting from overexuberant inflammation.

Finally, animals may be used for non-invasive imaging to help monitor in real-time, and over time, changes in the immune responses to infections. This will help reduce the number of animals in my experiments as the same animals will be used over time; it will reduce suffering as the investigations will be non-invasive and reduce the number of animals needed in our studies.

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

For the self-resolving models, which represent most of our work, these responses are asymptomatic except for transient (24-48h) weight loss of no more than 5%.

Intranasal introduction of pathogens to mice may cause animals to experience transient discomfort, for less than 5 minutes after administration of the inflammatory agent. Respiratory distress (1/200) and systemic inflammation may occur (1/200).

In cases where pro-resolution of inflammation pathways are experimentally interrupted or in mice where these pathways are dysregulated, as in old mice, it is our experience that these interventions have not caused severe adverse effects beyond transient weight loss of approximately 5%.

In studies where rodents are exposed to secondary infections as a means of testing the integrity of their post-resolved immune responses, the responses exhibited are mild/non-detectable and transient (bacterial clearance within 48h).

We will inject live tumour cells into mice to evaluate the impact of inflammation resolution in cancer predisposition. Tumours develop in size over the ensuing two weeks following injection alongside metastasis. To minimise suffering, we have a robust monitoring and clinical scoring system in place.
Nonetheless, tumours can cause both local and systemic effects that can affect the animal’s normal behaviour. Along these lines, mice will be monitored daily from the

injection of cancer cells and sacrificed upon signs of distress, which include weight loss as described below, breathing difficulty, abdominal swelling, paralysis and occasionally skin lesions.
For arthritis protocol, swelling of the affected limbs occurs within a week after disease onset. Forepaws and hind paws are affected with equal frequency. The knee joints are also involved. Most of the affected limbs progress to joint deformities. This is the only protocol with potentially severe adverse consequences. We plan to use this protocol as the final proof of concept in our study, minimizing the number of animals exposed to these stimuli.

Mice may have mini osmotic pumps implanted under their skin as a means of drug delivery. These pump-implanted animals can sometimes chew each other's wounds and expose the pumps (1/50). In addition, injection sites might result in infection (1/100). Re-suturing will be allowed once within 48h. In addition to pumps, mice may receive drugs by injection. Ulceration may occur at sites of injections at a frequency of 1/200.

Ablation of bone marrow by irradiation may also be used. The dose of irradiation will be calculated to ablate the bone marrow whilst minimising signs of radiation sickness. These include diarrhoea, redness and irritation of skin, eyes and mucous membranes and loss of appetite. While the signs of radiation sickness are usually self-limiting, animals exhibiting any of the above signs for more than 24h will be killed by a schedule 1 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)?

In all, I have eight protocols which includes breeding and maintenance of genetically altered animals which is the only mild procedure. Thereafter, I have six moderate protocols including establishment of a dorsal air pouch, pleuritis and peritonitis models as well as lung and skin inflammation and a tumour metastasis model all of which will have the option to be imaged noninvasively for optimal data collection. The only severe model is collage-induced arthritis.

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 2028

The PPL holder will be required to disclose:

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

Replacement

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

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

Immune responses to infection/injury are particularly complex events. They are best described as the movement of white blood cells into and out of sites of injury, coupled with cell death and proliferation alongside biochemical interactions between these white blood cells that aim to optimise the overall inflammatory response. All these overlapping events progress under the control of soluble mediators and hormones that are released by the affected tissue. Thus, it is the very nature of this highly evolved response that can never be adequately replicated in culture dishes using single cell suspensions.
Certainly, once the major cellular players have been identified and their nature in isolation or in collusion with other cells elucidated, then more complex cell systems can be used once the limitation of their usefulness and data interpretation is appreciated and taken in context of the bigger, clinically relevant data outcome obtained from animals and man.
For these reasons, rodent species have formed the backbone of much of the research into inflammation and inflammatory pathologies. They are also the first choice for the initial screening of compounds, which are purported to possess the ability to modulate these pathologies. That notwithstanding, my laboratory is also characterising a number of skin-window models of immunity in humans for the purpose of enhancing our understanding of leukocyte tracking and phenotyping as well as soluble mediator synthesis in young and aged individuals.

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

We are spending a great deal of time, money and effort in establishing and characterising human models of acute inflammation. These include the injection of killed bacteria and vaccines into healthy volunteers (to understand basic processes of inflammation/resolution) as well as patients with chronic disease (rheumatoid arthritis and ulcerative colitis) as well as aged individuals. Many of the stimuli that we plan to use in mice can also be used in humans. This brings a great deal of complementarity to our animal research programme. It also directly reduces the number of animals that we will be using as much more work can now be done in humans. In addition, to novel human models our in vitro expertise utilises live human cells to assess the effects of various agents on expression and/or release of inflammatory mediators/modulators and functional assays such as migration, proliferation and phagocytosis (all essential facets of the inflammatory response). Since molecules shown to be inactive in these in vitro assays are not examined further in vivo, these wider activities can be seen as a means of reducing the numbers of animals utilised in the project. Finally, the project utilises a wide range of inflammatory models and models of arthritis each of which in turn incorporates a variety of different treatments. The models are described in more detail below, but the options

available mean we can select the most appropriate and least severe model for the question being addressed and alter some of its properties to allow, for example, the establishment of immune and non-immune variations.

Why were they not suitable?

Using healthy humans exposed to killed bacteria, vaccines or chemical irritants in a controlled and localised manner on their forearm has provided unique insight into human immunity. Gaining ethical approval to extend these studies to older adults and patients with chronic inflammatory diseases has been invaluable to our mission of understanding the impact of age and diseases on human pathophysiology. Collaborations with the industry have taken our research to the next level, namely using their pharmacological tools to block biochemical pathways or agonise/antagonise receptors, affording us the privilege of understanding the role of those immune pathways in human inflammatory/immune responses.

That said, we are limited to using dead infectious agents as immune stimuli in humans, while pharmacological tools are considered blunt instruments due to the off-target effects. For these reasons, rodents allow us to use live infections, which drive different inflammatory responses with different

immune sequences than dead ones while also allowing us to determine the immune capability by measuring bacterial viability.

Besides, animals allow us to perform mechanistic investigations via the administration of drugs not approved for humans. Similarly, it will enable us to use genetically altered animals to address our mechanistic hypothesis. For the late, we generally use BALB/cj or C57BL/6J mice that have alteration on the biochemical machinery that synthesise and/or transduce (receptors or signalling pathways) the action of pro-resolution mediators, including lipids. These can be whole body transgenic, single-cell or conditional knockouts.

My research group has an active interest in developing and characterising models of inflammation in humans. However, we are still very much reliant on rodents to gain mechanistic insight into the role of cells and soluble mediators in innate and adaptive immunity. Mice, in particular, have emerged as the rodent of choice due to the ease with which their genome can be altered in order to label, delete and modify specific genes and cells. Indeed, such are the advances in this field that the scientific community have moved relatively quickly from global knockouts to conditional deletions where specific genes in specific cells can be targeted therapeutically. This level of precision is required to definitively elucidate cell function and origin during homeostasis and in disease states. It is for this reason that I plan to use mice for my research. In the field of inflammation research, we are increasingly finding many parallels between events occurring in murine resolving pneumonitis, for example, and resolving human skin blisters in terms of mononuclear cell profiles, phenotypes and long-term impact on tissues post-resolution. My research group is constantly drawing comparisons between our mice data and that

obtained from humans using similar stimuli. Our strategy is to use clinically relevant models and stimuli in rodents that best represent events that occur in man to generate meaningful data that helps understand basic processes, disease aetiology and ultimately treat diseases in man.

A retrospective assessment of replacement will be due by 10 February 2028

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?

In general, these estimates are based on 25 years of experience in animal research. More specifically, we have enumerated the average numbers of rodents used per protcol over the last two Home Office Project Licence, taking into account our increased use of human model of experimentation, which has seen a significant reduction (approximately 50%) in the numbers of rodents my research group have used over the past 8-10 years. With this, we have planned, in as much detail as possible, the experiments required to execute the aims of our research over the next five years thereby providing us with a broad estimate of the number of animals needed for this latest Project Licence.

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

Use of human model of experimentation

Perhaps the most impactful event on the reduction of animals in my experimentation has been the development of several models of local and systemic inflammation in healthy humans as well as aged individuals and patients with chronic inflammatory diseases. The models primarily use skin as a window into the human immune system and include those that cover tissue injury as well as innate immune mediated inflammation and adaptive immunity.

<u>Examples of human experimental models</u>

- Dermal E. coli, S pneumoniae – infectious innate
- Dermal cantharidin - nonspecific

- Dermal PTP antigen – adaptive
- i.v. endotoxin
- NIMP – mechanistic studies
- Use of ex vivo samples for in vitro experimentation form each of the above

In general, these use of these has resulted in a 50% reduction in our use of animals over the last 8-10 years.

Pilot studies and power calculations

Pilot studies are a good way to reduce the number of animals used is experimentation to estimate variability and evaluate procedures and effects. With these data (means, SD and n numbers), power calculations will be used to determine whether the experiment has a good chance of producing a statistically significant result if a biologically significant difference exists in the population. Or, in other words, whether the experiment has a high power, given a biologically significant effect size.

Consultation with a biostatistician can yield benefits to the PI and the experimental animals.

Use of quantitative experimental endpoints

While qualitative endpoints (e.g., dead/alive) often involve severe pain and distress and generally provide less information than quantitative measurements, more information can be found using quantitative endpoints and can, in some instances, lead to a reduction in the number of animals used during an experiment. To this end we developed the Murine Sickness Score to quantify humane endpoint before pain and suffering is experienced.

Animal sharing

We have frequently shared animals euthanized by one investigator to provide tissue for use by another to reduce animal numbers that should be explored by researchers.

Use of appropriate animals strain, sex and susceptibility to experimental disease

Correct choice of an animal strain for disease susceptibility where appropriate and model including those that use healthy, genetically similar animals generally decreases variability and, hence, animal numbers.

Auto Controls

Whenever possible we will design experiments in which animals serve as their own control. For example, using rodent skin models of inflammation, skin on the opposite flank will be used as control. Equally, imaging techniques will reduce the need for serial sampling of animals.

New Instrumentation and Techniques

We will keep abreast of new instrumentation or innovative techniques that can improve precision can reduce the number of animals needed for a study.

Appropriate Experimental Design

Careful experimental design by appropriate choice of control groups and standardising procedures to minimize variability.

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

Parameters of inflammation include knowing the numbers of cells present in tissues following a particular intervention of a pharmacological or genetic manipulation nature; the types of these cells (granulocytes, myeloid or lymphoid); the phenotype or inflammatory status of these cells as well as soluble hormones including lipids, cytokines, and chemokines that can all influence the severity and longevity of an immune response. In addition, we will use investigative platforms such as polychromatic flow cytometry, transcriptomics, proteomics, metabolomics, and lipidomics to generate large data sets that when used with the appropriate bioinformatics tools, will maximise information outcome from minimal animal experimentation. This will contribute significantly to the Reduction, Refinement and Replacement of animals in biological research. Lipidomics, proteomics and transcriptomic platforms, in particular, will generate data sets that will be shared with the broader academic and industrial community impact on other sectors of immunology.

In terms of efficient mouse breeding, when new mouse strains are generated attention will be paid to confirming the genetic alteration the strain experienced, while due consideration will be given to archiving the strain and controlling the integrity of the strain long term. Records of the breeding strains will be kept including litter size, litter interval, reproductive lifecycle, fertility and any welfare concerns.

We will try to predict how often each strain is used and whether it is more appropriate to archive the strain and rederive it in the future, or to maintain a breeding colony. Where the strain will not be used for longer than six months, for instance, consideration will be given to archiving the strain and rederive it in the future. This can reduce both genetic drift and animal use. In the case of a reduction in laboratory workload, an intermittent breeding strategy will be used to avoid animals being wasted.

A retrospective assessment of reduction will be due by 10 February 2028

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.

Pathogen-driven inflammation in the lung, peritoneum, created body cavity (air pouch) and skin, which represent the main organs that I will be using, is classified as Moderate. Where necessary, reversible anaesthesia will be used whereby animals will be injected with infectious material followed by licensee-monitored recovery. At specific time points, animals will be killed by schedule 1 procedure and the affected tissue digested and samples placed on ice for biological processing. From my long- term experience there are few unexpected side effects provided that animals are kept adequately hydrated especially in the case of bacterial/virus-induced pneumonitis and subsequent re-challenge at the pre-characterised pathogen titre. In fact, the very nature of my "pro-resolution of inflammation' hypothesis is, by definition, benign and transitory. To further minimise the numbers of animals used in this study, in terms of **Reduction and Refinement**, we have incorporated imaging techniques so that tissues can be non-invasively assessed thereby providing quantitative data whilst using the same animals as their own controls over time (auto-controls). As a safeguard, we have devised a Murine Sickness score to provide quantitative data from our experiments balanced against preventing pain and suffering. This is included each protocol as appropriate.

Collagen II induced arthritis has been the most widely studied model of rheumatoid arthritis. Severe in classification, it shares several pathological features with RA, and collagen is a major protein in cartilage, the target tissue of rheumatoid arthritis. Additionally, of the antigen-defined models that are based on cartilage proteins, it has the shortest duration between immunization and disease manifestation.

That said, diseases driven by chronic inflammation such as rheumatoid arthritis are characterized by a progressive erosive inflammation in joints leading to the destruction of cartilage and bone. The underlying mechanisms behind are still unclear but early therapy gives promising results. However, none cure the underlying diseases. It’s for this reason that we need to balance the outstanding need for new therapeutics for such a disease that exhibits severe clinical symptoms against the degree of discomfort that our experimental animals will experience. Its important to appreciate that the clinical signs in this model are a manifestation of the developing underlying disease pathology in the joint. Hence, no clinical signs means no joint pathology that fails to resolve, which is what we want to study and understand. Certainly, there may be some pre-clinical pathological changes occurring in the joint, but these don't inform on the aetiology of the full developed disease. And while

animals will experience a degree of pain and discomfort, this will be closely monitored and managed.

In our tumorigenesis studies the objective is to understand the role resolving inflammation may play in promoting cancer growth. For this reason, we are using stimuli such as low dose bacteria that trigger self-limiting inflammation (as described above) followed by the injection of tumour cells. Injected tumour cells are expected to home to the site of inflammation when administered systemically. The tumour cells chosen are robust, can be labelled for non-invasive imaging purposes and are easily distinguishable amongst murine immune and parenchymal tissues, hence contribution to the three Rs, hence contributing to the three Rs. Tumour size and metastasis are key readouts of our experimental design as they directly represent equivalent clinical readouts in humans with size and tissue distribution dictating lifespan. Secondary to these indices will be immune cell composition of the tumours to determine how white blood cells respond to the tumour burden. It's for this that we need to balance the outstanding need for new therapeutics for such a prevalent human disease against the degree of discomfort that our experimental animals may experience. Clearly, animals will be monitored closely for any signs of pain or discomfort. It is not in our moral or scientific interest to see animals suffer as scientifically we will have garnered a great deal of required information when clinical signs of mice being unwell become apparent.

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

Less sentient animals are less complex, immunologically. Heat, redness, swelling and pain are the cardinal signs of inflammation and its resolution, with immune mediators and diverse leukocytes collectively playing a key role in the inflammatory respsonse to infection and injury to affect complex outcomes; invariably interacting with one another to build immunity and improve barrier function. As we wish to have a model system that best represents the human system (bed to bench and back again), we need to use the most appropriate, representative and most characterised system and most conducive to scientific manipulation available which is rodents.

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

We are in full support of the aim of reducing to an absolute minimum the amount of distress experienced by animals used in our research programme.

Our research protocols are designed in such a manner that there are few unexpected side effects provided that animals are kept adequately hydrated especially in the case of bacterial/virus-induced pneumonitis and subsequent re-challenge at the pre-characterised pathogen titre. In which case, such animals will be monitored twice daily. We also plan to use transgenic mice whose innate and adaptive immune systems may respond differently compared to wild type controls. In which case Animal Unit staff will be notified of such studies and animals monitored twice daily. Moreover, as the experimental models

proposed are inflammatory in nature they may, at times, carry an element of pain, which will be controlled using analgesics providing these do not interfere with the aims of the experiment and are in agreement with the Named Veterinary Surgeon. The analgesic of choice, therefore, will be paracetamol. Where mortality is predicted, lower titres and concentrations of stimuli will be tried compared to equivalent concentrations in control and younger animals as appropriate after consultation with the Named Veterinary Surgeon.

We will pay attention to the correct choice of an animal strain for disease susceptibility where appropriate and model including those that use healthy, genetically similar animals generally decreases variability and, hence, animal numbers. Also as mentioned, and whenever possible we will design experiments in which animals serve as their own control whilst also seeking opportunities to share animals (e.g.controls) between experiments.

The use of imaging techniques optimises data outcome from relatively small animal group numbers when designing longitudinal experiments along with "omics" platforms to maximise data outcome from "same animal groupings" will collectively help to refine our experimental approach.

Perhaps our biggest contribution to refinement will be our reverse translation approach to experimental research. Here, we focus on data outcomes/observations from our human models of acute inflammation. We will then carry out pilot experiments in appropriate mice strains to determine if the observations made in humans are conserved in rodents. Then, and only then, do we progress to more complete experiments with pharmacological intervention and/or transgenic animals as appropriate.

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

For all experimental protocols, we will refer the PREPARE guidelines (Planning Research and Experimental Procedures for Excellence) published in Laboratory Animals in 2018 (DOI: 10.1177/0023677217724823).

In addition, the following resources will be accessed:

What about the following guidelines: ARRIVE Guidelines? NC3Rs guidelines on conducting a pilot study: https://www.nc3rs.org.uk/3rs-resources/conducting-pilot-study NC3Rs guidelines on humane end points: https://www.nc3rs.org.uk/3rs-resources/humane-endpoints NC3Rs guidelines on welfare assessments: https://www.nc3rs.org.uk/3rs-resources/welfare-assessment Workman et al guidelines for cancer studies: https://www.nature.com/articles/6605642 Non aversive mouse handling: https://www.nc3rs.org.uk/3rs-resources/mouse-handling GA breeding colony management: https://www.nc3rs.org.uk/3rs-resources/breeding-and-colony-management Aseptic technique and surgical best practice: https://researchanimaltraining.com/article-categories/aseptic-technique/ and LASA guidelines on this Administration of substances: https://researchanimaltraining.com/article- categories/administration-of-substances/ Guidelines on administration frequency and volumes

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

We will have regular discussions with the Named Veterinary Surgeon and animal technicians to review current approaches and whether there are any new 3Rs opportunities.

We will subscribe to the NC3Rs e-newsletter - a monthly update focusing on funding opportunities, 3Rs events and publications and maintain contact with local NC3R manager.

Animals in Science Regulation Unit newsletters

Attend NC3Rs events and workshops and registering for upcoming NC3Rs webinars.

A retrospective assessment of refinement will be due by 10 February 2028

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. An Education in Biomedical Research Methods

Project duration

5 years 0 months

Project purpose

- Higher education and training

Key words

education, experiential learning

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.

- 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?

The aim of this project is to provide high quality experiential education in using animals in research to the next generation of biomedical researchers.

A retrospective assessment of these aims will be due by 28 March 2028

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 to continue to educate future generation of scientists with the skills to appropriately use animals in research. Students with a particular interest in this area and are studying biomedical science, physiology, pharmacology or biochemistry can apply to our module, which focuses on providing this small group of highly motivated students with a good overview of how to plan and carry out research in animals. This includes hands-on experience in animal research and/or research using animal tissues to consolidate their theoretical understanding of this subject.

We have surveyed students on the impact of the experiential aspects of learning about using animals in research and 100% of students felt that their understanding of the processes and the experiments were heightened when they were able to carry them out themselves. In a Research Animal Science Education Scheme (RASES) survey which tested understanding before and after completing the course the students scored significantly better after the course on key in vivo concepts such as "do you understand how and why scientists take welfare of research animals into account as part of a research study" and "would you feel confident in giving an example of how inter-animal variability could be reduced in a particular type of experiment".

After completing this module, many students have chosen in vivo based projects in their professional placement year, final year project and/or PhD project. Through the experiential education they receive we produce students who have excellent understanding of using animals in research which is important in maintaining the skill set which is still heavily required in both academia and industry. Students have reflected:

"This module has given me great confidence that I will be able to provide the best possible treatment for animals in my care during my research career."

"I thoroughly enjoyed the module. It has taught me a lot regarding in vivo animal experimentation and since I am undertaking a professional placement year next year where I will need to have a PIL again, this module was a very good preparation for what will follow."

We also find that project supervisors and employers often favour these students, as students who have carried out these experiments have a deeper and realistic understanding of what will be expected of them.

How will course attendees use their knowledge or skills in their future careers?

Most students on these modules will go on to pursue a career in science either in academia or in industry. Our courses emphasise experimental design, consideration of the 3Rs and ethics as part of the in vivo learning experience. This provides a solid ground on which they understand not only technical aspects of animal research but how that links into good scientific practice. The experiential learning components in particular help students to troubleshoot and become more aware of how technical aspects can impact on experimental outcome. Critically students learn the extent of biological variability and how that can affect results in different models. They write scientific reports based on their experiments, where they are introduced to the ARRIVE guidelines and consider variability and of course statistical analysis. We believe this holistic training is of upmost important in starting a career in animal research.

Even those students who decide not to pursue a career in in vivo research can use the first-hand knowledge and experience they gained to better understand how to interpret in vivo science. Other career choices outside the laboratory setting can also benefit from first hand understanding of in vivo science such as scientific writing and journalism.

Overall, the students will have an overarching understanding of the preclinical scientific process which will stand them in good stead for a career in science or a scientific related job.

What are the principal learning outcomes from the course?

The principle learning outcomes are as follows

1. Know the 3Rs and how to achieve them.

2. Know the legal issues and practicalities of using animals in research.

3. Understand:

- why animals are used in biomedical research

- the advantages and limitations of using animals as models

- examples of animal models used

4. Have an understanding of what to consider when carrying out in vivo experiments

5. Understand biological variation and how best to analyse data derived from in vivo experiments

How are these learning outcomes important to the people on the course?

The students who are accepted on to modules containing in vivo components are specifically keen to pursue laboratory-based careers. The problem-solving skills they gain and understanding of what is involved helps their future career choice, as well as give them fundamental practical and theoretical knowledge that is useful for all scientists. The

demand for in vivo scientists for placement providers, PhD supervisors and industry remains high. We anticipate that we will be training around 300 students but this is less than 10% of our students studying on bioscience programmes at our institution so represents a select group of students with specific interest in this area.

Who or what will benefit from the transfer of knowledge, or acquisition of skills that this course will deliver?

Students on the course who decide to pursue a career in in vivo sciences

These courses provide rare opportunities for undergraduate students to gain in vivo skills and experiences making them highly desirable to future employers who run in vivo projects. Indeed students have reported that these experiences have directly helped get them a placement year position and/or PhD position. In addition the hands on in vivo experience enables them to better plan and carry out in vivo experiments as well as other benefits described below.

Students on the course who decide not to pursue a career in in vivo sciences

The skills the students gain go far beyond the technical skills of in vivo work. It teaches them, through experiential learning, concepts such as biological variation. Carrying out experiments themselves makes them think deeper about experimental design and when writing up the experiments they have carried out they are more aware of the ARRIVE guidelines. Their experience aids them in more critical evaluation of in vivo experiments described in the literature. These are skills that are beneficial for all scientists whether or not they are actively engaged in in vivo work.

It is difficult for a graduate who has had no experience of in vivo research to assess whether they would thrive in a job which contains animal work. This leads to an issue where graduates with no in vivo experience may apply for jobs or PhD projects that include animal work and only discover a few weeks in that they are not suited to or do not enjoy in vivo research. Therefore courses such as this can better place graduates in jobs that suit them.

Employers

For the same reasons as described above, the experience the students acquire benefits employers as it ensures, at least in these students, that they are making a choice to pursue in vivo research based on experience rather than aspiration. This avoids drop outs from applicants who, after starting, realise they are not suited to in vivo work.

The wider scientific community

As the experiments the students carry out are always linked to writing reports, the knowledge they acquire prepares the students for better scrutiny of the literature when reviewing papers. Experiments in the modules are designed to help students think more

about the 3Rs and ARRIVE guidelines in practice which will make better reviewers and journal editors in the future.

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

We aim to share resources to other educators in higher education through RASES (research animal sciences education scheme) funded by the British Pharmacology Society. This includes data and procedural videos which will reach a wider audience than our own students. We also, as part of that scheme, plan to publish our latest experiences in in vivo education.

Species and numbers of animals expected to be used

- Mice: 280
- Rats: 340

Predicted harms

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

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

We will use adult mice (mild) and rats (non-recovery). Mice are chosen to represent a model of mammalian blood glucose homeostasis and rats are suitable for tissue that we require as mouse hearts are too small which impacts on the ability to generate reproducible results.

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

Blood glucose will be measured in mice via a pin prick to the tail. Some mice will receive an i.p. injection of a drug (such as exendin-4). 30 min later an i.p. injection of glucose will be administered. Subsequently small blood samples (less than 1ul) will be used to measure blood glucose concentrations 15, 30 and 60 min later.

Rats will be terminally anaesthetised, heparin may also be administered at the same time. Once under deep anaesthesia the heart will be excised.

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

In mice the duration of the entire experiment is typically less than two hours. During this time the animal is handled around 6 times, of which it is restrained at most 1-2 times (for injections) and blood glucose is measured 5 times or less (pin prick to the tail).

The rats will be under terminal anaesthesia.

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
- Non-recovery

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

- Killed

A retrospective assessment of these predicted harms will be due by 28 March 2028

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?

Complexity of the in vivo systems and responses involved in the maintenance of normal body function cannot be fully and accurately reproduced in vitro and the action of drugs on the whole-body system requires the use living animals. Alternatives have not been rejected but instead form an integral part of the courses in which these protocols in this licence are carried out. These include lectures, videos and computer simulations.

Why can’t your aim be met by observing or by participating in ongoing research or clinical procedures?

During the Covid pandemic students initially carried out a "virtual" practical and when restrictions were lifted were able to come in and do it in person. We were therefore able to survey the students about their educational experience of both options and they strongly felt that carrying out the experiment themselves gave them a much better insight to in vivo research.

Due to timetable constraints we can not guarantee that students can participate in ongoing research.

A retrospective assessment of replacement will be due by 28 March 2028

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 modules are capped to a certain number of students so we can accurately predict how many animals we will need. On one module we generally accept 26 students per year. Each student carries out two experiments, each using one mouse per student. Occasionally we have more than 26 students if students have disrupted their studies and returned to studies the following year. I have therefore allowed for a maximum of 28 students per year in that module. The rats are used by separate 3rd year students in a different module . This module is capped at 32 students per year. 64 rats are used per year to generate data from two different protocols. Some contingency has been added to allow for failed preparations or small changes in student numbers

What in silico or ex vivo techniques will you use during training?

We have produced interactive online learning modules that students complete prior to attending in vivo based practical classes. These resources include videos of the procedures that students will use, which enables the student to see in close detail the techniques involved.

We have carefully reviewed animal use in our teaching practices. In some modules where the over- riding focus of the module is not in vivo science, we have now replaced in vivo practicals with alternative workshops based on ex vivo techniques and video demonstrations.

However, for some modules we believe the in vivo practicals are still important to learning outcomes. In these modules, we use a hybrid of online workshops as described above that students complete before the in vivo based practicals.

Will these techniques reduce animal numbers? If so, how?

We have reduced the number of animals used in teaching substantially by reducing in vivo practicals and reducing animal use in several modules. It should be noted that the current licence proposes using less than 30% the number of animals than our previous licence.

What other measures will you use to minimise the number of animals you plan to use in your project?

We have replaced a previous practical which used rats in regulated procedures with a practical that does not include regulated procedures. This practical, which studies burrowing behaviour in rats, also improves the students' understanding of normal rat behaviour. We offer researchers in our laboratory the mice to use as a source of tissue after the practical class (schedule 1 killing immediately after the practical class followed by removal of tissue). This does not reduce numbers in this project but it does mean that fewer mice overall are used by the lab.

A retrospective assessment of reduction will be due by 28 March 2028

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 in glucose tolerance tests and rats for the preparation of the Langendorff isolated heart perfusion. In the case of the rat, the animals are terminal anaesthetised at the time of heart removal.

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

Mice are the least sentient animals that have a similar blood glucose homeostasis as humans. In addition, they are widely used in the scientific community. All experiments using rats are under terminal anaesthesia.

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

In the glucose tolerance practical we teach students how to measure blood glucose without needing to fully restrain the animals. We also use small needles (27G or less) and the latest blood glucose meters (need less than 1ul blood) to keep blood samples as small as possible. Although fasting is generally used in glucose tolerance tests, we have decided to omit this step as a refinement.

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

Our research lab has carried out comprehensive studies of how to best refine the glucose tolerance test and we use a lot of this information in our practical. This also opens up discussions with the students and makes them thing about refinement as part of their training.

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

I am in close contact with NC3Rs and use some of their resources in teaching. I am a member of the research in animal science education scheme where we share best practice.

A retrospective assessment of refinement will be due by 28 March 2028

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. Modulation of Pathology and Repair in Autoimmune and Degenerative Conditions

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

Damage, Repair, Arthritis, Bone, Neuropathy

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

Retrospective assessment

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

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Contains severe procedures

Objectives and benefits

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

What's the aim of this project?

Autoimmune disorders and degenerative diseases are characterised by the interaction of joint swelling, nerves, blood vessels, muscles and bone, which cannot be studied in isolation. We aim to understand how the different systems interact and affect disease to develop future therapies.

A retrospective assessment of these aims will be due by 21 May 2028

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?

Autoimmunity, such as rheumatoid arthritis, directly affects approximately 1 in every 31 people and is a major-medical problem and, whilst great strides have been made in the diagnosis and management of the various autoimmune diseases, there remain many patients for whom the current licenced drugs are of limited effectiveness. Non-autoimmune diseases such as osteoarthritis affects 7% of the global population, more than 500 million people worldwide, with women disproportionately affected by the condition. There are still no disease modifying drugs to treat osteoarthritis and most treatments consist in life-style changes to better manage the disease. These autoimmune conditions and the non-autoimmune osteoarthritis are associated with substantial degenerative aspects that lead to disability. For example, arthritis in its many forms (e.g. rheumatoid or osteo) remains a significant problem for both animals and humans, as twice as many people suffering from arthritis receive disability living allowances as those with cerebrovascular, cardiac and pulmonary disease combined. Importantly, our understanding of how to reverse the damage caused to the joint and initiate potential repair mechanisms is limited.

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

In the short term, our studies will generate new information related to musculoskeletal autoimmune and degenerative disorders. This information will be shared with the scientific community through oral and poster presentations at national and international meetings, through publications that will be free for all to view (open-access) and through social media.

In the long term, this work is expected to provide novel insights into the regulations of the main systems involved in these diseases, as well as gain an understanding on how disease and failure of repair happens at the joint. This will provide us with an understanding of the pathways that contribute to the perpetuation of symptoms and the repair processes that are associated with arthritis (immune or otherwise). The development of novel treatments (via drugs, cellular or life-style changes) requires an advanced understanding of the roles and mechanisms of action of cells and proteins that are dysregulated. The important long-term benefit is that these studies will identify and validate new approaches that can be used to treat or manage these conditions and alleviate the burden of disease for both human and animals.

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

In the short-term, the main beneficiaries of our work will be other scientists as our data add to a body of knowledge about musculoskeletal diseases such as arthritis (autoimmune or non-autoimmune).

These data will help to move the field forward and generate new hypotheses relevant to how autoimmune and degenerative disorders develop and the intricacies of communication between systems and tissues involved.

In the long-term, the work produced under this licence will highlight pathways that can lead us to future treatments, identifying and validating new approaches that can be used to treat or manage these conditions and alleviate the burden of disease for both human and animals.

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

We will maximise the outputs of our research by ensuring that as much of our data as possible is published, either in primary scientific articles or via publications that are methods focussed. We will also share our data at national and international scientific conferences through oral and poster presentations. We will share our methods and data in discussions with former and current colleagues and collaborators, and will provide detailed step-step methods to any researchers who request this. Databases generated from these studies will also be made publicly available through the relevant platforms.

We will also make certain that the models will mimic clinical conditions to ensure a translational application, and thus we will cultivate collaborations with our clinical colleagues.

Species and numbers of animals expected to be used

- Mice: 7,300

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 complexity of these musculoskeletal disorders arises from the involvement of different interacting systems, which we cannot mimic together in the laboratory. In addition to this, the musculoskeletal system is dynamic and subjected to mechanical stimulations not present in the lab. It is only in the context of the living animal with the complexities of the different systems and environment that we can fully understand how diseases develop, can be modified and treated.

The models must represent the clinical observations, and age is one of the major components in musculoskeletal disorders. Whilst autoimmune diseases happen in young and old, there are differences in the type of disease and thus age must be considered and controlled. Osteoarthritis usually manifests clinically in old age, but it is a slow progression since youth, which ends in a degraded joint. Thus, we must study the early development of the disease, its progression and why it ultimately culminates in fast degradation of the joint tissues. Consequently, studies will be conducted in skeletally mature mice (10 to 14 weeks old) and aged mice (12 to 15 months old). Gender is also an important factor as women are more disproportionally affected by these diseases, therefore studies will be conducted in both male and female.

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

Wild type and genetically modified mice will undergo induction of disease via a injection of specified substances, sometimes followed by a booster injection, or surgical intervention. Surgical intervention aims to destabilise the joint by, for example, cutting a knee ligament and/or physically damaging the surface of the soft cushion of the joint. The overall health of the mice with be monitored by numerical scoring of pain/discomfort/distress using a Health Score scale. Pain relief will be administered during induction to ensure the welfare of the animals. If necessary, analgesia will be added to food that can be introduced in the cage so the animals can have longer treatment of pain if necessary. Manipulation of the disease may take place before or after induction where we aim to modify the immune compartment, hormonal background and/or the environment within the affected joint. Such manipulation may come in the form of, for example, viruses, molecules targeting specific proteins, diet, hormones, hormone inhibitors, surgical intervention, etc. Labelling of tissues may also take place, for example by intraperitoneal injection of calcein to label deposited calcium in the bone. Fluorescent probes/viruses to label specific cell types may be injected locally.

Duration of the experiments is variable. In autoimmune arthritis models, in which the full disease manifestation is rapid, the time scale from induction of disease to experimental end will be in the order of days or weeks. In milder non-autoimmune models where disease develops slowly, such as surgically induced osteoarthritis models there will be a full range of timepoints to understand how the disease initiates (weeks), progresses (months) and finally, how it becomes clinically relevant (1 year). We now have detailed preliminary data on the long-term assessment of these models, generated in our previous licence.

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

Chemical induction of arthritis causes joint swelling, pain and signs of ill health (hunched, reluctance to move, isolation and failure to groom) especially at the peak of inflammatory response. These side effects manifest a few days after induction and may last up to 48 hours.

Joint surgery results in temporary swelling and pain in the affected joint and temporary lameness is likely to occur post operatively. Animals may limp and exhibit decreased mobility immediately after surgery but normally recover within a day. Once recovered, mice are not likely to show changes in the way they walk (which is a manifestation of worsening disease) until months after induction.

Unrelated to the procedures, older animals may have other conditions related to age such as developing tumours, teeth problems and obesity.

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 or subthreshold: 82% (Protocol 1 + Protocol 3)

Moderate: 15% (Protocol 2 & 3)
Severe: 3% (Protocol 2)

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

- Killed

A retrospective assessment of these predicted harms will be due by 21 May 2028

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 are no laboratory models that can mimic the complexities of the joint and the many pathways that lead to arthritic disease, because the disease is a manifestation of several tissues and systems failing and interacting. Therefore, to test the mechanisms-of-action and efficacy of disease-modifying approaches it is essential that animals are used as it allows us to investigate the multidimensional nature of autoimmune and degenerative diseases. Furthermore, ageing, gender, obesity and exercise

have important effects and it is not possible to reproduce these in a laboratory setting. It is, therefore, not possible to duplicate these interactions in anything other than an intact mammalian model.

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

Many of our animal studies are combined with cell-based laboratory models. These laboratory experiments are useful for investigating mechanisms of disease and enable us to replace animals on many occasions. Growing different cell types together allow us to mimic specific tissue-tissue communications. For example, we can grow bone cells in the proximity of cells of the cartilage (the cushion in the joint that allows the movement of the joint), and we can stimulate either of them and measure the effect of that stimulation on the other cell type, thus mimicking the interaction between the two cell types.

Why were they not suitable?

These laboratory studies are limited by or even compromised by the artificial environments in which they are undertaken, which do not mimic the mechanical environment of the joint or the dynamics of all the tissues and systems involved as, for example, they are limited to two or three different cell types.

Also, some of our findings indicate that certain therapeutics are dependent on circulating proteins for their efficacy and that their interaction with cells cannot be adequately replicated/modelled in the laboratory. Whilst laboratory experiments can interrogate the effect on each cell type, they can not assess the global effect of the treatment.

A retrospective assessment of replacement will be due by 21 May 2028

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 on those numbers generated in the previous licence and the planned future work for this project.

The breeding programs will generate many animals of the undesired genotype hence the large number of animals estimated under Protocol 1 (5,000/5yr), which other than genotyping sampling will not

undergo any procedures. However, to minimise this surplus some of the mice will be maintained to act as wild-type littermate controls. An estimate of 300/5yr will be used under Protocol 2 (mice), and 2,000/5yr under Protocol 3 (mice).

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

Each protocol is based on extensive previous experience and has been optimised to ensure minimal suffering for the animals involved. Before starting an experimental study, we perform power calculations and randomisation approaches where appropriate. When planning these studies, we consider guidance from NC3Rs through the experimental design assistant. For experiments that involve new reagents or new analysis, we seek guidance from colleagues with experience in these areas.

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

As many experiments as possible are conducted on collected tissues, which minimises the number of animals undergoing licensed procedures.

Experiments will be conducted on both sexes, thus limiting wastage while addressing specific scientific questions on the effect of gender in disease.

Where possible experiments will be carried out in clean pathogen free cages to ensure reproducibility and to reduce the overall numbers of animals and experiments required to obtain statistically significant data.

Use of the information from the experiments to generate and use human cells/tissues in laboratory experiments to investigate the interaction of cells in the immune processes thereby minimising the number of animals undergoing licenced procedures.

Minimise number of animals necessary for valid statistical analysis, as determined by previous experimental data in these models and the use of power analyses to determine the lowest number of animals needed.

Non-invasive bioimaging to allow monitoring of specific luminescent or fluorescent markers in the same animal over time, thus reducing the number of animals used per measurement from 4 to 1.

A retrospective assessment of reduction will be due by 21 May 2028

The PPL holder will be required to disclose:

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

Refinement

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

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

We have developed considerable experience in these models over the last 10 years, optimising immunomodulation and induction of arthritis (autoimmune and non-autoimmune).

The wellbeing of the animal is a major consideration in our inflammatory models. To this end we administer analgesia prior to induction and, if necessary, provide additional food with pain relief treatment mixed in for a period of 3 days following induction when it does not interfere with the aims of the experiment; in addition the animals are monitored daily for the duration of the protocol. We have developed robust models to ensure that all animals respond to the induction protocol, thus limiting the number of animals used. We have also optimised the models to eliminate footpad injections, which are more harmful to the animals.

For the induction of osteoarthritis via the basic knee destabilisation surgical intervention we have optimised the surgery to minimise accidental damage to the joint during surgery. This results in less inflammation and cartilage degradation, thus resulting in a much slower progression of the disease with less pain and discomfort for the mice. Mice with induced osteoarthritis through the basic knee destabilisation surgical intervention do not show differences in hind limb weight bearing until 11 months after induction, indicating there is no significant discomfort until then.

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

The diseases we are trying to mimic require time to develop and, as the aim is to conduct translational research, we also seek to make it relevant to the clinic. Thus, we require skeletally matured mice, sometimes aged, and the necessary time for the disease to develop.

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

After induction mice are monitored daily for the first week. Monitoring is specific to each protocol. Depending on the model, close monitoring is continued when disease is known to appear. For example, in autoimmune arthritis models, there is a well-recognised articular inflammation score, that also contributes to management of disease severity. Mice are euthanised if footpad swelling or the total articular inflammation score exceeds set levels. Application of pain relief is limited due to anti- inflammatory effects. Use of these drugs will be discussed with the NVS where appropriate. In non- autoimmune models analgesia is given as a pre-induction measure to ensure good health and recovery. Self-administration of analgesia in mixed in additional food is also available post-op if the need arises.

We euthanise mice if they lose 20% of their starting weight or show unexpected clinical signs outwith the parameters established in each protocol.

We will also take a proactive approach to ensure welfare, ensuring that animals have warm comfortable bedding and easy access to food and water, especially during the peak of inflammation. For longer experiments we will ensure different types of enrichment are provided such as chewing toys, tunnels and shredding materials.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?
ARRIVE (Animal Research, Reporting of In Vivo Experiments) guidelines (www.nc3rs.org.uk/ARRIVE). Maximising the output of osteoarthritis research: the ARRIVE guidelines. N. Percie du Sert.
Osteoarthritis and cartilage. VOLUME 20, ISSUE 4, P253-255, APRIL 01, 2012.

Applying refinement to the use of mice and rats in rheumatoid arthritis research. Penny Hawkins, Rachel Armstrong, Tania Boden, Paul Garside, Katherine Knight, Elliot Lilley, Michael Seed, Michael Wilkinson, and Richard O. Williams. Inflammopharmacology. 2015; 23(4): 131–150.

Recommendations for the use of preclinical models in the study and treatment of osteoarthritis. R. Poole, S. Blake, M. Buschmann, K. Rudolphi, W. van den Berg, T. Yaksh. Osteoarthritis and Cartilage. VOLUME 18, SUPPLEMENT 3, S10-S16, OCTOBER 01, 2010

Local guidelines will be followed.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?
Up to date guidance will be monitored via the NC3Rs website (http://www.nc3rs.org.uk/), the monthly NC3rs newsletter and through communications from our animal facility as well as updates from our Animal Welfare and Ethical Review Board and Culture of Care subgroup.

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

A retrospective assessment of refinement will be due by 21 May 2028

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. Research on Bacterial Products used in Medicine

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

vaccine, bacterial, therapeutic, safety, potency

Animal types	Life stages
Guinea pigs	adult
Mice	adult, juvenile
Rats	adult
Rabbits	adult, 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 be able to provide assurance that products of bacterial origin and therapeutics used in medicine are safe and likely to be effective. it is also to allow the generation and evaluation of international, national and working standards necessary for the evaluation of these bacteriological products.

A retrospective assessment of these aims will be due by 17 May 2028

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 to understand whether a new vaccine or therapeutic product is likely to be safe for use in human and how it works. This will help us to inform regulatory processes in the UK (MHRA), in Europe (EMA and EDQM) and worldwide (WHO). In addition, the data will be used for the development of batch release tests and the output from such research would be published in peer reviewed journals to share knowledge with the scientific community.

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

1. An improvement in the quality of batch release procedures and hence the reliability of assurances provided about the safety and potency of existing and novel bacteriological products used in medicine.

2. Possible replacement of in vivo batch release tests with in vitro alternatives for traditional products which require validation. New and modified tests are ultimately incorporated into the pharmacopoeias and the Division’s regulatory project licence.

3. Provision of information if new generation therapies or new formulations / delivery systems for vaccines antigens are likely to be effective

4. The output from such research would be published in peer reviewed journals.

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

In the short term, the principal benefits from this research are:

The vaccines Division will have an improved understanding of whether a new vaccine or therapeutic product is likely to be safe and effective and how it works. Typically the output from such research would be published in peer reviewed journals.

In the long term, the knowledge gained will be used to inform regulatory process in the UK (MHRA), in Europe (EMA and EDQM) and worldwide (WHO). In addition, the data will be used for the development of an appropriate package of batch release tests.

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

The expertise gained from our work will allow us to improve on current testing methodology for vaccines and therapeutics and enable us to collaborate with other regulators to revise current guidelines or develop alternative in vitro methods where appropriate and possible. In addition, publishing and sharing our results with the scientific community will maximise the benefit and output from our work. This will also improve our position to set up collaboration with academia, research institutes, vaccine developers and regulators.

Species and numbers of animals expected to be used

- Mice: 17704
- Rats: 550
- Guinea pigs: 1130
- Rabbits: 65

Predicted harms

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

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

The project involves use of small animals only (mice, rats, guinea pigs and rabbits). These animal species chosen from information published in literature or as required from regulatory guidance, documents or monographs on the basis of generating relevant effect and suitable dose responses. Such information will often be supported by collaborative studies taking into account suitability per product, generating information on multiple antigens and with the least severe end points where possible. Extensive validation studies have contributed a significant body of information on the use of particular species or end point. Adult animals proven to be suitable will be used throughout the project, except in protocols 6 and 7 where juvenile mice will be used to evaluate vaccines and Protocol 13 where infant rabbits should be used to prepare complement from blood for use in bacteria killing assays, as complement prepared from adult rabbits is not effective.

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

Protocol 1 – Diphtheria Toxin Assay: This test involves injecting groups of guinea pigs intradermally with a series of dilutions of diphtheria toxin. Two guinea pigs are used for each toxin preparation. The test has a non-lethal end point based on skin erythema and has a moderate severity category. The duration of the test is up to 48 hours post-injection.

Protocol 2 – Diphtheria Antitoxin Assay: guinea pigs are injected subcutaneously with antitoxin or intradermally with a series of dilutions of reference or test antitoxin preparations, (or antitoxin/toxin mixes, or toxin alone).The intradermal test has a non-lethal end point based on skin erythema, has a moderate severity category and animals are observed 48 hours post challenge. The subcutaneous test for antitoxin preparation has a non-lethal end point and may last up to 4 weeks. Blood sampling may be done prior to and after injection with antitoxin to allow for measurement of circulating antitoxin concentration.

Protocol 3 – Tetanus Toxin Assay: this test involves injecting groups of mice subcutaneously with tetanus toxin. The test has a non-lethal scientific end point based on degree of local paralysis and has a moderate severity category. The duration of the test is up to 96 hours post-injection.

Protocol 4 – Tetanus Antitoxin Assay: this test involves injecting groups of mice subcutaneously with a reference or test tetanus antitoxin, each mixed with a fixed dose of tetanus toxin. The test has a non- lethal scientific end point based on degree of local paralysis and has a moderate severity category.

The duration of the test is up to 96 hours post-injection. The test may also be used to determine the in vivo potency of tetanus antitoxin samples.

Protocol 5 – Potency Tetanus Vaccine: this test involves subcutaneous immunisation of groups of mice with preparation of tetanus vaccine, followed by challenge 4 weeks later with tetanus toxin by subcutaneous injection and observed over 4 days for sign of local paralysis. The test has a moderate severity category and non-lethal scientific end points are applied.

Protocol 6 – Pertussis Toxin Assay (histamine or lymphocytosis): this test involves injecting groups of mice intraperitoneally with the test material/vaccine and then challenged with histamine to trigger the physiological response to pertussis toxin. The test has a severe severity category as mice that receive active pertussis toxin suffer anaphylactic shock (characterised by a marked reduction in activity and ultimately mobility, shallow respiration and eventually loss of consciousness) which can lead to death. Mice will be monitored for 24 hours for clinical signs of pertussis toxin.

Protocol 7 – Potency Pertussis Vaccine (Kendrick Test): this test involves immunisation of groups of mice intraperitoneally with a standard or test preparation of whole cell pertussis vaccine followed by intracerebral challenge 15 days later with a strain of the disease-causing bacteria, and then observed over a 14 day period for clinical signs of pertussis. The test has a severe severity category as mice that are not protected will experience

adverse effects as a result of overwhelming cerebral infection that include pilo-erection, loss of muscle control, convulsive motions, laboured breathing which may lead to death.

Protocol 8 – Pertussis Respiratory Infection: mice are immunised by the subcutaneous, intraperitoneal, sublingual, intramuscular, or intranasal route and then challenged with an aerosol or intranasal dose of a strain of the disease-causing bacteria. Mice are killed up to seven days after challenge and tissues harvested to confirm viability of the bacteria and the test has a moderate severity category.

Protocol 9 – Botulinum Toxin Assay (non-lethal): mice are injected with botulinum toxin by the subcutaneous route at the inguinocrural site and observed over 2 days for signs of localised flaccid paralysis characterised by abdominal ptosis (displacement of abdominal organs). The test has a moderate severity category.

Protocol 10 – Botulinum Antitoxin Assay (non-lethal): mice are injected with a mixture of botulinum toxin and antitoxin by the subcutaneous route at the inguinocrural site and observed over 2 days for signs of localised flaccid paralysis characterised by abdominal ptosis (displacement of abdominal organs). The test has a moderate severity category.

Protocol 11 Active immunisation: mice, rats, guinea pigs and rabbits are immunised using different routes (intradermal, subcutaneous, intramuscular, intraperitoneal, intravenous, sublingual, intranasal, oral/orogastric, rectal, transdermal or by inhalation) on up to five times at intervals of up to 14 weeks. The animals are bled for their serum at the appropriate times from a superficial blood vessel and at the end of the experiment under terminal anaesthesia. The test has a moderate severity category.

Protocol 12 Polyclonal antibodies: mice, rats, guinea pigs and rabbits are immunised using different routes (intradermal, subcutaneous, intramuscular, intraperitoneal, intravenous or oral/orogastric) on up to five times at intervals of up to 12 weeks. A further immunisation may be administered after an interval of up to 26 weeks to test for memory response. The animals are bled for their serum at the appropriate times from a superficial blood vessel and at the end of the experiment by cardiac puncture under terminal anaesthesia. The test has a moderate severity category.

Protocol 13 Terminal blood sampling: mice, rats, guinea pigs and rabbits are used to to withdraw the maximum volume of blood from normal animals to prepare serum or plasma components (e.g. complement or lymphocytes) for use in various immunological assays. A large volume of blood will be taken under general terminal anaesthesia. Infant rabbit serum is used as a source of complement for bactericidal and opsonophagocytic assays and therefore some infant animals may be required. The test has a non-recovery severity category.

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

Protocol 1- Diphtheria toxin assay: the guinea pigs will develop erythema at one or more of the injection sites and, rarely, some animals may develop localised necrotic lesions (dry, non-exudative necrotic scabs) at the site of injection. These effects will be seen for up to 48h.

Protocol 2- Diphtheria Antitoxin Assay: guinea pigs that are not completely protected will develop erythema at one or more of the injection sites and, rarely, some animals may develop localised necrotic lesions (dry, non-exudative necrotic scabs) at the site of injection. These effects will be seen for up to 48h.

Protocol 3- Tetanus Toxin Assay: the test has a non-lethal end point based on degree of local paralysis. Only those receiving the highest dose of toxin may experience increasing spastic paralysis due to tetanus intoxication. The clinical signs of tetanus intoxication are erect fur, with characteristic progressive spastic paresis, ultimately leading to paralysis, initially of the injected hind-limb, followed by the other hind-limb.

Protocol 4- Tetanus Antitoxin Assay: the test has a non-lethal end point based on degree of local paralysis. Mice that receive incompletely neutralised doses of toxin may experience increasing spastic paralysis. The clinical signs of tetanus intoxication are erect fur, with characteristic progressive spastic paresis, ultimately leading to paralysis, initially of the injected hind-limb, followed by the other hind- limb.

Protocol 5- Potency Tetanus Vaccine: mice that are incompletely protected (i.e. those receiving the lowest dose of vaccine) and/or those receiving the higher doses of toxin may experience increasing spastic paralysis due to tetanus intoxication The estimated duration of these effects is 1 to 3 days.

Protocol 6- Pertussis Toxin Assay (histamine or lymphocytosis): immunisation with some acellular pertussis vaccines may cause a short term reaction in approximately 50% of animals. The affected animals display a loss of coordination, a ‘straddling’ gait, and decreasing activity, leading to immobility. These animals normally recover during the following hour, although lethargy may persist for several hours. Following challenge with histamine, some animals will undergo shock (characterised by a marked reduction in activity which may lead to immobility, shallow respiration and eventually loss of consciousness and a proportion of these (approximately 34%) will die rapidly (within the first two hours) following the challenge with histamine. However, the proportion will also depend on the number of test groups. The mice in the two groups that receive the active pertussis toxin control are expected to undergo shock. Some of these mice will recover fully, including from loss of consciousness, usually within 3-5 hrs and depending on the degree and duration of adverse effects may be assigned a severe severity. It is not possible to define a humane end point because of the rapid and unpredictable progression of the reaction.

Protocol 7- Potency Pertussis Vaccine (Kendrick Test): following immunisation, some animals may show signs attributed to mild to moderate pain or discomfort, including partial

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

Protocol 8– Pertussis Respiratory Infection: Rarely (<0.2%) following challenge, animals may develop respiratory distress or endotoxin shock (characterised by fever, piloerection and diarrhoea), which could lead to death. This is obvious immediately after the challenge. In such cases animals will be killed immediately by a Schedule 1 method. Following immunisation with some adjuvants, animals (in our experience typically no more than 30%) may show signs attributed to mild to moderate pain or discomfort, including partial pilo-erection, orbital tightening, lethargy and abdominal contraction. Most effects will typically pass off within a few hours, but some may persist, usually to a lesser degree, for up to 24 hours.

Protocol 9– Botulinum Toxin Assay (non-lethal): most of the animals will develop abdominal ptosis with local palsy only for up to 48 h. This does not significantly affect mobility, activity or general health.

Protocol 10– Botulinum Antitoxin Assay (non-lethal): mice that receive incompletely neutralised dose of toxin will develop a varying degree of abdominal ptosis with localised muscular paralysis. This does not significantly affect mobility, activity or general health. These effects will be seen for up to 48h.

Protocol 11- Active immunisation: With Freund’s adjuvant local, non-painful swelling is expected. Rarely, temporary lameness, an abscess or ulceration may develop at the site of injection. Animals with no sign of improvement and with exudation lasting for 24 hours will be killed by a Schedule 1 method. Following immunisation with some other adjuvants, animals may show signs attributed to mild to moderate pain or discomfort, including partial pilo-erection, orbital tightening, lethargy and abdominal contraction. Most effects will typically pass off within a few hours, but some may persist, usually to a lesser degree, for up to 24 hours. It is possible that some adjuvant-adsorbed antigens will form a depot at the site of injection (nodule of a typical size of, but not restricted to 5mm) but this should not cause any pain or discomfort to the animals. Endotoxin present in some whole bacteria preparations may cause local inflammation or signs of endotoxic shock. Any animal developing unexpected reactions at the site of injection or other adverse effects will be killed by a Schedule 1 method which may be preceded by taking a larger volume of blood under general terminal anaesthesia. Possible adverse effects for physical enhancers include erythema at the skin site and lesions in the skin up to 48 hours.

Protocol 12- With Freund's adjuvant local, non-painful swelling is expected. Rarely, temporary lameness, an abscess or ulceration may develop at the site of injection. Animals with no sign of improvement and with exudation lasting for >24 will be killed by a Schedule 1 method. Endotoxin present in some whole bacteria preparations may cause local inflammation or signs of endotoxic shock. Any animal developing unexpected reactions at the site of injection or other adverse effects will be killed by a Schedule 1 method which may be preceded by taking a larger volume of blood under general terminal anaesthesia.

Protocol 13- No 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)?

Mice: approximately 3% non-recovery, 48% mild, 32% moderate, 17% severe

Rats: approximately 9% non-recovery, 82% mild, 9% moderate

Guinea pigs: approximately 5% non-recovery, 80% mild, 15% moderate

Rabbits: approximately 39% non-recovery, 55% mild, 6% moderate

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

- Killed

A retrospective assessment of these predicted harms will be due by 17 May 2028

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?

Protective potency and safety of biological medicines cannot be examined completely without the use of animal procedures, as multiple factors contribute to a protective immune response and the immune system itself is too complex to be modelled in vitro. Tests used in this project are performed according to methods described in regulatory documents

such as European Pharmacopoeia monographs and WHO recommendations, or are based on methods included in product licence dossiers or published in peer-reviewed journals. In all cases, no suitable validated non-animal alternatives are currently available. Also in vitro biochemical models for safety are often product specific and in vivo assays are still required for new products or formulation as well as for validation of product specific regulatory assays. In addition, polyclonal sera are still needed for use in various assays where monoclonal antibodies are not available or suitable. Where validated alternative test methods exist or developed, they have been introduced and this has resulted in a removal of some protocols from this project licence that were included in the previous project licence. Efforts are continuing to develop and validate alternatives to some of the methods that are retained in this project licence.

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

The Division has an ongoing programme exploring new technologies to potentially eliminate animal procedures for bacterial neurotoxins (protocols 3, 4, 9 and 10) by use of differentiated stem cell and pure human neurons in culture with a range of toxin specific read-outs. However, it could be a long time before these assays are implemented as alternative to animal testing.

The Agency is a partner in a large EU funded consortium working on development of non-animal methods for quality control of vaccines. An ongoing objective of this 5 year project is to determine whether the in vitro antigen content assay for diphtheria and tetanus can be used as a surrogate for potency with a view to future replacement of the current in vivo potency tests.

In addition, we will be participating in the ongoing efforts driven by DCVMN (alliance of vaccine manufacturers from developing countries) to replace the Kendrick test (Protocol 7– Potency Pertussis Vaccine) with a serological assay.

Protocol 6 is the safety test for acellular pertussis vaccine involves and is also used to calibrate the International Standard for pertussis toxin. The histamine test has a severe severity category. Non- animal alternatives such as the modified Chinese Hamster Ovary (CHO) cell-based method has recently been developed and validated for detecting residual toxicity in final vaccine formulations and measuring pertussis toxin activity. It has been incorporated into the European Pharmacopoeia monograph during the course of the previous project, but will still require an in-house validation study before it can be implemented and used as a replacement for the histamine assay. Other non-animal alternatives such as biochemical assays which detect binding and enzyme activity of pertussis toxin have also been developed but are still undergoing validation and are unlikely to be ready to replace histamine test during the life-time of this project. The International Standard for pertussis toxin is calibrated in both the histamine and CHO cell assays so there is still a requirement for the histamine test for standard characterisation.

Protocol 12 is for production of polyclonal antibodies. Although many assays for vaccine characterisation use antibodies, those antibodies generated by phage display library are usually monoclonals of limited specificities, of relatively low affinity and not always suitable for the required application in assays to characterise vaccines. Hence the need to produce polyclonal antibodies in animals.

Why were they not suitable?

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

A retrospective assessment of replacement will be due by 17 May 2028

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 estimates of the number of animals that will be used in the course of the current project are based on experience over the course of the previous project licence and use over the last 5 years. The introduction of new technology, reduction in animal testing and the refinement of existing tests have been taken into account (e.g. foreseen studies on validation of safety tests for pertussis in vitro alternatives). Similarly, the impact of changes in the vaccination programme and the introduction of new vaccines and antitoxin therapies (e.g. against C. difficile and streptococci groups A & B) have been considered when making these estimates.

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

The number of doses and group sizes used in some of the protocols (1-10) described in this license are based on well established and validated methods that have been laid down in reference documents issued or endorsed by scientific advisory bodies such as the WHO, licensing authorities and national or European Pharmacopoeias. In the absence of regulatory endorsed procedures, assays are designed taking advice from in house biostatisticians, with an aim to use the minimum number of animals that is expected to provide information of required accuracy and precision. Normally an experiment will

consist of a sufficient number of experimental and control groups, each containing an appropriate number of animals, to obtain statistically significant results. To reduce the number of animals used, several antigens, batches or components of a particular product are tested in the same experiment where a single control group will be used. Where possible, multiple vaccine batches are tested together in the same assay to maximise the use of the reference vaccine and/or 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?

During the validation or re-evaluation of existing regulatory tests, data obtained from work carried out on this licence may be combined with data from a regulatory test to give a statistically significant result. This reduces the number of animals required when validation or trouble-shooting assay problems can be combined with a regulatory test.

When a new protocol is introduced, a pilot study using limited number of animals is carried out to measure the variability of the response within the group. The generated data will help in the calculation for the minimum required number per group to achieve meaningful results.

Research to evaluate the potency and safety of new vaccines is generally hypothesis-driven and the interpretation of data therefore may depend on the statistical comparison of an experimental group with controls. Controls would include a group of unimmunised animals and may also include other similar vaccines or components for comparison depending on the hypothesis being addressed. Experiments will be powered to use the minimal number of animals required to produce significant data in discussion with in-house biostatistician.

A retrospective assessment of reduction will be due by 17 May 2028

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 work will employ animal species chosen from information published in literature with evidence of similarity between the chosen animal model and human infection/response, or as required from regulatory guidance, documents or monographs on the basis of generating relevant effect and suitable dose responses. Such information will often be supported by collaborative studies taking into account suitability per product, generating information on multiple antigens and with the least severe end points where possible. Extensive validation studies have contributed a significant body of information on the use of particular species or end point. In all probability the replacement or modification of an existing bioassay will ultimately result in the use of a less severe procedure for testing the potency or safety of bacteriological products. Thus, the existing pharmacopoeial protocol represents the “worst case” in terms of the severity of adverse reactions. To obtain the necessary comparative data for the validation of the new assay, it is necessary to perform the existing pharmacopoeial assay exactly as specified to obtain the definitive data. Prior to validation however, most, if not all, the development work leading to the establishment of the new assay can be carried out as standalone experiments without the need to include the existing assay for comparison. One protocol (Diphtheria potency) has been removed from the current project.

Efforts continue to refine procedures with severe or moderate severity categories. Challenge assays will only be used when there is no alternative. Protective animal models are considered more severe than serological models for the evaluation of vaccine potency. Where serological correlates of protection provide reliable indicators, serology is performed on a routine basis. The introduction of serology as alternative method to challenge means that the severity of the procedure is mild compared to the moderate category assigned to the challenge models. However, challenge models are likely to remain for use with products tested infrequently (as for the WHO) because of the lack of opportunity to validate refined or alternative assays for these products. A cell-based assay (CHO cell clustering assay) has been developed to replace the histamine assay (Protocol 6) for detecting residual toxicity in acellular pertussis vaccines and measuring pertussis toxin activity of reference materials. However, the current WHO International Standard for Pertussis Toxin is calibrated in both the histamine and CHO cell assays and therefore there is a need to retain the histamine assay for further characterisation. Potency for whole cell pertussis vaccines (the Kendrick test, protocol 7) is an example where protective efficacy by challenge remains the only accepted model, because of failure to identify and validate specific assays predictive of the protective immune response. For the assay performed, we have determined humane end points such that animals can be euthanased at the earliest stage at which it can be certain that they would otherwise go on to die. This is facilitated by increased monitoring including out-of-hours checks.

Production of immune sera that could be used to prepare reference standards and reagents requires induction of high antibody titre (protocol 12) and therefore the antigen is administered with a potent adjuvant such as Freund's adjuvant (FA). However, administration of Freund's complete adjuvant (FCA) and to a lesser extent of Freund's incomplete adjuvant (FIA) induces local and systemic lesions and has a great potential to

cause pain and distress in animals. It is unlikely to use Freund's adjuvant extensively in the new project and we plan to investigate the use of alternative adjuvant with less adverse effects during the lifetime of this project. When FCA is used it will be given subcutaneously only in the first immunisation and with FIA for the booster immunisations. Alternative methods to generate suitable antibodies e.g phage display will be explored. However, antibodies generated by phage display library are usually monoclonals of limited specificities, of relatively low affinity and not always suitable for the required application in assays to characterise vaccines.

Developmental vaccines against bacterial meningitis and septicaemia will largely be assessed by serological assays (active immunization, protocol 11) and in vitro bactericidal/opsonophagocytic assays have been developed and accepted as correlate of in vivo protection models, hence the removal of the protocol for infant rat bacteraemia/septicaemia model and from this application. In addition, in vitro assays have also been developed to assess bacteria binding to epithelial cells and effect of antibodies on blocking this binding, as alternative to the in vivo colonisation, hence the removal of the Group A streptococcus colonisation model from this application. For new products, however, the paucity of existing data on the correlation of serological data from animals with protection in humans may necessitate the use of protection by challenge models. We will use monitoring regimens that will mitigate the severe nature of the effects as far as possible.

During the course of the previous project we investigated the level of IgA antibodies in mouth washes and pilocaprine-induced saliva and found that mouth washes have higher level of IgA antibodies than saliva and therefore removed the saliva induction from the current application.

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

The majority of protocols used in this project require the use of mice and are based on well-established methods that are described in regulatory guidelines, monographs or in the published literatures.

Guinea pigs, rather than mice, are used in two protocols for the following reasons: the assay for diphtheria antitoxin potency cannot be done in mice because that species is insensitive to diphtheria toxin; the serological assay for diphtheria and tetanus vaccine potency can be done in mice in theory, but the very large differences in the level of immune response to the difference vaccine components means that additional vaccine dilutions would be needed (and therefore additional animals) to cover the dose response range for both vaccine components. The assay in guinea pigs can be done with the same dilutions of vaccine for both components, reducing the number of groups and animals to the minimum required to generate valid potency estimates. For immunogenicity studies (protocol 11), the most suitable species are used based on published literatures e.g. for Haemophilus influenzae b (Hib) vaccines, the rat is more suitable animal model (the mouse is not sensitive to Hib), while for group A, B streptococci and pertussis the mouse is

the most suitable species. Evaluation of immune responses and potency of vaccines and biotherapeutics usually require animals to be alive for a period of time to demonstrate effect of treatment and terminally anaesthetised animals are not suitable and cannot be used. Infant rabbits are used in protocol 13 to produce complement for use in bacteria killing assays as only baby rabbit serum have been shown to be the only good source of complement.

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

For all protocols, animals are habituated prior to commencement of procedures, typically for 7-10 days. Inoculation volumes are based on those described in the regulatory guidelines or monographs and do not exceed those recommended by LASA. We strive for continual improvement to animal housing, husbandry and handling aimed at minimising stress in animals and encouraging natural behaviours.

Animals undergo thorough pre- and during-study checks. Frequent monitoring by experienced staff, including out-of-hours checks for some procedures help to ensure that welfare costs are minimised wherever possible and analgesics will be administered where needed and where their effect will not interfere with the scientific outcome of the study.

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

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

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

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

A retrospective assessment of refinement will be due by 17 May 2028

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. Pathophysiology of Vascular Cognitive Impairment

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

cerebral vascular disease, memory, dementia, causes, treatments

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

Retrospective assessment

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

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Contains severe procedures

Objectives and benefits

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

What's the aim of this project?

Vascular cognitive impairment is a complex disorder caused by damage to blood vessels in the brain leading to stroke, memory problems ranging from mild to severe such as dementia. We still don't fully understand the mechanisms which restricts the development of treatments which remain limited. We will aim to provide more detailed information on the causes leading to Vascular cognitive impairment. Following on from these discoveries we

will aim to develop and test potential drugs in the most relevant models and determine whether they can delay or halt the progression of disease.

A retrospective assessment of these aims will be due by 15 June 2028

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?

There are currently estimated to be 850 000 people living with dementia in UK (2016) with an annual cost to the UK of £26bn per year. Cerebral vascular disease, which damages the blood vessels in the brain, including due to a stroke, is a major cause of cognitive impairment in the elderly and accounts for over half of all dementias (such as Alzheimer's disease). Numbers are expected to rise with an increasing aging population. Although the risk of death from stroke has declined due to improved treatment, this has led to an expanding population of stroke survivors (25-30%) that develop immediate or delayed cognitive impairment and dementia. This causes significant human suffering including to families and carers as the disease is protracted over many years. We still do not understand what causes this disease and there are no treatment options. Thus we require relevant animal models that mimic better the complexity of the human diseases in order to understand better what causes the disease and to identify targets to test and develop effective treatments.

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

The result of this work will lead to a greater fundamental understanding of the mechanisms associated with cerebral vascular disease, including stroke, and how this leads to cognitive impairment. These discoveries will provide a basis to identify key targets and/or modifiable pathways as a basis on which to develop and test potential therapeutic targets.

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

In the short-term the immediate benefit will be to researchers in the cerebral vascular field, including stroke, and related dementias as we share our findings. The project is directed to identify key targets and/or modifiable pathways as a basis on which to develop therapeutic targets. We work closely with clinical colleagues who are ideally placed to undertake follow-on early clinical trials of any promising therapies. Thus in the longer-term the findings will be of interest to clinicians and to pharmaceutical industry working on drug trials for cerebral vascular disease, including stroke, and cognitive impairment

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

We will aim to maximise outcomes of our research through collaborations and we have existing networks of researchers with whom we already collaborate. We will aim to publish datasets of all of our studies regardless of outcomes. We will make our data available for the scientific community through publications and additionally through a local website.

The information generated from our research will be published and be communicated through presentations at relevant scientific conferences to alert the research community of our findings. We have close interactions (and personal experience) with members of the public, including stroke and dementia carers or families. We will also communicate our findings to those individuals.

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.

Cerebral vascular disease, including stroke, leading to vascular cognitive impairment (VCI) is a complex physiological processes which can only be investigated in vivo. The use of animal models allows the systematic study of the pathophysiology of VCI. There are no alternative models in which to assess the effects of cerebral vascular disorders. We will use adult rodents and at increasing ages to study the progression of disease. Central to the disease is damage to cerebral blood vessels and blood flow disturbances. The effects of blood flow disturbances cannot be assessed in vitro. Animal experiments are critical in identifying disease mechanisms; the time at which they occur (also essential for knowledge about drug administration) and also provide considerable insight into the development of new drugs.

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

Rodents used in this project may be genetically modified to study genes that increase risk of cerebral vascular disease or that may modify the outcome. Inducible models of stroke or surgically induced stroke models may be studied. The animals may undergo surgery, injections of substances, cranial implants, neuroimaging, behavioural assessments and culling under anaesthesia. Experimental durations will range from acute (few hours) to several months. We would seek to minimise the number of procedures in each mouse to the minimum necessary to achieve the study aims. We would ensure mice are fully recovered before the next procedure (eg between imaging or behavioural sessions). We would not allow mice with cranial implants to undergo behavioural training.

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

Potential adverse effects include weight loss, possible death under anaesthesia in survival surgeries, pain following surgical procedures which will be significantly reduced with analgesia.

Spontaneous stroke models, are caused by gene mutations that damage blood vessels leading to stroke deep in the brain. The stroke are present from birth and mice are studied normally up to 1year. The mice with spontaneous stroke can be smaller than wild-type littermates but thrive similarly. These models are associated with low mortality post-weaning and clinical symptoms such as limb weakness is rare. Themice can develop retinal alterations due to vascular changes with advancing age but these do not affect ability to eat and drink.

For the surgically induced stroke models, we will mostly study small strokes and the longer term outcomes (weeks to months) including cognition and these models are associated with very low mortality, minimal weight loss and clinical symptoms.

We will rarely study surgically induced large strokes and these will be used mainly to study the effects in the short term (days) after a large stroke. The damage to the brain tissue will cause changes in behaviour that are reflective of the symptoms observed in patients. These include deficits indicative of stroke brain injury (e.g limb weakness) and reduced food/water intake.

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)?

Breeding is generally associated with mild and moderate severity.

Severities are considered to be moderate for most other protocols. We have refined our stroke models to minimise adverse effects to allow their long term study.

Rarely (<5%) will we use protocols that will result in severe adverse effects. Severe adverse effects will be encountered in the study of all large surgically induced strokes; in a small number of spontaneous stroke and a small number due to cerebral hypoperfusion.

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

- Killed

A retrospective assessment of these predicted harms will be due by 15 June 2028

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 experiments are critical to identify what causes disease; the time at which the brain changes occur (also essential for knowledge about drug administration) and also provide considerable insight into the development of new drugs.

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

Cell models may be useful to examine the cellular responses to brain injury (albeit in the absence of blood flow) and these models can make contributions to our knowledge. We have used these in the past, and are currently collaborating with groups studying human cell models, in parallel with our in vivo models and will strive to use these if deemed appropriate. 3D Organoids may be useful to study neural development and organisation but have key limitations for our research; they lack maturity, vascular endothelial and mural cells (and blood flow) and also lack immune cells all critical for normal brain health.

Why were they not suitable?

Cerebral vascular disease is a complex disorder that results from damage to blood vessels and blood flow disturbances. These conditions cannot be mimicked in cell model or 3D organoid systems in which blood flow is absent. Additionally there is a complex interplay between the many different cells in the brain that communicate with blood vessels. Stroke can lead to chronic, widespread inflammatory changes in remote brain areas connected with the lesion, and this cannot be modelled with culture systems.

A retrospective assessment of replacement will be due by 15 June 2028

The PPL holder will be required to disclose:

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

Reduction

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

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

We estimate the given numbers of experimental animals based on our previous experience as well as information provided from investigators within the same research field including publications. The numbers required for breeding and maintenance of lines are derived from our previous experience and that of estimates provided by the animal facility.

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

For each study, we will use the lowest number of animals required to give meaningful, statistically relevant results. Statistical power analysis is used to define groups sizes. All experiments will be conducted according to the ARRIVE 2.0 guidelines.

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

The majority of our animal numbers will be due to the breeding and maintenance of colonies. We will implement efficient breeding procedures and maintain our rodent colonies using as few animals as possible to generate sufficient numbers for studies without being wasteful. We will ‘bank’ tissues to use for multiple measures. In addition in some studies we will conduct longitudinal studies in the same animal which also reduces the numbers of animals required.

A retrospective assessment of reduction will be due by 15 June 2028

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 aim to study relevant animal models that mimic aspects of human diseases, stroke and vascular disease and the risk factors that may influence their development and progression. Over the last years we have sought to develop models that are more akin to

human disease. We have significant experience with these models and have over time refined our endpoints and strategies to reduce adverse effects wherever possible. We train researchers to the highest standards to be able to monitor and study rodent models of disease, to identify adverse effects quickly and to respond to these accordingly. We have rigorous monitoring procedures in place to minimise any adverse effects

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

The goal of our research is to understand complex brain disease processes caused by vascular damage or flow alterations, with age a major risk factor, and how these impact on cognitive abilities. It is not possible to examine these in less sentient species which restrict the ability to study a developed vascular system and cognitive behaviours.

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

There has already been considerable refinement in our studies where we have developed new models more akin to the relevant human condition of VCI. The mouse model of chronic cerebral hypoperfusion using bilateral carotid stenosis is now widely used to study VCI and replaced more severe models of global cerebral ischaemia. The stroke models have also been refined considerably. The new distal model of stroke has improved recovery and minimal mortality as compared to the intraluminal thread model of stroke. The latter we have also refined and reduced the period of occlusion (15 mins) to improve recovery and reduce mortality.

Since the procedures were first established in our group they have been subjected to considerable refinement in order to reduce levels of pain and distress. There have also been significant improvements in post-operative monitoring. Animals are monitored by licencees, animal care staff and vets more frequently and with a great awareness of indicators of pain and distress or disturbances of feeding and drinking. Clear endpoints are defined for intervention either to correct weight loss and dehydration or for termination of the experiment.

There is continual review of the duration of injury or the survival period (including age) required in each experiment with the intention of reducing these to the minimum required to address the specific hypothesis being tested.

In studying GA animals, the background strain is chosen carefully and if not able to be modified we have considerable experience of different background strains and effects on outcome after different injuries. Design (e.g. duration of ischaemia, survival period) is optimised to reduce mortality as animals which die before completion of protocol do not contribute to hypothesis testing.

Mice are regularly monitored and records kept of weight and phenotype to avoid mice encountering severe adverse effects.

Studies involving surgical or invasive intervention will adopt appropriate pain management via use of analgesia and post-operative care.

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

We will stay up to date on best practice guidelines set forth and regularly updated from the NC3Rs website (Guidance on the Operations of ASPA - https://www.nc3rs.org.uk/) and LASA. These have included guidance on record keeping and application of aseptic techniques.

Updates on best practise and guidance is also disseminated to animal users via our establishment.

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

We receive regular updates from the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) and will will stay informed by these communications and by attending informational events provided by them.

if there is a scientific advantage we consider seeking research funding to implement them in parallel with our studies in mouse. For example the Stroke research priority programme I lead was funded to support studies in a mouse model with a gene mutation that causes vascular disease and we study this in parallel with studies in human iPSCs (from patients with the same gene mutation) differentiated to vascular cells. However there are limitations to cell studies including human iPSCs . Vascular cells in a dish are phenotypically different to those found in brain in vivo.

A retrospective assessment of refinement will be due by 15 June 2028

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. The Exposure to and Effects of Second Generation Anticoagulant Rodenticides on Common Kestrels

Project duration

3 years 0 months

Project purpose

- Basic research

Key words

rodenticides, wild birds, population decline, nestlings, juvenile development

Animal types	Life stages
Falco tinnunculus	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 endangered animals

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?

Second Generation Anticoagulant Rodenticides (SGARs) are used to control rodent populations. Common kestrels eat rodents, so can ingest SGARs. This project will determine 1) the foraging range of adult breeding kestrels in relation to point sources of SGARs; and whether common kestrel nesting in areas differing in SGARs usage vary in 2) the concentrations of SGARs in their blood, feathers and faeces and 3) nestling health and development.

A retrospective assessment of these aims will be due by 22 May 2026

The PPL holder will be required to disclose:

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

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

Why is it important to undertake this work?

In the UK, the common kestrel population has declined 35% since 1995 and is now amber listed. This project will investigate whether exposure to SGARs could be contributing to this decline.

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

New information from this project will be the circulating levels of Second Generation Anticoagulant Rodenticides (SGARs) in common kestrels in the wild following the potential exposure to SGARs. Publications will identify measures of health in birds differing in their predicted and measured exposure levels. This will contribute to the evidence base required by policy makers and regulators involved in environmental protection decisions. The wider benefit is a contribution to our understanding of the factors resulting in the declines in many predatory bird populations

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

Short-term benefits during the project: Common kestrels might benefit from discussions between researchers and landowners about the impacts of rodenticides on raptors in terms of them using less rodenticides or in ways that might lessen the impact on non-target organisms.

Longer term benefits after completion of this project: This project will provide evidence of the impacts of SGARs on predatory species. Once outputs have been published beneficiaries will include Landowners, Bird conservation organizations such as the Royal Society for the Protection of Birds, regulators, academics and industry researchers who work on rodenticides and other pesticides. The birds themselves will benefit in two ways a) less invasive methods of assessing exposure to rodenticides will be established which will reduce the welfare costs to birds whilst allowing for a real time assessment of exposure; b) reduced exposure to rodenticides as a result of the planned output of guidance on reducing secondary poisoning of predators by rodenticides.

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

At the end of the project, we will disseminate our results via academic publications, press releases and a policy-briefing document (where applicable). Conservation agencies,

DEFRA and the Health and Safety Executive (one of the partners on the project) will benefit from this information to better assess the ecological risk posed by rodenticides.

Species and numbers of animals expected to be used

- Other birds: 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.

Raptors, sitting as they do at the top of the food chain, have been identified as sentinels for biomonitoring pollutants and their potential toxic effects. Common kestrels are declining in numbers in the UK for reasons that are not fully understood, but exposure to Second Generation Anticoagulant Rodenticides has been implicated in declines within some regions. We are focusing mainly on nestlings because we are interested in the impacts of SGARs on reproduction and also nestlings are potentially more vulnerable to rodenticides than adults. In this species, nestlings are altricial so cannot fly, undergo rapid development in the nest and are reliant on parents provisioning them with prey.

There is a considerable body of evidence showing that neonates are particularly vulnerable to the effects of contaminants during the critical window for perturbations to the developmental pathways and induction of persistent effects. A small number of adults will be sampled to investigate the exposure of parental birds compared to their offspring.

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

A bird will be blood sampled, weighed, measured, photographed, fitted with a BTO ring and released. An individual will typically only be subject to a single procedure during this project. Disturbance at the nest will be minimised.

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

The bird will experience mild discomfort and disturbance that will last a matter of 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)?

Mild for all animals.

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

- Set free
- Kept alive

A retrospective assessment of these predicted harms will be due by 22 May 2026

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?

This project focuses on measuring the circulating levels of SGARs in relation to exposure and also associated impacts on the physiology of birds exposed to SGARs in the wild, therefore there is no alternative to using live animals.

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

This project will investigate whether concentrations of SGARs in blood are correlated with concentrations in faeces or feathers.

As part of the rest of the wider programme of research, a spatially explicit model will be used to estimate the exposure of UK birds to SGARs and link this with changes in populations.

Why were they not suitable?

The spatial model first needs to be parameterised by data on circulating levels of SGARs and associated impacts on physiology in wild birds.

A retrospective assessment of replacement will be due by 22 May 2026

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 calculated sample size allows for sufficient power for the planned statistical analyses based on similar projects on different species of birds affected by different environmental contaminants. We will sample up to 300 individuals over the study, which is required to encompass the full variation in phenotype and fitness. For example, it ensures we sample those that breed or migrate early/average/late, and individuals of different sex - variation in which can influence foraging behaviour and prey types, and so SGAR exposure.

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

A pilot study in 2022 will indicate which areas are likely to have high and which low SGAR usage. There are no UK-wide records of SGAR usage. Such information can only be acquired via a combination of talking to land or property owners, surveying for bait boxes near kestrel nests plus sampling dead rodents and kestrel pellets (regurgitated parts of prey) in areas around active kestrel nests. Thus we can compare an exposed and control group of nests which will reduce the sample size required.

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

We plan to collect multiple different types of non-invasive samples from the animals used and their nests including pellets, moulted/lost feathers, faeces and prey remains. Pellets are the undigested parts of a bird’s food, such as hair or bones, which are regurgitated. They are commonly found in or under kestrel nests and roost sites

This will maximise the amount of data collected and also to determine whether non-invasive sampling methods can be used in lieu of blood sampling to assess exposure to SGARs.

A retrospective assessment of reduction will be due by 22 May 2026

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 studying Common kestrels Falco tinnunculus. Individuals will be captured within nestboxes, weighed, measured and blood sampled by trained and competent people. Experienced bird ringers, with BTO licences, will be involved with the capture, measurement and ringing of kestrels. Blood sampling will be done by appropriately trained and licenced individuals

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

There is no alternative to working on these wild animals. In terms of using a more immature life stage, it is illegal to collect and destroy raptor eggs. This would not allow us to test hypotheses linking exposure to effects on nesting success. Likewise, less sentient models or terminally anaesthetized animals would not enable us to address our questions of interest.

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

In the wild we will be working on a species, for which the limits to which they can tolerate disturbance are known. Importantly, we will minimise disturbance by streamlining our procedures and ensuring that highly competent individuals are handling birds. The British Trust for Ornithology (BTO) ringers who we collaborate with are all highly experienced in accessing kestrel nests safely and in handling nestlings and adults. Chicks will be kept in a warm dark place between removing them from the nest and being blood sampled to minimise heat loss and stress. All researchers participating in this experiment have had prior experience in all the techniques proposed in this application, so animals will suffer the minimum amount and duration of pain and distress during procedures. There are a number of studies showing that avian parents are very unlikely to abandon chicks following such short periods of disturbance (e.g. https://doi.org/10.3356/rapt-50-01-54-59.1)

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

We will continue to consult the species/taxon focused literature and the relevant Home Office wildlife research pages for new approaches and guidance. Likewise, there will be ongoing discussions about approaches with colleagues, veterinary teams, technicians, the HO inspector and the BTO for ongoing advice. We also follow the ASAB Guidelines for the treatment of animals in behavioural research and teaching (https://doi.org/10.1016/j.anbehav.2019.11.002).The British Trust for Ornithology produces guidance on the capture and handling of birds. We will also be guided by the PREPARE

(https://norecopa.no/prepare) and ARRIVE guidelines (http://www.nc3rs.org.uk/arrive-guidelines) for planning and reporting experiments using live animals.

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

We will stay current with the literature on advances in the methodological approaches used in the project and adapt any paradigms as feasible. In particular we will work closely with our NACWO and NVS to ensure that we are up-to-date with developments in other research groups within our institution, as well as in our collaborating institutions across Europe. We will stay current with the literature on advances in the methodological approaches used in the project and adapt any paradigms as feasible.

The university keeps all project holders informed of advances in the 3Rs via a regular newsletter. Also, I am on the mailing lists for organisations such as NC3Rs who provide updates on best practice.

A retrospective assessment of refinement will be due by 22 May 2026

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. Health and Wellbeing Requirements of Cats and Dogs Across Lifespan

Project duration

5 years 0 months

Project purpose

- Basic research

Key words

Cat, Dog, Nutrition, Health, Wellbeing

Animal types	Life stages
Cats	neonate, juvenile, adult, pregnant, aged
Labrador Retrievers	neonate, juvenile, adult, pregnant, aged
Beagles	aged, pregnant, adult, juvenile, 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.

- 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?

This project aims to study the interaction between nutrients, foodstuffs and/or other non-dietary interventions and the health and wellbeing of dogs and cats. Through investigation of individual or multiple body systems and biomarkers, it will reveal fundamental principles of good health across lifespan, and then determine the impact of varying diets, behaviours, and/or environments on maintaining health or preventing the progression of disease in these species.

A retrospective assessment of these aims will be due by 20 June 2028

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?

Domestic dogs and cats rely almost entirely on their human owners for their diet, care and environment, therefore understanding how these can be improved is necessary to improve their quality of life and wellbeing.

While research has been carried out over the years to establish nutrient, behavioural and environmental requirements for dogs and cats to live healthy, happy lives, there are still significant gaps in our knowledge. For example, why some cats and dogs find certain human environments stressful is not known, and therefore there is scant research into how best to mitigate their anxiety. A large proportion of the bacteria that live on and in cats and dogs are not yet characterised, and how this "microbiome" interacts with the animals and their diet to maintain health is not understood. The precise molecules in meat and fish that cats and dogs find palatable have not been fully described, and therefore we do not yet know why cats are such picky eaters. Common progressive conditions, such as chronic kidney disease in cats, are typically only diagnosed as late-stage disease. The progression from early to late-stage disease is not well understood, and whether this can be slowed to stopped through dietary intervention has been hypothesized but not yet proven. These are just some of many knowledge gaps in companion animal health today.

Due to the unique nature of both species, insights into human health, diet and wellbeing are often not generalizable to pets, therefore it is important that studies are carried out specifically for the benefit of our companion animals. For example, cats lack an ability to detect sweet taste, therefore most research into human food palatability is not relevant to cats. In contrast, both cats and dogs have many more flavour receptors that humans, giving them an ability to detect other flavours, well beyond human capability. Cats and dogs also have a different set of essential amino acids to humans, meaning they have different nutritional requirements to maintain good health. Consequently, they have very different microbiomes (bacteria that co-exist on and inside animals) to each other and humans, and so the extensive human research in the microbiome field is typically not generalisable to cats or dogs. They also suffer from different types of diseases and conditions. For examples cats and dogs typically don't develop dental cavities, unlike humans, therefore research into maintaining good oral health in humans is less relevant to

companion animals. These are just some of many differences in behaviour and physiology between cats, dogs and humans.

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

Objectives 1, 4, 5 and 6 aim to identify scientific knowledge of novel biomarkers of age, health status or wellbeing in dogs and cats. Here the expected outputs will be at least 8 scientific publications in peer reviewed journals and associated lay articles, 5 scientific conference presentations and 5 filed patents relating to candidate biomarkers and/or personalised care pathways.

Objectives 2, 3 and 4 are related to assessing dietary requirements and the impact of changes in nutrition on health status of cats and dogs. Here the expected outputs will be new knowledge communicated in at least 8 scientific publications in peer reviewed journals and associated lay articles, 5 scientific conference presentations and 5 filed patents relating to ingredient(s) to support specific aspects of health.

Objective 7 aims to establish the factors that enhance enjoyment of food in cats and dogs. Here the expected outputs will be new knowledge communicated in at least 3 scientific publications in peer reviewed journals and associated lay articles, 2 scientific conference presentations and 5 filed patents relating to dietary formulations and/or feeding approaches that influence food intake and enjoyment.

Objective 8 will identify candidate biomarkers and positive nutritional interventions in cats with early stage chronic kidney disease. Here the expected outputs will be new knowledge communicated in at least 1 scientific publication in a peer reviewed journal and an associated lay article, 1 scientific conference presentation and 1 filed patent relating to candidate biomarkers and/or personalised care pathways.

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

Objectives 1, 4, 5 and 6 aim to identify novel biomarkers of age, health status or wellbeing in dogs and cats. Here the primary beneficiaries will be pet care professionals, such as veterinarians, breeders or animal scientists. Knowledge and scientific publications can provide information that enable them to do their work faster or more successfully. Additionally, pet owners are expected to benefit, both because they will gain significant emotional benefits from having healthier, happier pets by applying the knowledge generated. These include websites, lay publications and digital tools and apps to provide information generated by this project. These outputs are expected between 1-3 years.

Objectives 2, 3 and 4 are related to dietary requirements and the impact of changes in nutrition on health status of cats and dogs. Here the primary beneficiaries will be pet dogs and cats, as the knowledge generated will be used by pet food formulators, nutritionists and owners to provide optimal pet foods or pet care products that improve their health, wellbeing or quality of life. The timescale for these are typically 3-5 years.

Objective 7 aims to establish the factors that enhance enjoyment of food in cats and dogs. Here the primary beneficiaries will be pet dogs and cats, as the knowledge generated will be used by pet food formulators, nutritionists and owners to ensure that their cat or dogs eat their food to obtain their nutritional requirements. Additionally, cat and dog owners are expected to benefit, because they will gain significant emotional benefits from knowing their pet is enjoying their food. The timescale for these are typically 3-5 years.

Objective 8 will identify candidate biomarkers and positive nutritional interventions in cats with early stage chronic kidney disease. Here the beneficiaries will be pet care professionals, such as veterinarians. Knowledge and scientific publications will provide information to enable them to do their work faster or more successfully. Pet cats will also benefit from veterinarians applying this knowledge to improve the management of this chronic disease. Additionally, pet owners are expected to benefit, because they will gain significant emotional benefits from having healthier pets. The timescale for these are typically 1-3 years.

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

Much of the scientific data collected from this work will be stored in digital repositories for other scientists to reanalyse for additional purposes, thereby increasing the scientific value of the knowledge gained. Outputs that contain new knowledge will be shared via publication in some form, through scientific publication, patents, lay publications or websites. We will also actively draw attention to those publications through blogs, social media and soliciting media interest. Approximately 40% of projects will be expected to be conducted in collaboration with other researchers from Universities, institutes or companies; data and samples will be shared with these collaborators.

Species and numbers of animals expected to be used

- Cats: 420
- Other dogs: No answer provided
- Beagles: 100

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.

Knowledge, products or services aimed at improving the wellbeing of cats and dogs will be most effective if collected through studies using cats and dogs. This is because many aspects of cat and dog behaviour, physiology and metabolism are unique to their species, and so scientific studies using other types of animals are unlikely to provide the same accuracy. Likewise, because the needs of cats and dogs differ by life-stage, it is important we use the appropriate life-stage to conduct research to meet those needs.

For instance, it is widely known that a number of foods routinely consumed by humans are acutely toxic to cats or dogs (e.g. chocolate, grapes, onions). However, there are more recent reports of other ingredients and/or nutrients associated with unexpected health concerns in cats and dogs when fed daily over prolonged periods (e.g. legumes, inorganic phosphorus). Additionally, a wide range of bioactive compounds that are safe in other species can cause liver damage in cats due to differences in liver metabolism and, the causes and progression of some common diseases are unique to these species (e.g. dilated cardiomyopathy in dogs, hyperthyroidism in cats). Finally, the sense of taste is very different in cats compared to most other animals. Cats do not have an ability to taste sweet, so most research into making food more attractive is not relevant for cats.

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

An animal in this project will typically be fed a new diet or ingredient, or interact with a new toy or chew, or experience a different type of interaction with a human, or be introduced to a new environment.

Before, during or after these interventions, we will typically analyse small biological samples (for example, urine, faeces, DNA, plaque, saliva or blood) to assess health and wellbeing. We may occasionally capture body scans, such as x-rays, and use anaesthetic for restraint purposes only. Many measures of health will be determined without the need for regulated procedures e.g. through collection of faeces or urine or through assessing behaviour, but others such as blood sampling will require the use of regulated techniques.

Some experiments may last only minutes with only one procedure, others may last up to six months with samples taken once a month. Similar samples may also be taken from animals intermittently across their lifespan to investigate how they naturally change.

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

The procedures on most animals in this project are not expected to result in lasting harm, and are classified as mild severity. For example, some animals may experience mild transient discomfort when blood samples are taken or exhibit transient stress behaviour when experiencing a new environment. At the end of this project these animals will either be used for additional studies or rehomed.

A smaller number of cats have been diagnosed with progressive kidney disease. These animals will be studied in the same manner as their disease develops. Due to the progressive nature of the disease, these cats are likely to experience moderate severity at some point and will be euthanized before their symptoms become severe. Symptoms that some animals may experience immediately prior to euthanasia include weight loss, reduced grooming and subdued demeanor.

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

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

We expect less than 5% of cats may experience moderate severity due to their disease diagnosis, the remaining cats should experience no more than mild severity. We expect all dogs to experience no more than mild severity.

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

- Killed
- Rehomed
- Used in other projects

A retrospective assessment of these predicted harms will be due by 20 June 2028

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 ultimate aims of this project are to understand the impact of dietary and non-dietary interventions on the health and wellbeing dogs and cats. We need to use animals to understand how the body translates the constituents of a diet into metabolites that can influence enjoyment or health, or how a non-dietary intervention interacts with the body via neuronal or hormonal processes. These processes involve organs such as the mouth, nose, stomach, intestine, blood, skin, liver, kidney and brain, and the interplay between them. They also involve interactions between the animal and the bacteria that live within the intestine, mouth or skin. These processes are so complex that currently no in vitro system is able to replicate this.

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

For some aspects of this project it is possible to use non-animal approaches and wherever possible this route will be followed. For example, the development of an in vitro model of gut fermentation; computer modelling of taste and smell receptors; or artificial intelligence analysis of veterinary records. These alternatives were identified from reviewing the literature and partnering with collaborators with expertise in non-animal approaches (for example, artificial intelligence and molecular modelling).

These non-animal approaches often inform in vivo studies and/or are used to "rule out" interventions that are unlikely to improve health or wellbeing, thereby refining experimental

designs and reducing the number of interventions used on animals. For example, medical records of hundreds or thousands of cats with chronic kidney disease were retrospectively analysed to identify the blood and urine biomarkers that indicate different stages of the disease. However, retrospective analysis itself cannot assess the efficacy of a novel intervention. This knowledge was critical to determine which biomarkers to use to track the progression of the disease in cats in response to interventions, an objective in this project.

In another example, hundreds of thousands of potential flavour molecules were screened against computer models of cat smell and taste receptors to identify a short-list of around one hundred that cats may enjoy. Likewise, dozens of ingredients that may modify the gut microbiome was screened for bioactivity in vitro, using an "artificial gut". However, these in silico and in vitro alternatives are significantly simplified models compared to assessing interventions in complex living mammals like cats and dogs. Therefore to fully meet the aims of the project, it is necessary to confirm bioactivity in an animal model to ensure it is indeed beneficial to cats or dogs.

Why were they not suitable?

Some aspects of the work cannot currently be effectively modelled using non-animal alternatives yet. For example, understanding the combined impact of taste and smell on food preference; measuring how a pet food can impact the complex immune system of a cat, understanding how maternal milk influences the network of bacteria living in the gut of newborn puppies.

A retrospective assessment of replacement will be due by 20 June 2028

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 employ a team of dedicated statisticians who calculated the number of animals and experimental design (e.g. a cross-over or intervention/control study, longitudinal or cross-sectional) for earlier studies that were conducted under the authority of previous licences. This approach has enabled us to use the minimum number of animals while ensuring that the results are statistically significant. Sample sizes for future experiments are always estimated from past experiments (otherwise smaller pilot studies are conducted, first).

Calculations show that minimal group size requirements are typically ~10 animals per intervention, and maximal group sizes are typically ~40 animals per intervention to achieve the results necessary to meet objectives. Some experiments involve two interventions (e.g. diet and control) and others way involve up to four interventions (e.g. exposure to three different toys and a no-exposure control).

We also have significant experience in reusing cats and dogs in multiple iterations of a protocol, which enables us to calculate the appropriate time-frames between re-use, and therefore estimate the total number of animals required to meet the objectives of the project within its length.

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

Whenever possible we will study change in the same animals over time (longitudinally), rather than compare one set of animals to another (cross-sectionally). This typically requires the use of fewer animals. As the impact of most procedures are mild, we will use the same animals in a number of different investigations under the same protocol, but not between protocols. This means that, overall, fewer animals are needed to meet the objectives of the project.

The factors that typically influence the number of animals used in an experiment are breed, age range, technical variance in analysing the primary measure, and effect size of the intervention. These are controlled in the experimental design phase by fixing the variable whenever possible (e.g. using a single breed) or by balancing across groups (e.g. by ensuring the age ranges are similar across intervention and control groups). We have implemented harmonized protocols for data collection across all our projects. This reduces technical variance in capturing measures, and ensures the data an experiment is powered from is not different from the data that will be collected. Finally, by pre- screening potential interventions using in vitro or in silico methods, we will select those that are likely to have the largest effect size, therefore reducing the number of animals required to detect the impact of the intervention.

These steps were taken in consultation with a dedicated team of statisticians employing NC3Rs experimental design guidance. They will also advise on appropriate randomisation and blinding strategies, and the appropriate methods of statistical analysis for each experiment.

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

Whenever possible historical data from the exact same animals using the exact same technical approaches can be used to calculate how many are required for subsequent studies, this improves the accuracy of power calculations, and means less animals will be required.

When historical data is not available, pilot studies will be conducted to collect data and ensure the minimal number of animals will be used. Whenever possible, experimental data (or pilot data) will be generated from previously biobanked or residual samples to reduce the number of procedures animals are exposed to.

Genetic analysis will be used to source animals, to ensure they are free from potential disease-causing variants and that the genetic diversity of the research colony is maintained. This will minimize the number of animals that are unsuitable for use in this project.

A retrospective assessment of reduction will be due by 20 June 2028

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 are specifically interested in understanding the impact of diet and simple non-dietary interventions in cats and dogs, therefore the use of these species is necessary. All the methods we use are optimised to reduce pain, suffering, distress, or lasting harm to the animals. For example, we use non- regulated methods to collect samples whenever possible, when a regulated procedure is necessary, we will train animals to participate in the procedure using positive reinforcement, which reduces the likelihood the animal experiences stress.

When animals are under anaesthesia for veterinary purposes (e.g. when having their teeth cleaned), we will endeavour to perform other regulated procedures required by the study e.g. blood samples, so that the animal does not have to experience this event while conscious. This both minimises the number of anaesthetic episodes across the life of the animal and reduces potential stress or discomfort.

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

Our aims are to improve the health and wellbeing of cats and dogs across their life-stages. Using less sentient species or immature life-stages will not support that aim.

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

Our standard procedures for animal training, sampling, veterinary care and analysis are harmonised so that they happen the same way each time. This enables efficiency, ensures that high standards are maintained and permits data collected from different studies to be directly compared and reused. Each harmonised procedure has a review schedule, which involves an assessment of changes in current best practice, available technology and learnings from our own experiences. This process will enable us to continually refine our procedures to minimise its impact on each animal's welfare.

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

We will closely monitor governmental and industry bodies that issue pet food and ingredient nutritional guidelines, to ensure that our experiments meet regulatory needs. We will also follow guidance from professional veterinary bodies and literature published in their journals. We will also closely follow bodies such as NC3Rs. While much best practice is optimised for research using rodents or fish, we will consider whether the same principles or approaches can be adapted for experiments using cats and dogs.

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

We have dedicated roles in our organisation that are employed to protect and improve the welfare of animals. They engage with external bodies (such as NC3Rs) and routinely disseminate that information among project leaders. They also participate in the governance and review of research proposal, ensuring any advances they are aware of can be implemented as new studies are developed.

A retrospective assessment of refinement will be due by 20 June 2028

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. Safety Evaluation of Industrial and Agricultural Chemicals

Project duration

5 years 0 months

Project purpose

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

Key words

Toxicity, Safety, Toxicokinetics

Animal types	Life stages
Mice	adult
Rats	adult
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

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 generate toxicological and safety data in animals following exposure to industrial and/or agricultural chemicals and veterinary products that Man may be exposed to. The studies performed will be designed to reveal any effects on mammalian toxicity and the main studies will be based on the most up to date ECHA or OECD regulatory guidelines.

A retrospective assessment of these aims will be due by 26 April 2028

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?

Chemicals play an important role in daily life. Therefore, their safety for Man, other animals and the environment has to be considered carefully. By establishing sufficient toxicological and other safety data in animals, safe handling precautions may be determined thus protecting the health and welfare of hundreds of humans and animal species which may contact the materials concerned, as in the case of site limited industrial chemical intermediates with limited potential for human exposure to millions of humans as with industrial or agricultural product with world-wide market. and facilitate their safe use of world-wide.

The projects performed under this licence will also provide safety data which facilitates sound regulatory decisions to be made worldwide that protect the public and the environment from possible hazards.

Safe, regulated products have the potential to improve and enhance the health, well-being and quality of life of people and animals, by improving crop-protection thereby, increasing food security, whilst the development of safer chemicals and chemicals with reduced environmental impact is clearly beneficial for humans, animal health and the environment.

The projects undertaken in this licence use methodologies that are well established and known to produce accurate and reliable results that can be used in regulatory risk assessment. Furthermore, the studies can rapidly identify any overt toxicity which would cease the development of the test item and therefore enable the Sponsor to make a decision at the earliest opportunity to cease production: reducing the risk of possible human exposure and avoiding unnecessary expenditure and use of resources. The work performed under this licence will be undertaken in a GLP compliant laboratory thereby ensuring data integrity and accuracy.

Unfortunately, the use of alternative methods, including the use of dead animals, at this moment in time cannot generate relevant data to support the submission of safety data to international regulators.

Alternative methods such as in-vitro techniques will be used as much as practicable to supplement the work involving protected animals.

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

The outputs of this project will support the development and continued use of safe chemicals that do not impact on the health or well-being of humans and the generation of robust data, in the form of study reports, to enable the safety assessment. Study reports will be included in regulatory submissions to allow regulatory authorities (e.g. OECD, EPA, ECHA) to make judgements on whether to permit the licence of a chemical.

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

The projects performed under this licence will initially benefit manufacturers throughout the world who invest heavily in the production of chemicals. However, once safety has been established and regulatory approval has been given for world-wide distribution and use then end-users and the general public alike will benefit from the use of safer chemicals.

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.

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

The majority of studies will follow a similar paradigm; All animals will be dosed with the test chemical via a route that would mimic accidental exposure in man, typically in food, water or contact with skin at normally three dose levels and monitored closely for signs of toxicity. 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 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 Veterinary Surgeon if considered necessary. No animal will be allowed to endure pain for long periods and remedial action which may include euthanasia 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 26 April 2028

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 interacts with, or is metabolised and distributed by a living mammalian body, has a significant effect on its potential toxicity. At this time, effects on complex interacting biological systems cannot yet be replicated in in-vitro in-silico or ex-vivo tests and, as a consequence, for the majority of chemicals it is imperative they are tested on living animals in order to assess for toxicity of tissue, organs and systems e.g. the cardiovascular, neurological, respiratory and reproductive systems following repeated exposure.

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

In-vivo studies will only be performed where there are no validated in-vitro alternatives available to us, including in-silico modelling to predict toxicity. Where necessary, guidance pertaining to the necessity of a study will be sourced using sources such as the European Commission’s Tracking System for Alternative Methods (TSAR).

Why were they not suitable?

As the use of alternative methods, including the use of dead animals cannot, currently generate relevant data which supports the submission of safety data to international regulators, alternative methods such as in-vitro techniques will be used as much as practicable to supplement the work involving protected animals which are a legal requirement of worldwide regulatory authorities.

A retrospective assessment of replacement will be due by 26 April 2028

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 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. For preliminary studies, small groups are acceptable because of the potential use of overt toxicological endpoints. 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 26 April 2028

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. 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; in most cases, this will be the rat or mouse.

Most chemicals 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, legislative requirements and availability; 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?

It is important that the life stage of the animals used are equivalent to the expected life stage of humans to whom the substance will be administered. For the studies performed under the authority of this licence tests using animals at immature life stages will not produce the safety information required to fully assess the safety of materials under evaluation. Where there is a risk that embryonic or foetal humans may be exposed to a test substance then this work will typically be conducted under separate authority.

In addition to this, the majority of studies performed require changes in physiological 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. Where necessary, current, industry accepted techniques for dosing and blood sampling for example, will continue be refined under separate authority i.e. under another project licence, thus ensuring existing 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 26 April 2028

The PPL holder will be required to disclose:

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

21. Neuromodulatory-Frontal Cortical Interactions

Project duration

5 years 0 months

Project purpose

- Basic research

Key words

prefrontal cortex, cingulate cortex, decision making, learning, motivation

Animal types	Life stages
Rhesus macaques	adult

Retrospective assessment

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

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

Uses non-human primates 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 understand the role of brain areas in decision making, learning, and motivation. We are interested in two sets of brain areas; the first set includes the prefrontal and cingulate cortex and the second set includes the neuromodulatory systems.

A retrospective assessment of these aims will be due by 22 June 2028

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 now established that the prefrontal and cingulate cortex are important for learning, decision making, and motivation. We do not, however, understand how such brain areas come to have these roles. Learning, decision making, and motivation are fundamental to normal biological and psychological functioning in humans and other animals and so it is important to understand them and the way in which these processes are mediated by the activity and interaction of brain areas.

When prefrontal and cingulate cortex activity leads to learning, decision making, and motivation it does not do so in isolation. Instead, these brain regions accomplish these roles, via their interactions a second set of brain areas – the subcortical neuromodulatory systems. We do not understand how the information contained in these different brain systems is exchanged and when and how the information is different in nature or specialized in one system as opposed to the other.

While the primary interest is in understanding learning, decision making, and motivation mechanisms in the healthy brain, our results are likely to lead to new insights into how these processes go awry during psychological and neurological illnesses. For example, illnesses such as depression are sometimes characterized by a failure to learn or estimate either the value of the environment that a person finds themselves in or the values of the choices and courses of action that are available to a person. A person with depression may underestimate the value of the choices available to them. If someone underestimates the value of a choice that is available to them then they may fail to take a good choice when it is available. In addition, models of the neural mechanisms of learning and decision making have inspired computer and artificial intelligence (AI)-based advances and this may also be a consequence of our work.

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

At the end of this project we will have the following outputs:

1. An understanding of prefrontal and cingulate cortical systems and the interactions and interdependencies that they have with subcortical brain areas such as ascending neuromodulatory systems, during various aspects of learning, decision making, and motivation including behavioural flexibility (objective 1), information seeking (objective 2), acquisition and use of knowledge of task structure to guide decision making (objective 3), and in order to learn how to learn more efficiently (meta-learning and the ability to generalize from one context to another; objective 4). We will do this not just when animals are learning from their own experience but when they learn from observing others (social

learning; objective 5). In some cases it may be possible to examine the welfare impact of the procedures (objective 6).

2. An understanding of how neural representations (patterns of neural activity that stand for an environmental feature, an action, memory or plan in the internal workings of the brain) differ in subcortical as opposed to prefrontal and cingulate systems and how they bring about learning, decision making, and motivation. Again we will do this while examining various aspects of learning, decision making, and motivation including behavioural flexibility (objective 1), information seeking (objective 2), acquisition and use of knowledge of task structure to guide decision making (objective 3), and in order to learn how to learn more efficiently (meta-learning and the ability to generalize from one context to another; objective 4). We will do this not just when animals are learning from their own experience but when they learn from observing others (social learning; objective 5). Again, in some cases it may be possible to examine the welfare impact of the procedures (objective 6).

3. An understanding as to whether transcranial ultrasound stimulation (TUS) can be used to deliver selective neuromodulation to specific brain areas. This is a relatively new technique that will be employed to address many of the research objectives (objectives 1-6).

The information is likely to be made available principally through the publication of articles in peer- reviewed scientific journals; over the last five years we have published 40 articles in well known peer- reviewed scientific journals such as Nature, Nature Communications, Nature Neuroscience, and Neuron. In addition, as in previous projects, we expect to release data sets that can be used by other scientists to analyse themselves. In some cases this may also allow other scientists to address new scientific questions. We have actively contributed to the development of data repositories, including the PRIMatE Data Exchange (PRIME-DE) and Collaborative Resource in Computational Neuroscience (CRCNS). One or other of two papers that initially reported details of the repositories are typically cited by research teams that go on to publish further work and re-analyses of the data. The fact that the two papers have been cited just under 200 times indicate that the data are widely shared, re-used, and published in peer-reviewed journals by other scientists.

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

Initially it is anticipated that other scientists will benefit from the knowledge contained in the scientific papers that we will publish in peer-reviewed scientific journals. In addition to established scientists, trainees, research students, and undergraduate students taking courses on brain function will benefit when they are taught about the work in the papers we publish during courses on cognitive, behavioural, and computational neuroscience. That the topics that we focus on, behavioural flexibility (objective 1), information seeking (objective 2), the acquisition and use of knowledge of task structure

to guide decision making (objective 3), learning and generalization (objective 4), and social learning (objective 5) are all ones in which scientists are currently interested and this is for several reasons. This is because, first, they are believed to be important for understanding higher brain function.

Second, understanding how learning processes occur in the brain has often had an impact on how we build artificial intelligence systems that can learn or make decisions. Third, it is widely thought that knowledge of these processes is a prerequisite for an understanding of changes and alterations in learning, decision making, and motivation in psychological and neurological illnesses and so it may benefit clinicians working with such patients and ultimately it may benefit the patients themselves. For example, apathy is a symptom that is reported in many psychological illnesses such as depression. We now know that apathy is characterized not simply by an inability to exert the effort needed to make an action but by diminished ability to choose the most effective course of action (objective 1) and to find out about which is the most effective course of action (objective 2).

A part of the planned work that we will perform is concerned with new minimally invasive techniques, such as TUS, that we will use to assess and manipulate brain function. Partly because of some of our previous work, these techniques are increasingly of interest to researchers and surgeons working with human patients. Our results may therefore be of benefit to patients. Our objective is not to establish precise parameters for regulatory agencies to employ when governing the use of these techniques with people (although this may well be a by-product of our research) but rather our aim is to establish the ways in which the techniques can be used to manipulate neural activity.

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

In addition to publishing the results we will translate the investigations into research into human brain function where this is possible. Typically we will do this by we collaborating with researchers who build computational models of the brain mechanisms and cognitive processes that we investigate. Data sets that we generate using animal models are used not just by us on one occasion but repeatedly to answer new questions. In many cases this is done by releasing the data to colleagues in other institutions; in the past our data sets have been widely shared by sending the data in an electronic format to other scientists. In other instances we have uploaded electronic data to PRIMatE Data Exchange (PRIME-DE) so that they can be downloaded by other independent research groups. The data have been used not just by colleagues but by independent research groups to produce new scientific results and conclusions that have been published in journals.

We contribute to public engagement events, typically at schools and museums, during which we explain how we conduct our research, the results that it produces, and the various types of benefits that such results have.

Species and numbers of animals expected to be used

- Rhesus macaques: 26

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 adult macaques to investigate these brain areas because the brain areas are either unique to or especially well developed in primates such as macaques and previous investigations of this type have shown that the anatomy and function of the macaque brain is very similar to the human brain. We are using macaques rather than another primate species because other aspects of brain function and anatomical connectivity are well characterized in macaques but this is not the case for other species. The brain areas take time to develop and so we investigate them in adult animals.

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

Animals will be trained to perform behavioural tasks that allow us to assess cognitive processes related to learning, decision making, and motivation. They will learn to perform these tasks by choosing between visual patterns and objects that are presented on a computer monitor. At the same time, we will measure which choices they take by recording the movements that they make on touch sensors placed near the objects. Animals will then be trained to perform the same tasks while in a magnetic resonance imaging (MRI) scanner so that measurements of brain activity can be taken at the same time. In order to do this, the macaque is trained using food rewards, to lie down in a special type of chair which is then put into the scanner. The macaque's head needs to be kept completely still.

This is done by implanting a device to the animal's skull (under general anaesthesia) which is then used to prevent the animal moving its head while the scan is taken. Recently the National Centre for the 3Rs (NC3Rs: National Centre for Replacement, Reduction, and Refinement) funded a group of researchers to produce an alternative approach for keeping the heads of animals still, for example, by training the animals to put their heads in a mask that was placed at a particular location in the scanner. However despite investing considerable time and effort, the researchers were unable to develop the alternative procedure and so the approach that we are taking is the only one that is currently viable.

The animal is gradually trained over several months so it becomes accustomed to having its head restrained in this way. Typically, MRI scans taken when animals are performing behavioural tasks, last for less than an hour but they never exceed two hours.

Sometimes animals will be given transcranial ultrasound stimulation (TUS) when they are performing the tasks. This involves a short train (usually less than a minute) of ultrasound

pulses that temporarily alter brain activity in a small part of the brain. We can measure the alteration using MRI scanning and careful measurements of the animals' behaviour. Typically this takes less than an hour but never exceeds two hours. In some cases TUS is combined with delivery of a neuromodulatory drug. We can test whether the TUS makes it possible to deliver the drug more directly to the brain with fewer peripheral side effects. In summary, the maximum time animals are in the MRI scanner performing behavioural tasks (whether or not they are exposed to TUS and/or neuromodulatory drugs) will be 2 hours, with most sessions lasting around an hour.

For a subset of experiments we will need to record the activity of individual brain cells. To do this we need to insert very fine, delicate, electrodes into the brain. This is done through a small hole in the skull, a craniotomy, which is created whilst the animal is under general anaesthesia. The hole is kept open by implanting a metal cylinder over it (a chamber). Animals are given analgesics when they wake

up from the craniotomy surgery and are expected to adapt quickly to the presence of the chamber. Once the animals are fully recovered from the surgery, electrodes are carefully inserted into the brain. The brain does not have any pain receptors so the animals do not feel any pain from the insertion and subsequent withdrawal of the electrodes. These procedures are similar to ones used in people.

Experiments using these electrodes require the animal's head to be kept completely still so, like the TUS experiments, animals' heads are fixed using the head post when they are in the scanner.

Experiments using electrodes can take several hours (typically 5-6 hours but exceptionally up to 7 hours). Such recording are usually performed 4-5 days per week but occasionally 6 days week for up to a two week period. Therefore, the macaque typically receives 2-3 days off in any period of 7 days but may have only one day off per week for a two week period. Typically, the total number of sessions for an animal would be 30 but the maximum would be 45. Devices that are implanted through the skin of an animal need to be regularly cleaned and treated. This is because the skin does not integrate with the device and so does not fully heal over. Animals need to be restrained for this care to be provided. This is usually done by the animal voluntarily entering a container (a primate chair) that allows access to head. We use the electrodes to record activity in individual brain cells (neurons) while animals are performing behavioural tasks. Making sure that the electrodes are recording properly can take time and so the recording sessions may take up to seven hours. We think, however, that we can obtain all the data of this type that we need within 30-45 recording sessions.

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

There is a risk of pain when the head fixation device is inserted and when the craniotomy is made and the chamber placed on the skull. We minimize this risk by performing these procedures while the animals are anaesthetized and given analgesia.

There also a risk of infection when the head fixation device is inserted, when the craniotomy is made, or when the chamber is placed on the skull. These risks are minimized by the careful use of aseptic procedures.

There is a risk that the animals will be fearful of the laboratory equipment. We minimize this risk by acclimatizing them to the laboratory and the procedures that are used in it in a very careful and gradual manner. This also ensures that the animals continue to perform the behavioural tasks well.

There is a risk of discomfort if the animals are in a fixed position for a protracted period of time. We minimize this risk by training animals and carrying out MRI and TUS experiments in short sessions that are typically under an hour in duration. It is more difficult to keep the sessions short in electrode recording experiments and so we take care to limit the number of these longer recording sessions. We therefore train animals to tolerate periods of restraint. We do this by providing positive reinforcement to the animals while they are restrained and by gradually increasing the level of restraint and increasing the time for which it is applied. If the animal becomes uncomfortable during the period of restraint then it stops performing the behavioural task and the experiment is stopped. All stages of the training are managed with positive reinforcement. One of the first ways in which animals are restrained is by the placement of a neck plate that keeps their head in approximately the right position during the experiments. Animals are given positive reinforcement such as food treats when they put their head in the correct position so that the neck plate can be closed. After a short period of such training, sometimes just a single day but sometimes approximately a week in length, the animals tolerate the placement of the neck plate. There is a risk that animals may be thirsty prior to the daily testing session because it is sometimes necessary to control the amount of fluid to which they have access prior to the start of the testing session. However, we always either give them free access to water after the session is finished or else we carefully measure the total amount of fluid they are given to ensure that they do not receive less than is advised in guidelines for laboratory animals that are used in the UK (Prescott and colleagues, 2010: Refinement of the use of food and fluid control as motivational tools for macaques used in behavioural neuroscience research: report of a Working Group of the NC3Rs; J Neurosci Methods. 2010 Nov 30;193(2):167-88).

There is a risk that a brain area may be damaged by the application of TUS. We have always completely avoided this in the past by carefully calibrating the intensity and duration of the TUS and expect that this will be possible in the current programme of work.

Long-term cranial implants can be associated with infections, though the implants are bio-compatible and regularly inspected and cleaned, such that any infections are quickly detected and appropriate antibiotics are used.

Although extremely rare, there are a number of possible adverse effects that could occur during/following surgery and anaesthesia (brain haemorrhage, seizure, partial paralysis

[for example weakness in a limb or a lack of dexterity], breathing obstruction, failure of respiratory or cardiac systems) or from insertion of recording electrodes into the brain (brain haemorrhage, seizure, partial paralysis or weakness). Of these rare events, seizures are the most common, though these typically subside immediately with medical treatment. While they remain a possibility, we think that the techniques that we have developed mean that it is very likely that all these adverse effects will be avoided.

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 experiments are all expected to be of moderate severity. In general, this is the classification that has been made when previous projects in the applicants' laboratories have been reviewed, after completion, with the named veterinary surgeon and named animal care and welfare officer. However, if there is an unexpected adverse effect on the animals then there is a chance (<1%) that a procedure will be deemed to have reached the severe category.

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

- Killed

A retrospective assessment of these predicted harms will be due by 22 June 2028

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 use animals to study brain function, rather than humans, because the experiments require an awake, behaving primate and the techniques we need to use to record and alter brain activity require surgery to the skull and run the risk of brain damage (albeit that we have avoided this in the past).

Consequently, it would be unethical to use human volunteers. The need to study behaviour also means that in-vitro and ex-vivo models are not suitable.

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

We also considered carrying out the work with human volunteers. In a number of cases we found that it was possible to use non-invasive methods such as functional magnetic resonance imaging (fMRI) to record neural activity in humans and so we are using this approach where it is possible. In some cases we found it was possible to use transcranial magnetic stimulation (TMS) to transiently change human brain and so we are also using this approach where it is possible

Why were they not suitable?

It was not possible to record brain activity in humans at the level of individual neurons using non- invasive neuroimaging processes and so we need to use an animal model to record such activity patterns. It also was not possible to transiently inactivate human brain activity deep in the brain using TMS and for the times periods necessary. TUS will allow us to do this but using TUS in this way is not currently considered safe in humans and so we need to use an animal model for experiments requiring TUS.

A retrospective assessment of replacement will be due by 22 June 2028

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 plan to use animals in six groups of four to address six lines of enquiry outlined in the project plan. This means that we will need to work with at least 24 animals. In addition, In case any animal enters the procedure but then is found not to be appropriate for the techniques we need to use, we have included an additional two animals taking the total to 26. For example, in the past an animal was found, at the moment of its entry into the procedure, to have a cardiac arrythmia that precluded its use. We focus next on the justification for groups of four animals in each experimental line of enquiry.

When several recordings of brain activity are taken with functional magnetic resonance imaging over the course of several days in each individual then it is possible to obtain a statistically reliable measurement and to assess that it is reliable across individuals. Similarly, we can assess whether the impact of transcranial ultrasound stimulation on brain activity or behaviour is statistically reliable.

When recordings of activity in individual neurons are made then it is possible to obtain statistically reliable estimates from two individuals. The key measurements are now no longer the behaviours of individual animals but of the individual neurons. By having data from two individuals, it is possible to demonstrate the replicability of the patterns of neural activity recorded in the population of neurons drawn from one individual in the second individual.

In summary (and as explained above), what this will mean is that a group of four animals will be used to test a line of enquiry. The group of four will first, be investigated using functional magnetic resonance imaging (fMRI) and transcranial ultrasound stimulation (TUS). The fMRI provides a picture of activity across much of the brain and the TUS allows us to test its causal importance for a behaviour. The final stage of the investigation involves the use of targeted recordings in a specific brain region.

We will investigate two brain regions or circuits so two animals will be used for one set of targeted recordings and the two animals will be used for the other set of targeted recordings.

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

We have reduced the number of animals used in this project by:

1) taking repeated measurements of brain activity from each individual. This makes it possible to establish the consistent features of brain activity patterns in each individual.

2) using a reversible technique for altering brain activity (transcranial ultrasound stimulation) that means that each animal can serve in both control and experimental conditions. The within-subject design also makes comparison of control and experimental conditions more reliable by reducing variation due to individual animals.

3) consulting on-line tools such as the NC3R’s Experimental Design Assistant.

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 optimised the number of animals that we plan to use by:

1) carefully examining the results and reliability of statistical effects in experiments using analogous designs.

2) carefully examining the reliability of behavioural and brain data in the experimental paradigms used in the current programme of research prior to initiating the study.

3) sharing the data that we obtain in on-line data repositories so that other scientists can examine the data without having to conduct further experiments of their own.

A retrospective assessment of reduction will be due by 22 June 2028

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.

Model species

We will use rhesus macaques in this study because:

1) they possess the brain structures that are the focus of our investigation. The frontal cortical brain structures are not found in non-primate species or non-mammal species. However, it has been established that macaque brains and behaviour are good predictors of human brain function and behaviour.

2) there is precise knowledge about key features of the rhesus macaque brain including the anatomical connections of the areas that we investigate. Such information can be used to guide the investigations we are planning now. Such knowledge both guides the development of our hypotheses, constrains our interpretation of results, and determines practical aspects of the study such as exactly which brain regions we examine. Such information, however, is not available for other primate species.

3) they are bred in the UK and so they are not caught in the wild or transported over long distances.

4) they are known to be able to perform behavioural tasks that share features with those that we intend to use. There is, therefore, a high chance that we will be able to train the animals on the behavioural tasks that we plan to use. Whether we would be able to train other primate species to do the same is, however, not clear.

Anaesthesia

A small part of the investigation involves surgical procedures to make an opening in the skull to record brain activity with an electrode or to implant onto the skull a post to hold the

head still while brain activity is recorded. At these points in the study there is a clear risk of causing pain. To avoid this, we only conduct such procedures while animals are anaesthetized. They will also be given analgesia during their post-operative recovery.

On some occasions it might be necessary to conduct a magnetic resonance imaging (MRI) scan under anaesthesia. When MRI scans are obtained in this way they yield especially high quality images of the brain.

Procedures that require administration of anaesthesia are conducted rarely and are spaced apart, allowing the animal to recover from each anaesthetic. We would not do more than one of these on average per year but, typically, not even one surgery would occur in any given year.

fMRI

On some occasions we will record neural activity using functional magnetic resonance imaging (fMRI). FMRI allows us to examine activity in many areas across the whole brain at the same time. This can be done while the animal is anaesthetized and also when it is awake and either awake or performing a behavioural task. The fMRI approach itself is non-invasive and can be used in humans. However we need to use an animal model because of the way we combine it with other techniques such as transcranial ultrasound stimulation (TUS). In summary, fMRI constitutes a refined method for recording brain activity.

TUS

TUS is used to temporarily disrupt brain activity in a minimally invasive manner. The ultrasound wave can be used to alter neural activity even at a distance so it can be used to alter activity even deep in the brain without affecting more superficial tissue. We employ TUS in an animal model because the amount of ultrasound stimulation we need to use exceeds the ultrasound limits that have been defined for humans. We nevertheless carefully calibrate the TUS's intensity and other features and all our tests so far indicate that its effects are fully reversible and do not cause any permanent change to the brain. TUS therefore represents a very refined technique for manipulating brain activity.

Minimally invasive neural recording and manipulation techniques.

The section on project harms, above, lists various adverse effects, that could occur during/following surgery and anaesthesia including brain haemorrhage, seizure, partial paralysis or weakness, breathing obstruction, failure of respiratory or cardiac systems, or from insertion of recording electrodes into the brain (brain haemorrhage, seizure, partial paralysis, or weakness). However, by using minimally invasive techniques for neural recording (fMRI) and manipulation (TUS) we ensure that these risks are almost completely avoided. While they remain a theoretical possibility, to date none of these adverse effects have been observed in our laboratory when these approaches have been used.

Neurophysiological recording

We will record neural activity using electrodes inserted into the brain. This approach makes it possible to record the neurophysiological activity of individual neurons or small groups of neurons albeit in a limited number of specific regions. The recording process is not, in itself, painful or harmful but it requires an opening to be made in the skull so that the electrodes can be put in place. There is, however, a risk of causing of brain haemorrhage if a blood vessel is hit when the electrode is inserted. In addition, there is a risk of infection when a skull opening has been made. For these reasons, we use an animal model to conduct the research but even still we believe that we will be able to avoid these adverse effects. Scientists use this approach when it is necessary to characterize the activity of individual neurons or small groups of neurons or when there is a need for extremely precise measurements of the timing of brain activity that are not possible with fMRI. It is for these reasons that we are using the neurophysiological recording approach in our experiments.

Restraint

In order to record brain activity, it is currently necessary for the head to be still. When the recordings are made with electrodes there is a risk of injury if the head, and consequently the brain, were to move. We therefore train animals to tolerate periods of restraint. We do this by providing positive reinforcement (food and/or juice) to the animals while they are restrained and by gradually increasing the level of restraint. Initially we train animals to sit in a specially designed box that constrains the degree to which their body can move then we train animals to tolerate their head being held still. We keep the times for which animals are restrained as short as possible depending on the type of brain activity recordings we are making

Behavioural training and juice rewards

Where possible we will use a wireless touch screen training system that is installed in the home cage. The system utilizes real-time facial recognition to start subject-specific tasks without the need for separation of the monkeys. Each animal selects their preferred time to work and chooses for how long they will do so. This is a significant refinement over previous methods of initial training. Other researchers have found that monkeys consistently use the system on a daily basis to quickly learn complex behavioural tasks.

Following the initial training animals need to become accustomed to the greater confinement needed to enable recording to be made from the brain whilst the animal does the behavioural tasks. The chair is placed in the home cage room for several days before training starts so that the animals become accustomed to it. Chair-training is based on providing rewards for entering the chair and sitting in it for increasing lengths of time. Food and fluid restriction is not used for chair restraint training

We use behavioural tasks with particular features to probe specific cognitive processes which we then relate to brain activity. Typically, these tasks involve the animal learning to respond to one stimulus or location, on a computer monitor, rather than another. if the

correct choice is made then a juice reward is given. However, by making small changes to the behavioural tasks, we can assess different cognitive processes to different degrees. For example, we might assess behavioural flexibility (objective 1) by changing which is the correct stimulus or location or by switching between stimulus identity and stimulus location being the determinant of the correct response. We might assess, information seeking (objective 2) by giving the animal access to more information about the set of features that identify a particular stimulus by responding to it repeatedly (for example, first its shape and then its colour might be revealed. We might examine acquisition and use of knowledge of task

structure to guide decision making (objectives 3 and 4) by creating sets of stimuli with shared features; when these have been learned, animals can infer which is the correct choice to take, even when they encounter new stimuli, by generlizing from past experience. We can do this not just when animals are learning from their own experience but when they learn from observing others (social learning; objective 5). We train animals to perform the tasks by gradually increasing their difficulty over time.

For example, initially it is important just to teach the animals very simple things such as to touch the apparatus used in the test. We might, therefore, teach them to touch a touch screen or a response sensor. In general, we do this by giving them a food or juice reward for making the necessary response. Over time animals learn to make more complex response – for example touching one stimulus rather than another in a specific context. In this way they gradually learn to perform specific tasks to obtain the rewards.

We give animals juice rewards to motivate them to perform the cognitive tasks when recording brain activity. In order for the juice rewards to be effective sources of motivation, we take care to ensure that animals are sufficiently thirsty to want the juice when they begin performing the task. When we are using fMRI or TUS to investigate the brain then the periods of behavioural task performance are relatively short and it is often not necessary to do anything special to ensure that animals are thirsty enough to complete the session; the juice delivery alone is sufficient to motivate enough responses from the animals for us to have usable data. Sometimes, however, we remove the animals' home cage water prior to the testing session and then give them free access to the home cage water only once they have finished. This provides a simple and straightforward way to ensure that animals are thirsty when they begin the test but in addition it ensures that the animals have enough fluid each day. When we are recording individual neuron activity, however, it may be necessary for testing sessions to be longer. In some cases, we very carefully regulate the amount of water available prior to, or following, the testing session to ensure that the animal receives most of their daily fluid while performing the task. When this is necessary, we gradually build up to such a procedure and we carefully measure the total amount of fluid received in the testing session and then after the testing session when the animal is back in the home cage. This is the approach that we try first.

Sometimes, however, this approach is not sufficient to ensure that they are motivated to perform the task. If we find that this is the case and more care needs to be taken to ensure

that the animal is thirsty at the beginning of the session, we, therefore, remove the animals' home cage water prior to the testing session. However, subsequently we give them free access to the home cage water once they have finished. This provides a simple and straightforward way to ensure that animals are thirsty when they begin the test but in addition it ensures that the animals have enough fluid each day because the animals are able to regulate their own fluid intake each day. In the past, we have found that no further steps need to be taken to enable fMRI and TUS testing sessions proceed properly.

There is one further degree of water control that might be used and this is employed when the approaches outlined are not sufficient to ensure that animals are motivated to complete the testing session. As explained above, while, it might be used during fMRI and TUS testing, it has not been needed in thepast. It is, however, likely to be needed when we are recording individual neuron activity because then it may be necessary for testing sessions to be longer. In order to ensure that animals are motivated to work throughout the session we sometimes need to carefully regulate the amount of water available prior to, or following, the testing session to ensure that the animal receives most of their daily fluid while performing the task. First, we work out the amount of water an individual animal normally drinks in a day when it has a free choice. We provide this amount to the animal during each testing

session. If the testing session finishes and the animal has not received all the fluid that constitutes its normal daily self-chosen amount then the animal is given the remainder after the end of the session. If the animal does not complete the session then the additional amount is reduced in size by 100ml. If, again, subsequently the animal does not complete the task then the amount is reduced again in the same way. If, however, the animal subsequently completes the next testing session then then amount is increased by 50ml on the first day and then another 50ml on the next day and so on. By repeated use of this procedure, we can titrate the water intake to be at the highest level that ensures good task performance. If sufficient performance is maintained with a 1000ml supplement, the animal will be returned to the procedure describe in the previous paragraph (free access to water after the testing session but not before). The absolute minimum daily fluid intake will be no less than 20ml/kg/day.

Because this is lower than frequently observed ad libitum intake levels of 30ml/kg/day, we would only use such an amount with careful monitoring of the animal's health.

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

The work cannot be done with non-mammal species because the cortical areas that are the focus of our investigation are only fully developed in mammalian species. The frontal cortical areas that are the specific focus of our study do not exist or are not developed in the same way in non-primate species such as rats and mice. As a consequence, such species cannot be used in the experiments that we plan. It has been argued that some of the frontal brain areas under investigation are, in fact, only present or fully developed in Old World primates such as the macaque and not in New World primates such as the

marmoset. The macaque is known to be capable of performance of the behavioural tasks that will be used in the programme of research but similar evidence that New World monkeys can do the same does not exist.

We cannot use animals that are terminally anaesthetized in our experiments because we do not just want to examine brain activity in isolation. Instead, we aim to relate the brain activity that we record to behaviour. In order to produce behaviour, the animals that we investigate must be awake and not anaesthetized.

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

A major aim of the approaches we use for brain recording and brain activity manipulation that we have outlined above is the reduction of harm to each animal. In addition, we carefully monitor each individual animal for signs of pain, especially in post-operative and post anaesthesia periods and administer analgesia when necessary.

Our institution and research teams are fully committed and equipped for highest quality experiments in macaques. We place special emphasis on pre- and post-operative care of the highest possible standard, including use of analgesic regimes designed in consultation with the Named Veterinary Surgeon (NVS). Animals are routinely injection trained for experimental or veterinary procedures as standard to reduce the need for restraint. Food and fluid intake will be constantly monitored and recorded to verify a stable weight and the health of the animal. Animals are also monitored daily, so any changes in their body condition, behaviour or overall health can be rapidly detected and any necessary changes to their schedules or veterinary treatments made promptly. We have developed effective methods for pair/group housing that promote the social well-being of animals. We take great

effort to ensure that monkeys are comfortable in the testing chairs (when needed), and that the tasks are interesting and motivating to the animal, by providing a variety (and choice) of preferred reward types, changing the stimuli or providing new task problems to solve. We also typically reward animals with fresh fruit following good performance during training sessions. As a result, NHPs voluntarily participate and work well on our tasks.

We also continually refine our methods to promote best practice. We survey the scientific literature closely and refine implant designs to promote biocompatibility and long-term health and stability. We have also interacted with various working groups to develop better implants and surgical approaches. We discuss our surgical approaches with human neurosurgeons and human craniofacial surgeons, and they have attended our surgeries and advised on best practice. Our electrophysiological methods are also continually refined as new technologies emerge, so that we can obtain better neural data with fewer electrodes, and fewer recording sessions; this reduces the duration an animal remains on protocol and this, in turn, reduces the risk of any adverse effects.

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

Any guidance from the Animals in Science Regulation Unit (ASRU) that is available is followed very carefully; it is usually a legal requirement that we do so. In addition, we follow the advice of the NC3Rs and the Laboratory Animal Science Association (LASA) when it is available unless it conflicts with ASRU guidance or is not approved by ASRU. We also follow any guidelines they are produced by learned societies such as the Society for Neuroscience or the Federation of European Neuroscience Associations unless it conflicts with ASRU guidance or is not approved by ASRU.

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

Many of the major advances in the 3Rs that we have made in the past have been based on our careful reading of the scientific literature and developing new methods for performing our investigations. This happened most recently when we began to use TUS to manipulate brain activity instead of making permanent brain lesions. We will therefore attempt to continue to do the same in the future.

In addition, we have learned about other advances by talking to colleagues conducting related research. For example, we have learned about techniques that have been developed for in-home training of behavioural tasks in this way. We will continue to maintain our awareness of potential refinements in this way.

We have monthly meetings with all colleagues in our institute including other researchers, veterinary staff, and animal welfare staff that have a major focus on welfare and welfare improvement. Every three to four months we have “gold standard” meetings at which we consider how best to ensure the highest welfare standards.

In some cases advances in the 3Rs are promoted by the UK’s National Centre for the 3 Rs (NC3Rs). This was the case, for example, when in-home cage training techniques were developed recently; these approaches were supported by grants made by the NC3Rs. We will hear about the development of future techniques by attending, or asking a group member, to attend the NC3Rs non-human primate research days which have usually been held annually and by reading the NC3Rs newsletter. The NC3Rs have a regional manager who attends the institute’s gold standard meetings and advises on additional welfare developments.

A retrospective assessment of refinement will be due by 22 June 2028

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. Tissue Functions of Lymphocytes

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

Diabetes, Respiratory infection and inflammation, Influenza, Multiple Sclerosis (MS, Traumatic brain injury (TBI)

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

Retrospective assessment

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

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Contains severe procedures

Objectives and benefits

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

What's the aim of this project?

The aim of this project is to understand the role of blood cells in the disease processes impacting tissues (e.g. lung, pancreas, brain, etc.). We seek to understand how these blood cells change when they are in the tissues, how they alter the disease process in those tissues, and whether we can exploit their biology to prevent or reverse disease.

A retrospective assessment of these aims will be due by 05 April 2028

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?

Intensive research over decades has given us a comprehensive understanding of the components of the immune system and their function in health and disease. However, almost all of this research has taken place on circulatory cells: blood cells or accessible tissues such as tonsils in humans, and blood, spleen, or lymph nodes in mice. Arguably, however, the most important immune reactions are those that occur in the tissues: lung infections, diabetes, autoimmunity and brain disorders are all examples of diseases of the tissues. In each of these pathologies it is thought that tissue-resident and tissue- infiltrating immune cells either contribute to pathology or protect against pathology by promoting healing, however knowledge is incomplete. Tissue-resident and tissue-infiltrating immune cells can help clear infections, but also some of these cells drive the pathology associated with infections.

Likewise, tissue-resident and tissue-infiltrating immune cells can drive autoimmunity or injury- dependent inflammation in a tissue, but can also protect against damage and even initiate repair processes. It is therefore critical that we start to understand what is different about tissue-resident and tissue-infiltrating immune cells during injury or disease pathology. Furthermore, we need to understand which features of tissue-resident cells are specific to one tissue (e.g., brain only, lung only, pancreas only) and which features are pan-tissues properties that are conserved across different tissues and different types of pathology (e.g., injury versus autoimmunity). By understanding both the unique and shared features of these cells, during different types of pathology in different tissues, we will be able to develop therapeutics that tap into this system to prevent pathology and drive a return to health.

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

Key output of new information: a comprehensive understanding of the cellular and molecular basis of tissue immune cell biology during disease processes occurring in the brain, lung, and pancreas.

Key output of new information: understanding of how tissue immune cell biology during disease processes changes with age.

Key output of resources: we will create online resources for the major datasets generated through this study

Key output of publications: we will seek to publish all results from this study

Key output of patents: where recommended by our knowledge exchange and commercialisation team we will patent findings.

Key output of products: this project will develop immune modifying reagents that modify the immune context of specific organs. These products may have potential for clinical application.

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

Other Researchers:

Our work will create a unique data resource pertaining to the functional biology of tissue-resident lymphocytes during pathological processes of the brain, lung and pancreas. This resource, through both publication and the provision of online interactive datasets, will directly inform the research of other researchers. The work will identify candidate pathways for investigation in biomedical research, and in silico data mining resources to substitute for animal research.

Industry:

Tissue-specific immune modulation remains the key objective of many drug development programs. Targeting systemic pathways not only reduces efficacy when treating organ-specific disease, but it increases the chance of unwanted side-effects, from systemic immunosuppression to off-target inflammation. Tissue-specific targeting, by contrast, increases efficacy and reduces unwanted side- effects. In this project, we will directly test potential therapeutics for the ability to modulate the disease process occurring in the tissues. Results will be commercialised, where possible, through collaboration with industrial partners.

Patients and Clinicians:

We will continue to ensure that our research findings in mouse models are translated to the human immunology context. >50% of our research publications in the past 5 years have included data derived from human samples, ensuring, wherever possible, that our results are relevant cross-species and result in translational advances. We have patents filed to cover the generation of immune-modulating substances altering tissue regulatory T cells, allowing us to directly work towards translating this pre- clinical research tool into clinical practice.

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

We will publish all data and also create online data resources of the complete dataset. This will enable other researchers to access and mine our data for incidental findings that we did not identify. We will share our research tools, methods developed and datasets freely. We will patent findings when advised that this approach will increase uptake by industrial partners.

Species and numbers of animals expected to be used

- Mice: 38250

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 because they are currently the best non-primate model for human immunology. The immune system comparison of mouse and humans shows strong parallels between cell types and functions, in both health and disease, although some details vary across the species. For any other mammalian species, the level of basic immunology knowledge is much lower, and an enormous amount of validation work would need to be run before the project could be initiated. Furthermore, many tools are already developed for mouse work, including optimised immunomodulatory drugs and processes for genetic modification.

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

In protocols 1-3, we will perform the injections and surgeries required to generate new mouse models. These surgeries are similar in nature to those involved in IVF, and while the surgery does result in mild to moderate discomfort, pathologies are generally avoided.

In protocols 4-6, we will breed and maintain mouse strains for experimentation. Protocols 4-5 will cover maintenance until a maximum of 15 months of age, covering most mice used in this study. Protocol 6 will cover a small subset of mice that will be aged out past 1 year, as some immunological processes change with age and this needs to be accounted for. Protocols 4 and 6 cover the maintenance of mouse strains where no or mild adverse effects are predicted. Protocol 5 covers the maintenance of mice that develop adverse effects that are moderate.

In protocols 7-9, pathologies of the central nervous system will be induced. Protocol 7 will induce Experimental Autoimmune Encephalitis, which is the gold-standard model of Multiple Sclerosis, with autoimmune pathology of the brain and spinal cord. This will result in severe discomfort for the mice, with ascending paralysis of the tail and hind limbs over the course of weeks, prior to euthanasia. This procedure was selected as it is highly validated as a read-out for immune modulation of autoimmune reactions in the central

nervous system. Protocol 8 will induce traumatic brain injury, using the open- skull controlled cortical impact. This will result in moderate discomfort for the mice during the days following the impact. In the weeks following impact, mice develop behavioural changes that can be observed in behavioural challenge tests. The model reflects the type of traumatic brain damage that is associated with accidents, for example, motor vehicle accidents, and allows the testing of therapeutic agents we have developed. Protocol 9 will induce concussive brain injury, using the closed-skull repetitive impact model. This will result in moderate discomfort for the mice during the days following the impacts. In the weeks following impact, mice develop behavioural changes that can be observed in behavioural challenge tests. The model reflects the type of multiple concussive disorder that is associated with victims of domestic violence, sports injuries, or accidents and allows the testing of therapeutic agents we have developed. The combination of Protocols 8 and 9 allows us to determine the shared similarities and differences between a single large impact and multiple small impacts.

In protocol 10, we will inject mice with substances to modify their immune system, using ex vivo analysis. The impact on mice will be mild, comparable to an injection, in 90% of mice. 10% of mice will also receive partial irradiation, resulting in a moderate experience.

In protocols 11 and 12, we will study the early stages of diabetes, with moderate adverse effects experienced by mice. In protocol 11, we will use diabetic mice (spontaneous or induced) and inject mice with substances to modify their metabolism (via metabolic cages and glucose/insulin testing following challenge) and immune system (using ex vivo analysis). This allows us to test therapeutics that we have developed for type 1 diabetes prevention. In protocol 12, we will use islet transplantation to study the effect of an intervention on transplant survival.

In protocol 13, we will create bone-marrow chimeras and challenge their immune system. The bone- marrow chimera procedure is very similar to bone-marrow transplantation in humans, and while it results in moderate discomfort and severe weight loss in some mice, pathologies are generally avoided. We then give mice an attenuated flu virus or a dead vaccine/synthetic immunogen. This results in some inflammation, similar in duration and severity to that of a patient receiving a vaccination. This procedure was selected due to the physiological relevance of flu vaccination to humans, as well as the highly-refined low-pathology nature of the protocol in comparison to other respiratory infection models. The protocol will also address differences between the tissue and immune system interaction observed in young and old mice, as ageing is a key parameter altering the outcome of respiratory infection in humans.

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

The majority of mice in this project (>50%) will experience no or subthreshold adverse effects. Another 25% of the mice in this project will experience only minor adverse effects, similar to the transient pain and inflammation caused by an injection.

Less than 15% of mice in this project will experience adverse effects that are rated as moderate, and 3% as severe. In most cases the moderate effects are transient, with pain, weight-loss, discomfort or minor diarrhoea, similar to recovery from a standard surgical procedure and lasting several days. Other mice will have moderate adverse effects from disease induction, traumatic brain injury or multiple concussive disorder (similar to the corresponding injuries in humans), diabetes development (similar to type 1 diabetes in humans) and exposure to attenuated influenza (similar to a human cold in severity). Severe adverse effects will be experienced by mice induced with experimental autoimmune encephalitis, to study multiple sclerosis. These mice will develop an ascending paralysis that limits mobility. The development of symptoms in these mice is necessary to achieve the scientific objective of understanding the interaction of the immune system with the tissue during disease: as the nature of interactions changes during the course of pathology, some mice need to be assessed at each of the clinical stages. Severe weight loss, in the absence of other health conditions, is also transiently observed in a subset of mice undergoing bone-marrow chimerism and antibiotic treatment. The need for a severe threshold in this protocol is to allow the reduction in total numbers of mice used, by not euthanising otherwise-healthy mice with transient weight loss prior to scientific assessment.

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)?

Total animals (mice) used: 49175

Total animals used (mild phenotype): 12335 (25%)

Total animals used (moderate phenotype): 5990 (12%)

Total animals used (severe phenotype): 1600 (3%)

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 April 2028

The PPL holder will be required to disclose:

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

Replacement

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

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

Immune responses are complex processes involving multiple cell types and interaction with the tissue microenvironment. When tissue-resident immunity is involved, further complexity is generated, with modifications to the system in primary lymphoid tissues, secondary lymphoid tissues, circulation, and the tissues. To date, an in vitro model of such a complex system is not available, and it will not be possible to develop such a model until we have a full understanding of the in vivo context we seek to model. The complex interactions between different cells of the immune system cannot be modelled adequately in tissue culture or by computational methods. This is because immune cells are highly sensitive to the environment, responding to cellular, extracellular matrix, and soluble mediator cues, which differ in minute but important ways between tissues, between locations within tissues, and even temporally within the same tissue location. Furthermore, even the successful modelling of a single anatomical location (a scientific feat not yet possible) would negate the highly migratory nature of lymphocytes, which differentiation and activate in multiple tissues, linked by complex blood and lymphatic migratory patterns. Ultimately, while we can model simple distinct processes in vitro, complex immune reactions need to be modelled in vivo.

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

We have considered the use of in silico modelling, cell line work, in vitro cultures, organoid systems and non-vertebrate animals.

Why were they not suitable?

For in silico modelling, there are no datasets currently available that cover the experimental designs needed. A key output of this project will be the generation of these datasets and in silico models. For cell line and in vitro culture work, the systems do not adequately recapitulate the complexities of immune regulation. Certain validation experiments can and will be performed in cell lines and in vitro, however the in vivo experiments described here cannot be performed with the same degree of scientific accuracy without an in vivo system. Organoid systems are showing increasingly interesting results at modelling single anatomical sites, however the study of immune responses in organoids is limited by the need for primary MHC-matched donor cells and the lack of normal vascularisation.

Added to this is the multi-organ nature of the questions here being assessed. Non-vertebrate animals do not have adaptive immune responses and therefore cannot be used in this project.

A retrospective assessment of replacement will be due by 05 April 2028

The PPL holder will be required to disclose:

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

Reduction

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

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

The numbers of mice required for the generation of modified mouse strains are based on the standard operating procedures extensive experience and literature review. The numbers of mice required for the breeding and maintenance protocols is based on estimations of mouse strain numbers, experience at sustainable colony management practice and the frequency of required genotype combinations. The numbers of mice required for individual experiments are based on power calculations and statistical modelling. We input the known statistical properties (phenotype average and variation), decide upon the minimal effect size acceptable from the experiment from a biological perspective and hence calculate the appropriate group size. The number of experiments required within each protocol is based on the assumption of all go-no go decisions being positive and successful grant funding achieved.

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

The key steps taken to reduce the number of animals being used are: 1.) use of controlled animal facilities to reduce biological and environmental variation, 2.) use of optimised internal standard operating procedures to reduce technical variation, 3.) consultation with a full-time mathematician embedded in our research group, 4.) design of experiments to allow large-scale measurements from each individual mice (e.g., parallel assessment of lymphocytes inside multiple tissue per mouse, rather than running one experiment for the brain, one for draining lymph nodes, one for peripheral immunity, etc), 5.) design of multiplexing screening experiments, such as CrispR screening, where many candidate genes can be functionally tested in the same mouse.

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 we will share mouse strains rather than generate new strains. This bidirectional exchange will reduce net mouse use during mouse generation. Breeding strategies are designed to minimise the number of mice experiencing mild or moderate

severity, e.g., by only bringing together harmful combinations of genetic modifications when required for experiments, rather than constantly maintaining the combination. We have a dedicated mathematician embedded in the group to generate mathematical models from the data that can guide future experiments with greater levels of precision. We will stock frozen stores of biological samples (bone-marrow, serum, tissue sections) so that certain experiments can be performed on previous samples rather than using new mice. Stocks will be generated using excess tissue taken down at regular experimental end-points.

A retrospective assessment of reduction will be due by 05 April 2028

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 proposed as their immunological responses bear a high degree of similarity to that of humans. The genetic background and immunoreactivity of mice is very well characterised and there are a wealth of reagents and research tools available that are compatible with mice.

Suffering will be minimised by provision of analgesia where appropriate, provision of diet-gel food on cage floors should animals have difficulty accessing water due to mobility impairment, sub-cutaneous hydration if dehydration is apparent, housing in heat room/on heat pad if temperature drops significantly (e.g. following anaesthesia).

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

The choice of species is limited by the fact that invertebrate species do not have an adaptive immune system that is comparable to humans. Mammals share key aspects of immune biology with humans and have formed the basis of the modern immunological synthesis. Immune responses change markedly with age, and thus studying adult mice is the appropriate life stage. Terminal anaesthesia is used when experiments are of sufficiently short-term (minutes to hours) to allow for it, however, when the immune

processes to be study are in the range of days to weeks terminal anaesthesia is not feasible.

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

Given the impact on the well-being of the animals, this project has carefully selected protocols where the majority are mild or moderate in severity, avoiding any studies classified as severe where possible. For the moderate or severe severity models, our work focuses on understanding the course of pathology and key pathological stages or reducing the degree of pathology. While it is necessary to induce pathology in order to understand and treat pathology, input will be encouraged from the NVS (Named Veterinary Surgeon), animal technicians, and NACWOs (Named Animal Care & Welfare Officer, responsible for overseeing the day-to-day husbandry, care, and welfare of the protected animals held at their establishment) following studies to identify possible areas for refinement. Clinical score sheets linked to humane endpoints and cumulative severity limits will be developed in collaboration with BSU staff and put in place for all diseases models.

Administration of compounds is performed according to the route and dose that minimises toxic effects. All recovery and long-term non-recovery surgery will be done aseptically to HO Minimum Standards of Aseptic Surgery. Peri-operative and post-operative analgesia will be given when necessary, using advice from the NACWO and NVS (Named Veterinary Surgeon), responsible for, monitors and provides advice on the health, welfare, and treatment of animals). Choice of analgesic, duration, and dose will be adjusted to the clinical signs observed, prioritising animal welfare, while taking into account possible impacts on the experimental plan while observing clinical score observations and cumulative severity limits.

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

All experiments which will integrate refinements from the NC3Rs (e.g., the ARRIVE guidelines), the LASA aseptic guidelines, LASA Diehl guidelines on volumes and frequency limits (Diehl et al. A good practice guide to the administration of substances and removal of blood, including routes and volumes. 2001 J. Appl. Toxicol. 21, 15-23) and the most up-to-date veterinary knowledge.

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

We will actively stay updated with our field of research through collaboration, conference attendance and reading the literature. We will take particular note of any technical advances that enable reduction, refinement or replacement in our experimental design. The local mouse facility is also a key source of knowledge, transmitting the latest

information on the 3Rs to researchers. Internal protocols are shared across the institute, enabling rapid uptake of any improvements to the method across groups.

A retrospective assessment of refinement will be due by 05 April 2028

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. Safety and Efficacy Testing of Veterinary Products

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)

Key words

Safety, Efficacy, Livestock, Veterinary, Medicines

Animal types	Life stages
Cattle	neonate, juvenile, adult, pregnant, aged, embryo
Sheep	neonate, juvenile, adult, pregnant, aged, embryo

Animal types	Life stages
Goats	juvenile, adult, pregnant
Pigs	neonate, juvenile, adult, pregnant,
Domestic fowl (Gallus gallus domesticus)	embryo, neonate, juvenile, adult
Quail (Coturnix coturnix)	embryo, neonate, juvenile, adult
Ducks	embryo, neonate, juvenile, adult

Retrospective assessment

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

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Contains severe procedures

Objectives and benefits

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

What's the aim of this project?

The aim of this project is to provide a testing service for the animal health industry to independently assess the safety and / or efficacy of a range of veterinary products, in a quality environment, prior to registration of the products for use in production livestock and birds.

A retrospective assessment of these aims will be due by 29 February 2028

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?

Veterinary product development incorporates a number of different steps. The initial stages of the work are often carried out in the developers own facilities, however before veterinary products can be licensed for commercial use, or in order to add additional specifications to a current marketing authorisation, it is first necessary to confirm the safety and efficacy of the products, in the target animals, in a controlled independent quality environment. As a contract research organisation (CRO), our role is to provide an independent assessment of the safety and / or efficacy of products in line with appropriate guidelines such as the European Pharmacopoeia monographs, VICH guidelines (including VICH GL 44 - Target Animal Safety - Biological) and Regulation (EU) 2019/6 of the European Parliment and the council of 11 December 2018 on veterinary medicinal products and repealing Directive 2001/82/EC. Studies carried out at CRO's are vital to the successful registration of veterinary products.

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

At the end of this project it is expected that a number of the products assessed under the licence will progress to registration by the UK / EU veterinary medicines boards, or at least data generated under this project licence will be included in dossiers prepared and submitted to the authorities for review.

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

The benefits of the outputs will be the provision of safe and effective veterinary products for use in the agricultural industry to reduce / prevent suffering of farmed livestock. This will include disease control products such as vaccines and other novel immunomodulatory products as well as therapeutic products, such as antibiotics and anti-inflammatory products, which will allow effective treatment of disease / injury.

The outputs may include novel products, where no similar products are on the market, optimised products, for which other similar, but less effective products are already on the market, or a wider range of products of a similar type which will allow increased selection, and potentially will aid in reduction of antimicrobial resistance. It may also include additional label claims for products already on the market to include a broader age range for use, use in pregnant animals or against a wider range of pathogens.

Other benefits may include a better understanding of the pathology of specific diseases as a result of development of pathogen challenge models and development of new assays to support detection of the pathogens. This information may be shared with the wider scientific community.

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

The majority of the work carried out under this project licence will be covered by confidentiality agreements which limit public disclosure of the results of the work, prior to registration, unless approved by the clients. On occasions, however, with client approval or collaboration, it is possible that the results of some studies can be shared with the wider scientific community, prior to registration of the products. Where possible information generated during studies, specifically in relation to challenge models which may be of interest to the wider scientific community and where the data may result in a better understanding of a particular disease or in-vitro assay, refinement of an established model or reduction in the numbers of animals required to produce viable data, will be disseminated to the wider community.

Species and numbers of animals expected to be used

- Cattle: 1500
- Sheep: 700
- Goats: 100
- Pigs: 2500
- Domestic fowl (Gallus gallus domesticus): 4000

- Quail (Coturnix coturnix): 50
- Other birds: 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.

The type and age of animals used in studies will be determined by the product that is being tested and the intended label claims for the product following registration. For example, a product destined for use in pregnant animals will, at some point, require testing in pregnant animals to confirm both the safety and efficacy of the product.

For each listed species there may, on occasions, be a requirement for both product safety testing or challenge model efficacy testing. For efficacy testing there is a variety of species specific challenge models available for each species. Some examples for each species are shown below;

Bovine - Escherichia coli, Bovine Viral Diarrhoea Virus (BVDV), Bovine Parainfluenza virus Type 3 (PI3), Bovine Herpes Virus Type 1 (BHV-1), Mannheimia haemolytica, Pasteurella multocida, Mycoplasma bovis, Cryptosporidium parvum, Streptococcus uberis, Staphylococcus aureus

Porcine -- Streptococcus suis, Pasteurella multocida, Actinobacillus pleuropneumoniae, Salmonella typhimurium, Escherichia coli, Porcine Reproductive and Respiratory Virus (PRRSV).

Poultry -- Salmonella Enteritidis, Campylobacter jejuni

Ovine -- Neospora caninum, Toxoplasma gondii, C. parvum

Other models are also available and additional models may be developed during the course of the licence as indicated in protocol 4 (challenge model development).

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

Animals used under this project licence will be administered test products by a range of different routes, in line with the intended label claims for the product following registration. These will include; intramuscular, intravenous, subcutaneous, intranasal, intradermal, intraperitoneal, topical, oral, intramammary, or transdermal routes. Challenge material (live bacteria, virus or parasites) may also be administered by these routes as required to induce clinical / sub clinical disease to the required level. In addition to the procedures detailed above, a range of samples will be collected from animals on individual studies in line with the study design and set objectives. Sample collection will include; blood

samples, faecal samples, milk samples, nasal swab samples, nasopharyngeal swab samples, laryngeal swab samples and bronchial alveolar samples.

The maximum volumes, frequency and routes of administration and sampling will be in line with local guidelines. Test items will be administered at volumes and frequencies that are not expected to result in more than transient and mild harms, although safety testing may on occasions present more acute unexpected reactions in a small number of animals.

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

The majority of animals undertaking procedures under this licence will experience only mild to moderate clinical signs as detailed below. On a small number of occasions however it is possible that some animals will experience reactions which are classified as substantial. These will however generally be few in number in protocols 1 (safety of products), 2 (efficacy of products), 4 (validation of challenge models), and 5 (in-vivo propagation of challenge material) but with a possible increased frequency in protocol 3 (efficacy of products in a porcine Streptococcus suis disease model).

For example;

1) Mild reactions would include transient pain at the time of blood sample collection or administration of injections.

2) Moderate reactions could include painful swelling at the injection site or high temperatures on more than one occasion.

3) Severe reactions could include anaphylactic shock or sudden death.

While the products under test are in the advanced stages of development, there is still a small risk that more severe reactions may occur in a small number of cases. Such cases will be managed in conjunction with animal care and veterinary staff to ensure appropriate endpoints are applied.

In the sections below the adverse effects expected to occur following administration of products under this licence are detailed.

1. Mild discomfort and inflammation may be associated with sites of injection / administration of Test Materials or challenge materials or sites of sampling.

2. A transient pyrexia may occur in some animals following administration of veterinary products or following challenge administration. in general such pyrexia is not expected to last for more than 48 hours post administration although in some animals this may be extended, especially following challenge administration.

3. The infection of animals with pathogens can result in a range of symptoms depending on the pathogen in use and the age and status of the animals. Effects on the

animals can range from respiratory signs (pyrexia, increased respiratory rate / effort, nasal / ocular discharge, coughing) to enteric signs (pyrexia, reduced demeanour, abnormal faeces) to lameness (reduced mobility, joint swelling etc). All models used will be validated prior to use in studies with clear endpoints. Body weight loss / loss of condition is also expected in a number of challenge models in use at Moredun but will be carefully monitored in line with local guidelines for endpoints.

This licence contains one protocol which has is classed as severe. This protocol is specific for experimental Streptococcus suis disease in pigs. While the majority of the animals which are challenged with S. suis will show mild/moderate progressive signs of the disease, some animals may develop an acute reaction which can result in sudden death. It is not possible in a small number of cases, to predict / prevent sudden death despite frequent monitoring, therefore a severe protocol has been produced to identify this risk.

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 this licence severity in all species will range from under threshold to severe depending on the models being used. The majority of the animals will experience only mild or moderate severity, with all animals under supervision of scientists, animal care and veterinary staff throughout studies to ensure that the welfare of the animals is maintained and endpoints are monitored and acted upon. Only a small number of animals, for one specific model (porcine Streptococcus suis model), are expected to reach severe levels, however with effective monitoring during critical phases, this number is expected to be extremely low.

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 29 February 2028

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 regulations for registration of new veterinary products currently still require at least a proportion of the testing to be carried out in in-vivo models. While it is likely that the number of studies requiring the use of animals will reduce over the duration of this licence, it is still expected that in-vivo testing will continue to be required for the majority of products being assessed in the coming years. For safety studies there will continue to be a requirement for in-vivo testing as currently it is not possible to determine the safety of most products in an in-vitro model, with the exception of products which have the same active ingredients / excipients (i.e. generic products) where some aspects of both safety and efficacy testing may be waived with approval of the regulatory authorities. Some efficacy testing can however be carried out in in-vitro models (such as minimum inhibitory concentration MIC) or minimum bacterial concentration (MBC) testing) which will reduce the use of animals in some cases.

Discussions will be carried out with clients to determine whether there is a need for invivo testing or whether some or all can be replaced by invitro assays. The Establishment Animal Welfare and Ethics Review Board (AWERB) will review all studies to ensure that no appropriate replacements are possible.

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

Non animal alternatives will be considered for all studies carried out under this licence. The type of non-animal alternatives which will be considered will be dependant on the individual studies being reviewed.

Why were they not suitable?

Non animal alternatives will be considered as unsuitable if they do not allow the specific regulatory requirements for individual products to be met.

A retrospective assessment of replacement will be due by 29 February 2028

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 which are expected to be used has been based on usage under previous licence (PFA7E7AD6) and based on estimations of the type of work that is likely to be contracted in the coming years. Based on the previous licence there has been a reduction in the number of sheep / goats that are expected to be used and removal of horses / ponies as well as mice. The usage of cattle is expected to remain consistent or increase slightly, from previous years and we expect to see an increase in the number of both pigs and poultry since new infection control initiatives for these species are high priority for the animal health industry due to a reduction in the use of antibiotics.

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 used in individual studies under this licence will be determined based on regulatory requirements (where monographs / guidelines detail a specific number of animals that should be used) as well as power calculations determined during challenge model developments. Statistical advice will be sought when reviewing model validation data and specific study objectives to determine the appropriate number of animals which will be used on studies, to increase likelihood of objectives being met, while reducing the number of animals that are used.

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 provide data which can be analysed to provide more accurate assessments of the number of animals that will be required to meet objectives for larger studies. A review of published data will also be carried out to advise on optimisation of animal numbers for specific disease models. Informed animal selection will be used to limit variability and improve likelihood of objectives being met (for example type age / weight ranges, specific breeds etc).

A retrospective assessment of reduction will be due by 29 February 2028

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.

A range of different animal models will be used this licence which can broadly be categorised as ; enteric, respiratory, reproductive, mammary or systemic. For each species a range of different validated challenge models are available for use. Examples of species specific models are detailed below. This list is not exhaustive for each species / category and additional models may be added following development (detailed in protocol 3 - challenge model validation). Any new models added to the list will be reviewed by the local animal welfare and ethical review board, following development, to determine suitability for use.

Enteric - Pathogens / parasites which affect the gastrointestinal system resulting in clinical signs such as diarrhoea, dehydration, depression, loss of body weight / condition, pyrexia, loss of appetite.Generally signs would be mild / moderate severity. Routes of infection for enteric models would normally use the oral route (gavage) or by using stomach tubes to deposit challenge material directly to the stomach.

- Bovine -- Escherichia coli, Cryptosporidium parvum, nematode infections including Ostertagia ostertagi, Cooperia oncophora, Trichostrongylus spp.
- Porcine -- Escherichia coli, Salmonella typhimurium, Ascaris suum
- Ovine -- Cryptosporidium parvum, nematode infections including Haemonchus contortus, Teladorsagia circumcincta, Trichostrongylus spp.
- Poultry -- Campylobacter jejuni, Salmonella Enteriditis

Respiratory - Pathogens / parasites which affect the respiratory system resulting in clinical signs such as pyrexia, nasal discharge, ocular discharge, coughing, increased respiratory rate, increased respiratory effort, depression, loss of appetite, loss of body weight / condition. Generally signs would be mild / moderate severity. Routes of infection for respiratory models would normally include intranasal, intratracheal or using nebuliser masks.

- Bovine -- Mannheimia haemolytica, Pasteurella multocida, Bovine Herpes Virus Type 1 (BHV-1), Mycoplasma bovis, Parainfluenza virus type 3 (PI3), Dictyocaulus viviparus
- Porcine - Mycoplasma hyopneumoniae, Porcine Reproductive and Respiratory Syncytial virus (PRRSV), Actinobacillus pleuropneumoniae)

Mammary - Pathogens / parasites which affect the mammary glands resulting in clinical signs such as pyrexia, swelling, discolouration, pain / discomfort, reduction in milk volume produced, loss of appetite, loss of body condition, depression. Generally signs would be

mild / moderate severity. Routes of infection for mammary models would include direct inoculation of the mammary gland via teat canals using teat cannula.

- Bovine - Staphylococcus aureus and Streptococcus uberis mastitis
- Ovine - Staphylococcus aureus and Streptococcus uberis mastitis

Reproductive - Pathogens / parasites which can affect the reproductive success of animals including abortion, reduced litter size, reduced litter weight, health of offspring. Generally signs for pregnant animals would be mild (no lasting effect following abortion or production of reduced / poorer quality litters), however the effect for the offspring is obviously more acute. Routes of infection for reproductive models would normally use the intravenous, intramuscular or subcutaneous inoculation routes.

- Bovine -- Neospora caninum, Bovine Viral Diarrhoea virus (BVDV)
- Ovine -- Neospora caninum, Toxoplasma gondii, Chlamydia abortus
- Porcine - PRRSV

Systemic - Pathogens / parasites that have a systemic effect on the animals which include clinical signs such as depressions, lameness, central nervous system abnormalities, loss of appetite, loss of body weight / condition. Generally signs would be mild / moderate severity., however in some cases signs can be acute / severe and without intervention can result in death. Routes of infection for systemic models would normally use intranasal, intravenous or subcutaneous routes.

Animals will be challenged by the route or routes most appropriate for the individual models in order to induce the required level of clinical disease, sufficient to satisfy regulatory requirements. Where appropriate a more natural, less invasive route of infection will be utilised (such as intranasal delivery), but in order to induce the required level of disease for some pathogens it will be necessary for more invasive routes such as intratracheal or intravenous administration to be utilised. During the course of the licence it is intended that more natural challenge models will be investigated / validated to replace the more invasive models.

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

The products which are being assessed during this licence are specifically aimed at veterinary livestock and in order to meet the regulatory requirements for product development, at the clinical stage, it is necessary that the products are tested in the stage and species of animals for which the product is intended to be used.

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

For new challenge models, small pilot studies will be carried out to allow assessment of the most appropriate infectious dose and volume to induce the required level of disease.

During these studies clinical data will be generated which will allow determination of endpoints which will be used for future efficacy studies. For current models, clinical data will be assessed on completion of each study to determine whether further refinements to the models are possible. This may include refinements to challenge concentrations, routes or volumes, changes to individual pathogen isolates or changes to clinical or welfare observations.

Refinements to procedures including veterinary product or challenge administration, sampling techniques or other licenced procedures will be undertaken based on local or national guidance, or following review of publications.

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

To ensure that the studies are carried out in line with the established best practice guidance, the following publications / resources will be utilised.

- Home Office code of practice
- Relevant articles from the 3Rs website
- Laboratory Animal Science Association (LASA) Website / publications
- Large Animal Research Network (LARN) Webiste / publications
- Animal Research: Reporting of In Vivo experiments (ARRIVE) website / guidelines
- Norecopa Website
- NC3Rs Experimental Design Assistant
- European Pharmacopoeia

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

The Establishment AWERB committee in conjunction with the 3R's committee actively works on refinement of animal studies, by external discussions with other research establishments, by maintaining ongoing communication with regulatory and ethical review bodies, by review of papers / publications, attending relevant conferences as well as internal reviews and implementation of refinements developed at the Establishment.Prior to the conduct of animal studies, the Establishment will ensure that the individual protocols cannot be refined further with regard to the number of animals, the scientific objectives, the statistical significance and the expected outputs.

For new procedures or models, or where data is not available on the use of the products in animals, it may be necessary to conduct validation studies to generate data relating to disease model success, and onset, duration and severity of clinical symptoms. This data will then be analysed by the Establishment in conjunction with the NVS/Animal care staff

and statisticians with a view to refining the model / study for future use for determining clinical endpoints and generating power calculations for future studies.

On completion of studies, further reviews are undertaken to assess the quality of the data produced, the welfare of the animals on the study and to determine whether any future refinements to the design of future studies can be incorporated.

In all cases, refined animal experimentation will be done under best practice principles to alleviate or minimise potential pain, suffering or distress. Animal handling and sacrifice will be conducted using methods approved by current legal directives. Personnel working with animals will be trained in good practice and housing facilities will fulfil all requirements observed by current legal directives. Policies for animal husbandry and challenge infections will be monitored closely.

In all cases, the minimum number of animals will be used to obtain the most informative statistically significant data. Reduced animal experimentation will be achieved by optimized methodological design. Power calculations will be used for representative trial and immunological experiments, keeping in mind that these will continue to be refined and optimized throughout the project. As data becomes available from efficacy trials, animal numbers required to yield statistically significant and meaningful data will be reviewed using statistical methods applied by suitably qualified statisticians.

A retrospective assessment of refinement will be due by 29 February 2028

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 Neurodegenerative Diseases

Project duration

5 years 0 months

Project purpose

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

Key words

imaging, tool compounds, experimental autoimmune encephalomyelitis, drugs, amyotrophic lateral sclerosis

Animal types	Life stages
Rats	juvenile, adult
Mice	juvenile, adult

Retrospective assessment

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

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Contains severe procedures

Objectives and benefits

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

What's the aim of this project?

The aim of this project is to provide unique in vivo/ex vivo/in vitro data using imaging and related technologies in animal disease models of severe severity harms, on an "as and when required" basis for this establishment and on behalf of Sponsors. 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 04 May 2028

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?

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 two disease areas in which we will employ imaging techniques, namely multiple sclerosis (MS) and amyotrophic lateral sclerosis (ALS) (also known as motor neurone disease). There are currently no cures for these diseases and the benefit of current treatments is limited. Furthermore, ALS is difficult to diagnose. Therefore, there is an unmet need to develop new and better biomarkers of disease and effective 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 expression of a drug biological 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 few established imaging tool compounds available that may be used to investigate aspects of multiple sclerosis and amyotrophic lateral sclerosis pathology. These compounds have limitations and are not suitable in all cases. The data obtained through the work under this project licence will support the development of new imaging tool compounds for multiple sclerosis (MS) and amyotrophic lateral sclerosis (ALS) by providing important information on their distribution and binding properties within the body. 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 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 measured 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. As the tool compounds and drugs are being evaluated for later use in MS or ALS, animal models, displaying aspects of the relevant disease, will be needed. This is because 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. 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 multiple sclerosis or amyotrophic lateral sclerosis. 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 a Sponsor to assess the suitability of their potential drugs at an early stage in development. Where the Sponsor has collected previous data 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 such as quantification methods. This will provide a unique opportunity for our Sponsors, collaborators and/or ourselves to perform pharmacological, anatomical, and physiological studies in humans.

Additionally, it will support our Sponsors in allowing them to assess 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 MS and ALS patients and as such have potential to benefit a significant patient population. These disease areas are of particular interest to our Sponsors 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):

Experimental Autoimmune Encephalomyelitis (EAE):

Rodent models of EAE are the most commonly used experimental models for multiple sclerosis (MS) research as they mimic many aspects of the clinical disease. They involve

administering substances that cause inflammation within the central nervous system resulting in degradation of the fatty tissue that surrounds and protects the neurones. They also make the blood brain barrier 'leaky' thus allowing the influx of further inflammatory cells into the brain. The rodent models inevitably experience clinical signs, which cannot be avoided as they are directly driven by the biological mechanisms under study. The animals start to exhibit progressive paralysis and weight loss, starting with a flaccid tail and eventually leading to complete hind limb paralysis, which may last for 1-3 days, and then the animals start to recover. During the paralysis stage, the animals are able to move around using their fore limbs and will be provided with food on the floor of their cage to make feeding easier. Saline injections will also be administered to avoid dehydration.

The effectiveness of novel treatments or the evaluation of novel imaging tool compounds for MS and related diseases cannot be fully characterized without in vivo testing in these types of models as the target binding sites are either not present or are present at very low concentrations in naïve animals. There are a number of different models of EAE that have been used worldwide, exhibiting various pathological characteristics of EAE and different levels of disease severity. However, a single rat model and 2 mouse models will be used here as they are well established and well characterised by paralysis and inflammation of the central nervous system. Two mouse models will be used as they induce EAE by different inflammatory mechanisms and therefore, the choice of which model to use will be dependent on the experimental question.

The rat model is self-limiting, and the rats recover after 1-3 days, without relapse. The mouse models partially recover from the paralysis after the initial peak of disease, but this recovery is limited to a modest improvement in hind limb movement and around 25% of the mice will exhibit a relapse in disease severity, although it is expected that the animals will exhibit some movement in one or more of the hind limbs at this stage. Any animal exhibiting a significant level of suffering will be humanely killed. All animals will be humanely killed when the experimental goal or, if sooner, a humane endpoint has been reached. The relapsing-remitting nature of these mouse models is highly indicative of the early human condition, which is also associated with relapsing-remitting episodes of symptoms, and they are, therefore, very valuable models for exploring the progression of the disease.

The models to be generated under this protocol typically exhibit a consistent disease onset and severity, together with a short clinical course. The model to be used for individual projects will be the most appropriate to answer the experimental question. Both the rat and mouse models allow the possibility of evaluating both prophylactic and therapeutic treatments and also, in the case of the mouse models, allow the effect of treatments given during the remission phase on subsequent disease relapses to be explored. In order to minimise adverse effects as much as possible, the duration of experiments will be limited to the minimum required to achieve the aims of the study and refinement measures such as fluid replacement and extra bedding will be used.

Amyotrophic Lateral Sclerosis (ALS) (also called Motor Neurone Disease):

Genetically modified mice that are well established and internationally accepted models of ALS may be used. Approximately 5-10% of all clinical ALS cases are familial, with one of more genetic mutations being passed down by a parent. The remaining, sporadic, cases are also linked to genetic mutations that may be both contributing to and/or causing the disease. The mouse models to be used under this licence have genetic mutations that are commonly seen in ALS patients and are, therefore, highly relevant to the disease process. They are some of the most studied ALS mouse models world-wide and have been used to explore many potential drug-target binding site mechanisms. Each model allows an individual mechanism of disease to be explored and therefore, the choice of model to be used will be dependent upon the requirements of the individual studies requested by our Sponsors.

Both the human disease and mouse models are associated with neuronal cell loss leading to progressive paralysis. The clinical signs cannot be avoided because they are directly driven by the biological mechanisms under study. Young animals will be used whenever possible to minimise the development of clinical signs as a result of a more severe disease. However, depending upon the experimental question, it may be necessary to use older animals with more severe disease in some cases. For example, where we are investigating the presence of a novel drug biological target over the time course of the disease and imaging is required during the pre, early and late symptomatic phases of the disease. In this case, some animals are expected to exhibit paralysis and weight loss. Where adverse effects do occur, these will be carefully managed to minimise their effect on the wellbeing of the animal. All efforts will be made to minimise suffering of the animals, including regular observation and monitoring, together with refinements such as soft food being placed on the cage floor for easy access and saline injections to prevent dehydration. In all cases, the suffering of animals will be kept to a minimum by using the genetically altered model with the mildest disease severity for the shortest experimental duration possible.

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. 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. Much of our work is conducted on behalf of Sponsors, however, where results are not commercially sensitive, data will be submitted for publication in peer-reviewed scientific journals.

Previously, the work carried out under the authority of our last 2 project licences led to the publication of 7 manuscripts in several high ranking scientific journals including Molecular

Psychiatry and Angewandte Chemie. We also presented the outcome of our work on 16 occasions at international conferences including BrainPET, Society of Nuclear Medicine and Molecular Imaging, and Neuroreceptor Mapping.

Species and numbers of animals expected to be used

- Mice: 550
- Rats: 300

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 and rats 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 that shows a relatedness to humans and the disease models that we will use in the areas of ALS and MS are well established in mice. Rats are immediately after mice in the evolutionary tree and will also be used for MS studies, as there are well established disease models in rats for MS.

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 limitations of preclinical imaging, rats as larger species, provide a better opportunity to quantify biological processes within regions of organs. In addition, the blood volume of rats 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 or degree of senescence 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 or adult animals may be used under this licence depending upon the disease model and study objectives.

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

There are two protocols under this licence. Under protocol 1, a rat or mouse disease model of multiple sclerosis will typically be generated by the subcutaneous administration of substances under general anaesthesia. Mice may also receive 1-2 intraperitoneal

injections of another substance whilst conscious to complete the model. The substances administered cause inflammation and neurodegeneration in the brain.

The animals may also be placed under general anaesthesia on 1-3 other occasions and undergo the administration of a substance(s) by intravenous injection and imaging on a scanner especially designed for scanning rodents. Imaging sessions will typically last for up to 3 hours. Other procedures may be carried out on occasion such as blood sampling, the administration of additional substances and/or behavioural assessments. Animals will typically be on study for up to 1 month and will be killed at the end of the series of procedures.

Under protocol 2, a genetically modified mouse model of amyotrophic lateral sclerosis may be placed under general anaesthesia on 1-3 occasions and undergo the administration of a substance(s) by intravenous injection and imaging on a scanner especially designed for scanning rodents. Imaging sessions will typically last for up to 3 hours. Other procedures may be carried out on occasion such as fasting of the animal overnight prior to the imaging, blood sampling, the administration of additional substances, single housing and behavioural assessments. Animals will typically be on study for up to 6 months and will be killed at the end of the series of procedures.

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

The development of clinical signs is expected in the animal models of disease to be used under this licence as it is not possible to separate clinical signs from the biological changes required for the model induction and progression of disease. The disease models to be used involve alterations to the brain, either by experimental procedures or by altered genetics. These alterations will typically lead to changes in the movement and behaviour of the animals, including the gradual onset of hind limb paralysis. All animals will be closely monitored at least daily until the onset of clinical signs, after which they will be monitored more frequently, at least every 7h whilst an animal experiences complete hind limb paralysis.

Experimental Autoimmune Encephalomyelitis:

Both a rat model and two mouse models of multiple sclerosis may be used. The rat model will typically start to experience a flaccid tail at around 10-12 days after administration of a disease inducing substance(s). The effects of the substance(s) will then progress, resulting in abnormal stature and eventually paralysis of the hind limbs. In some animals, the onset of disease may result in signs of ill- health such as hunched posture, rough hair coat, unkempt appearance or lethargy. During this time there will also typically be significant body weight loss (<25%). The peak of disease is expected to occur at around 3 days after onset of symptoms and last for 1 to 3 days, then the rats will start to recover. All rats are expected to recover fully within 20 days after induction.

Within 9-14 days after the administration of a disease inducing substance(s), the mouse models of multiple sclerosis will typically start to experience body weight loss and the progressive onset of neurological signs as described above for rats. They may also show signs of feeling unwell such as having a hunched posture, unkempt appearance or lethargy. The peak of disease is expected to occur at around 3-5 days after onset of symptoms and last for 1-3 days and then the mice should start to recover. Approximately 25% of the mice are expected to relapse and experience partial paralysis a second time. Animals would be kept for no more than 4 weeks following the induction of disease.

The administration of the disease-causing substances may sometimes result in ulceration or necrosis (skin cell death) at the site of administration. We will limit the possibility of ulceration or necrosis by using appropriate, sterile techniques but if any ulceration/necrosis develops, it will be treated under the advice of the named veterinary surgeon.

Amyotrophic Lateral Sclerosis (ALS):

The genetically altered mouse models of ALS to be used under this licence will typically develop progressive paralysis in one or more limbs, with varying degrees of age of onset and disease progression rates. Animals are also likely to experience significant body weight loss (up to 25%) and other signs of ill-health such as muscle atrophy, abnormal limb reflex, rough hair coat, hunched posture, and piloerection.

Following the onset of paralysis in both the EAE and ALS models above, urine moisture might appear on the hindlimbs of the animals. Left untreated, urine moisture could result in urine 'burn' and skin lesions. Urine moisture will be treated by clipping the hair, applying warm water to remove the urine, and gently blotting dry. Any skin lesions would be treated under advice from the named veterinary surgeon.

Animals exhibiting the onset of limb paralysis will be given soft food on the floor of the cage, long sipper tubes to facilitate drinking water and extra bedding. The severity of their disease will be closely monitored using a defined scoring system to ensure that it can be clearly recognised when a humane endpoint has been reached. Animals with complete hind limb paralysis will be given at least daily injections of saline to prevent dehydration and will be fed by hand where necessary. If the paralysis was to continue for 3 days (ALS animals) or for 4 days (EAE animals) or impede an animal moving forward or if the animal was unable to right itself from being placed on its side, then the animal would be humanely killed. Any animal exhibiting ≥25% body weight loss would also be humanely killed.

The animals may undergo further procedures such as the administration of substances, anaesthesia, imaging, blood sampling or behavioural assessment, however, these procedures are expected to result in no more than mild transient pain or distress and no lasting harm to the animals. Some models of ALS may be fasted overnight and/or singly housed. Single housing will typically be for up to 5 days at a time 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.

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	0
	Mild	0
	Moderate	40
	Severe	60
Rat	Non-recovery	0
	Mild	0
	Moderate	30
	Severe	70

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

- Killed

A retrospective assessment of these predicted harms will be due by 04 May 2028

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 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 04 May 2028

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?

As a Contract Research Organisation, the number of animals that we use will depend upon the studies performed on behalf of Sponsors over the duration of the licence. The number of animals to be used for each protocol has been estimated based upon previous experience of typical study designs and number of sponsor requests, together with predicted growth in both study requests from our existing sponsors and growth in additional new sponsors. The number of animals used for each type of study (e.g. occupancy, biodistribution) 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 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 requested by our Sponsors, and we always use the minimum number of animals for our studies, whilst ensuring that robust scientific data are collected. Where permitted by our Sponsors, 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, the experience of our Sponsors and/or the literature may be used to help establish animal numbers.

In the event that a Sponsor requests the use of a higher number of animals than we would typically use, the rationale for this would be requested from the Sponsor and a discussion would be carried out between the Sponsor, study leads and mathematicians in order to ensure that the rationale is robust.

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 or 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 04 May 2028

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 rodent disease models to answer key experimental questions in the areas of multiple sclerosis and amyotrophic lateral sclerosis (please see below). These models are widely accepted and used by international experts in the relevant field.

Experimental Autoimmune Encephalomyelitis (EAE)

The EAE model is a gold standard model that has been used in multiple sclerosis (MS) research for over 50 years. EAE is induced in rats and mice by the administration of substances that cause inflammation within the brain leading to the animals experiencing the progressive onset of paralysis in the limbs similar to that seen in multiple sclerosis patients. Recently, a genetically modified mouse model has been developed that has optic

nerve damage but does not manifest paralysis and is considered to be an alternative model for MS. However, this model is not suitable for our purposes. We are aiming to develop novel imaging tool compounds for use in clinical studies using imaging techniques such as positron emission tomography and single photon emission computed tomography. Due to the spatial resolution limitations of these techniques, it may not be possible to reliably detect optic nerve damage in rodents. However, the effects of EAE on the brains of rats and mice may be studied using these preclinical imaging techniques.

Due to the induction of inflammation within the brain in the EAE model, approximately 30% of animals are expected to experience neurological effects of a moderate severity, i.e., limp tail, partial hind limb weakness. In 70% of animals, we expect a more severe effect including complete hind limb paralysis. The peak of disease will last for 1-3 days, and animals should remain alert and able to feed. The animals will then start to recover from the symptoms. Rats will recover fully by day 20 after induction of EAE. However, around 25% of mice will show a relapse of symptoms after an initial partial recovery, at around 20-27 days post induction of EAE. We will closely monitor the wellbeing of all animals and apply a scoring system to assess disease progression. Supportive treatments such as soft food and saline injections will be provided that will aim to minimise weight loss and dehydration. Any animal exhibiting a significant level of suffering will be humanely killed. All animals will be humanely killed when the experimental goal or, if sooner, a humane endpoint has been reached.

Amyotrophic Lateral Sclerosis (ALS)

Genetically altered mouse models of ALS may be used that mimic the genetic mutations seen in the clinical disease. As in the clinical disease, these mice typically develop progressive paralysis in one or more limbs, together with other signs of motor impairment such as abnormal stance. They may also exhibit signs of pain or distress such as rough hair coat and weight loss. Unfortunately, the clinical signs are not mechanistically separable from the biological mechanisms involved in the disease progression. Zebrafish may be used as an alternative, less severe model to study aspects of ALS, however, there are significant limitations to imaging these animals in relation to the resolution of the scanners and quantification of the data.

The suffering of the ALS mice will be kept to a minimum under this licence by using the genetically altered model with the mildest phenotype for the shortest experimental duration possible. All efforts will be made to minimise suffering of the animals by using young animals before advanced disease occurs, wherever possible, and where adverse effects do occur, these will be carefully managed to minimise their effect on the wellbeing of the animal. We will closely monitor all animals and apply a scoring system to assess disease progression. Supportive treatments such as soft food and saline injections will be provided that will aim to minimise weight loss and dehydration of the animals. Any animal exhibiting a significant level of suffering will be humanely killed. All animals will be humanely killed when the experimental goal or, if sooner, a humane endpoint has been reached.

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 described above for EAE 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 transient 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 Sponsor 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, to facilitate the administration of substances to the CNS in mouse models of ALS, will be conducted under

aseptic 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?

Rodent models of disease will be used to replicate the pathology of human disease. 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. Terminally anaesthetised animals cannot be used as the disease models develop over time.

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 and Sponsors.

All animals will be provided with environmental enrichment 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 using temporary cannula. 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 and weighed at least twice weekly. The severity of neurological signs will be assessed daily using a defined scoring system. Animals displaying adverse effects will be observed more frequently, at least twice daily and weighed once daily. Once animals display complete hind limb paralysis, they will be observed at least every 7 hours. Where an animal starts to show partial paralysis of the hind limbs, they will be provided with soft food gel on the cage floor for easy access.

Long sipper tubes will also be provided to facilitate the drinking of water. When complete paralysis of the hind limbs has been reached, at least daily injections of saline will be given

to prevent dehydration. Animals with significant weight loss (>10%) will be provided with warming. Hand feeding will also be carried out as necessary.

Any cage bedding and/or nesting material and/or enrichment material will be changed if found to be wet to the touch. Urine moisture will be treated by clipping the hair, applying warm water to remove the urine and gently blotting dry.

During any 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 undergo post anaesthetic oxygenation and 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.

The possibility of ulceration/necrosis at the adjuvant administration site in the EAE models will be limited by using aseptic techniques and using smaller volumes.

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. Although there is no specific guidance published covering the 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 04 May 2028

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. The Development and Optimisation of Antimicrobial (Antibacterial and Antifungal Agents) to Address the Challenge of Infectious Diseases and Antimicrobial Resistance

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

Antimicrobial resistance (AMR), Pharmacodynamics, Antibacterial, Antifungal, Infection

Animal types	Life stages
Mice	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?

The aim of this project is to establish the pharmacokinetic and pharmacodynamic (PK-PD) relationships for new antibacterial and antifungal agents that enable them to progress from the laboratory to first-in-human, early and late phase clinical studies.

A retrospective assessment of these aims will be due by 03 February 2028

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?

New antimicrobial agents and therapeutic strategies are urgently required to address the societal and biomedical crisis of antimicrobial resistance (AMR). An in-depth understanding of the PK-PD properties of a new antimicrobial agent are a regulatory requirement. PK-PD enables the right regimen (i.e. antimicrobial dose and schedule) to be studied in patients the first time. PK-PD substantially de- risks antimicrobial drug development programs.

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

This licence will enable multiple (approximately 20-25) PK-PD packages that support the dose, schedule and indication of new antibacterial and antifungal agents against target pathogens that represent an unmet medical need. These outputs will appear as study reports (for sponsors), which will be submitted to regulatory agencies (Medicines and Healthcare Regulatory Agency (MHRA),

European Medicines Agency (EMA); Food and Drug Administration (FDA)) as part of dossiers supporting filing of new agents.

Wherever possible and appropriate, new information will be presented in national/ international scientific meetings and published in peer reviewed literature. Typically, the outputs of this program of work will include (but will not be necessarily limited to)

- Detailed understanding of the pharmacokinetic and pharmacodynamics of new antimicrobial agents

- Identification of antimicrobial regimens suitable for further study in Phase I-IV clinical studies

- Decision support for setting in vitro susceptibility breakpoints (i.e., whether an antibiotic is classified as “susceptible” or “resistant”)

- Evidence of activity of new antimicrobials against specific resistance mechanisms

- Evidence of combining antimicrobial agents to maximise effect and prevent the emergence of antimicrobial resistance

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

The principal beneficiaries include sponsors (biotechnology companies, small pharma, large pharma), not for profit organisations (e.g. DNDi, Global Antimicrobial Research and Development Partnership; GARDP); academics (e.g. medicinal chemists); and ultimately patients in the UK and throughout the world. The principal output is new knowledge that guides antimicrobial development (e.g., provides a candidate regimen for study in a clinical Phase II/III trial and/or provides the preclinical evidence that a new agent is likely to work against a pathogen with a particular resistance mechanism).

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

All output of this work will be captured in the form of study reports (some of this work will contain negative results for compounds or model systems that are inactive or sub-maximally active), presentations at national/ international meetings and in peer reviewed literature. Our work from previous licenses has been reported at publicly accessible workshops held by the US Food and Drug Administration (FDA). Studies (or approaches) that are negative but contain important information will be published, if possible, even if in the form of a short note or letter.

Species and numbers of animals expected to be used

- Mice: 20,000
- Rabbits: 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.

Mice are used for most of the work since two experimental models using this species (murine thigh and murine pneumonia model) represent well-accepted gold standards for the assessment of new antibacterial agents. The murine thigh and pneumonia models are known to be highly predictive of complicated urinary tract infection and pneumonia, respectively in humans. Similarly, murine models of disseminated candidiasis, cryptococcal meningitis and invasive pulmonary aspergillosis have been used extensively to develop novel antifungal drugs for humans.

In specific contexts, the rabbit model provides key information that cannot be obtained from mice and hence offers important complementary information. The rabbit enables a more faithful mimic of human disease for: (1) invasive pulmonary aspergillosis; (2) cryptococcal meningitis; (3) bacterial pneumonia (to enable the emergence of antimicrobial resistance to be modelled); and (4) neonatal fungal and bacterial meningoencephalitis. Hence, to complement data packages primarily based on mice or to pursue specific clinical questions that cannot be addressed mice, the rabbit provides a critically important additional species for this program of work.

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

Generally, most (but not all) animals require some level of systemic immunosuppression with a cytotoxic agent and/or corticosteroid to enable infection to be reliably established and maintained. Infection is established via the route that is relevant to the pathogenesis of the disease that is being mimicked. This may include inoculation into the bloodstream, cerebrospinal fluid, subcutaneous tissues, or the lung.

Treatment with a novel or standard antimicrobial agent is initiated after a short delay (depending on the specific model system) which is typically 2-6 hours, but is 24 hours for some model systems. Test agents are injected subcutaneously (s.c.) or intravenously (i.v.) and determined from prior information or from preliminary tolerability studies. The specific regimen that is used depends on the pharmacokinetics of the test agent. The schedule of administration is rarely more frequent than q4h.

The duration of the treatment depends on the model system and is always as short as possible to enable the relevant information to be acquired (and therefore may not necessarily be completely concordant with duration of therapy in clinical contexts). In mice, the duration of therapy is rarely >24 hours. In rabbits, the duration of therapy in generally several days and rarely >7 days.

At the end of antimicrobial therapy, all animals are culled using a Schedule 1 procedure. The relevant study samples (e.g., microbial densities in tissues and/or fluids) are obtained after death (with the exception of all rabbit models where repeated plasma sampling is performed on live animals and rabbit models of cryptococcal meningitis where CSF sampling is performed from live animals under general anaesthesia.

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

The impacts and adverse events can be grouped as follows: (1) those related to infection in untreated controls or animals receiving submaximal antimicrobial therapy. Depending on the model system (and the primary site of inoculation), this may result in abnormal behaviour, difficulty moving [thigh infection model], pain [thigh infection model], laboured breathing [pneumonia models] and central nervous system disease [meningitis models]; (2) those related to the test antimicrobial agent. These generally manifest as phlebitis, infusional toxicity, weight loss, loss of condition and behavioural changes.

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)?

For severe protocols, approximately 60% of mice and rabbits will reach a severe endpoint. This has been estimated from the currrent licence and experience with the models and protocols from the past 10 years. For mice, we anticipate the % of mild, moderate and severe to be 20%, 20% and 60%, respectively. For rabbits we anticipate the % of mild, moderate and severe to be 10%, 30% and 60%, respectively.

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

- Killed

A retrospective assessment of these predicted harms will be due by 03 February 2028

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 routinely used a variety of in vitro models of infection that provide alternative platforms to establish antimicrobial PK-PD relationships. Unfortunately, however, these in vitro models may not always be complete replacements for laboratory animal models for the following reasons:

1. Absence of any immune effector cells or molecules that may have an important impact on the drug exposure-response relationships

2. Inability to faithfully mimic or simulate human pathogenesis and biology that have an important impact on the ultimate clinical outcome (e.g. areas of tissue infarction) that are important determinants of the ultimate drug effect.

3. Inability to simulate tissue sub-compartments that are of relevance for human disease (especially sanctuary sites such as the eye, lung and central nervous system)

4. Binding of some drugs to plastic tubing means human-like antimicrobial concentration-time profiles cannot be simulated using in vitro models.

We do have expertise and a strong record in running hollow fibre infection models (HFIM) The HFIM program complements this program of work. The HFIM has clear benefits. The most obvious example of this is the ability to model the emergence of drug resistance, which is difficult in laboratory animal models because of the high infectious densities that are required.

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

Hollow fibre infection models are routinely used in our work and is planned to complement the laboratory animal program. We have also used a range of other in vitro models and systems that include time kill assays, combination assays and novel cell culture models to mimic early invasive fungal diseases.

Why were they not suitable?

The information obtained from in vitro models complements that obtained from laboratory animal models of infection. These models provide additional information that is clinically relevant but cannot be easily obtained from laboratory models.

A retrospective assessment of replacement will be due by 03 February 2028

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 an estimate of the number of animals required to deliver each program of work for a new drug or a specific question based on multiple examples where compounds have arrived in our laboratory as test agents and exited with high impact publication and a dossier presented to regulatory agencies to support clinical licensing. Approximately 100 and 2000 rabbits and mice, respectively are required per program. These estimates are derived from the programs as listed below:

F2G (olorofim): 3000 mice; 60 rabbits

Allecra (cefepime-enmetazobactam): 1100 mice

Spero (tebipenem) : 2200 mice

Antabio (meropenem-ANT2681) : 2700 mice

Pfizer (oral avibactam) : 1000 mice

Neonatal bacterial meningoencephalitis: 130 rabbits

We anticipate 10-20 programs or work in the next 5 years and therefore estimate we require at least 20,000 mice and 1,000 rabbits in that time.

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

We use block designs using the same protocols, challenge strains and experimental design. This enables us to combine all data and comodel the combined pharmacokinetic and pharmacodynamic dataset using state-of-the-art mathematical modelling approaches. Where there is uncertainty early in drug development programs, we use small studies to provide a more complete understanding of the pharmacokinetics and exposure response relationships.

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

We continuously fit mathematical models to the data as they emerge and use these models for subsequent designs and experiments. We understand and use D-optimal design. We use mathematical to set hypotheses that can be prospectively tested in limited and focused experiments. This approch is highly efficient and optimises the number of animals that are required to describe the pharmacodynamics of new agents and make safe predictions for clinical regimens that are likely to be safe, effective and prevent the emergence of resistance.

A retrospective assessment of reduction will be due by 03 February 2028

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 predominantly use mice for this program of work. Murine models are well characterised and have been extensively used for antimicrobial drug development. There are multiple examples of successful bridging from mice to humans. Murine thigh infection models are used to identify clinical regimens for skin and soft tissue infection and complicated urinary tract infection. Murine models of pneumonia have been used for clinical pneumonia programs. Comparable model systems (and therefore pathways) in other species have not been developed. Hence, the mouse is the most commonly used model system in this licence, which is reflective of the antimicrobial drug development field.

For some clinical questions and drug-pathogen combinations rabbits are required because they are a better mimic of human disease in the lung or central nervous system. The rabbit enables certain tissue subcompartments that are especially relevant to human pathogenesis to be accessed (e.g. CSF). The rabbit may also better reflect human infection by enabloing multiple sampling from the same animal.

A majority of protocols in this licence are classified as severe, which recognises a relatively small proportion of animals will significantly depart from normal health and functioning. Models are designed to be a rigorous test of new compounds—this is necessary to ensure appropriate conclusions are drawn about candidate regimens for humans and identification of dosages and schedules that are safe and prevent the emergence of resistance. The design of models that are classified as mild-to- moderate risk significantly underestimating antimicrobial efficacy and therefore the utility of a new agent for patients. Severity stems from several factors that include: (1) using an inoculum that is high enough to enable the emergence of resistance to be modelled (as occurs in patients); (2) provide a mimic of damage to normal tissues, which then is an important factor in the efficacy of a new antimicrobial agent (e.g. penetration of a new drug into infarcted lung caused by invasive pulmonary aspergillosis); (3) prevent spontaneous clearance of infection by innate or adaptive immune responses as typically occurs if the inoculum is too low; and (4) the requirement to use some form of systemic immunocompromise as mimic for the underlying host features typically seen in many clinical infections.

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

The least sentient animals are used for this program of work. Ex vivo models and invertebrate models (e.g. Galleria) do not enable dose response relationships in severe clinically relevant disease to be estbalished. The decision to use rabbits (even though they have equivalent sentience to mice) is based on the following: (1) providing a focussed assessment of critical findings from murine experiments where it is imperative for drug development programs that key conclusions are confirmed;

(2) when there are critical questions about tissue sub-compartments that cannot be addressed in mice. This predominantly related to diseases of the central nervous system where the cerebrum, meninges and CSF can be reliably distinguished in rabbits; (3) where a higher inoculum and longer study duration is required to observe the emergence of antimicrobial resistance. This predominantly relates to questions related to nosocomial pneumonia where resistance is a major issue that compromises treatment outcomes.

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

We have considerable experience in safely conducting standard murine thigh and lung models of infection. For newer model systems and especially those where the experimental conditions are designed to promote the emergence of resistance, then we will ensure appropriate levels of monitoring are used to ensure the welfare of the animals that are used. This may include increased frequency of manual checks, monitoring of temperature, ensuring timetabling so that staff are available when animals become sick, installing cameras and potentially assessing behaviour via movement tracking and predictive algorithms.

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

We will refer to the ARRIVE 2.0 Guidelines to ensure experiments are conducted in a refined manner.

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

We will ensure we follow the principles of the 3RS in the following ways: Maintain a close relationship with NAWCO, NVS and NC3Rs officer Follow advances from NC3Rs

Maintain close academic collaborations with other laboratories using and developing lab animal models. For example, we are currently collaborating with investigators from GSK, CARB-X, University of Uppsala to better characterise and standardise laboratory animal models of bacterial infection.

A retrospective assessment of refinement will be due by 03 February 2028

The PPL holder will be required to disclose:

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

26.Cardiovascular Protection, Repair and Regeneration

Project duration

5 years 0 months

Project purpose

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

Key words

Gene Therapy, Myocardial Infarction, Heart Failure, Biological Drugs, Gene Editing

Animal types	Life stages
Mice	neonate, juvenile, adult, pregnant, embryo
Rats	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 project studies cardiovascular disease and mechanisms of repair and regeneration of the heart, protective factors, genes and processes contributing to myocardial infarction (heart attack) and heart failure (failing of the heart to provide adequate pump function). These studies will aim to overcome a number of challenges associated with recovery of cardiac function and the development of effective treatment and therapies. The project will support the discovery of novel therapeutic treatments and novel targets to treat a heart

attack and heart failure and the development of more effective delivery methods for cardiovascular gene therapy.

A retrospective assessment of these aims will be due by 01 February 2028

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 World Health Organisation estimates more than 17 million people die each year from cardiovascular disease, what stands for approximately 31% of all deaths worldwide. While available pharmacological and invasive therapies for myocardial infarction and heart failure may reduce symptoms and slow disease progression, there remains an urgent need for novel therapeutic approaches to effectively treat or cure these conditions. In particular, no therapies are available to protect cardiac cells from death, to stimulate their proliferation and thus achieve cardiac regeneration, or to correct their inherited defects leading to cardiac disease. This project will generate basic information on cardiac function and dysfunction, advance knowledge of molecular mechanisms and provide translational potential for the development of biotherapeutics (proteins, viral vectors, nucleic acids) for the treatment of these conditions.

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

Anticipated results include the identification of novel treatment protecting the heart against a heart attack and for heart failure, and the development of methods to correct the genetic information leading to inherited cardiac disease such as cardiomyopathies (e.g., hypertrophic cardiomiopathy, dilated cardiomyopathy). It is vital to achieve because these conditions impose a major disease burden on a significant section of the adult population and lead to very substantial costs for the health service both in the UK and worldwide.

Outputs will include important new information and publications in peer-reviewed scientific journals, which will extend our knowledge on cardiac biology and dysfunction, together with the development of therapeutic leads for therapy. The project will also generate novel approaches and selection methods to search for therapeutic factors and aims to develop advanced technologies for cardiovascular research.

As our projects have a strong translational aim (e.g. to develop new drugs and treatments for cardiovascular disease), we already have ties, and plan to expand them in the future,

with pharmaceutical companies for the subsequent development of the therapeutic leads we identify.

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

Ultimately, the impact of the project outputs will be far-reaching, as they will contribute to important progress in the development of effective treatments and therapies for myocardial infarction, heart failure and other cardiac diseases. This promises to serve to benefit a significant proportion of the global population in the UK and abroad, given that these conditions are a major cause of death worldwide. As well as this, several of the expected outputs will offer further scientific and technological benefit in a range of various research areas and ways. For example, innovative approaches and improved methods may be applicable to future studies towards the treatment and prevention of different types of diseases and conditions, and not specific to only cardiovascular disease studies. This is particularly true for the identification of factors that protect the heart from damage, the development of methods to correct genetic defects in the heart and the identification of novel therapeutic factors.

Results obtained in this project will be published in open-access journals through the 5-year period and will therefore add to the body of information for the wider scientific and clinical community. Findings will also be disseminated to the scientific and medical community by presentations at seminars and conferences. They will be of value to other research groups working in the field of cardiovascular disorders, including other groups developing new therapies.

The intellectual property on the most important discoveries will be protected and will represent an asset for the investment by pharmaceutical companies and venture capital funds, aimed to further develop the therapeutic molecules we discover towards effective clinical application.

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

The outputs of this project will be maximised by collaborating with several other research groups, and the distribution of new methods and knowledge, including dissemination of unsuccessful approaches to research to avoid unnecessary duplication of work. Our group has extensive national and international collaborations which will further enhance dissemination. To maximise visibility of our studies, we will aim to publish our research in open-access journals. New findings will also be promptly disseminated at national and international conferences and seminars. In terms of collaboration with pharmaceutical companies, we already have collaborative agreements with companies for the clinical development of therapeutic proteins and small RNAs we have discovered.

Species and numbers of animals expected to be used

- Mice: 20000
- Rats: 5000

Predicted harms

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

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

Mice and rats of both sexes at various ages will be used for experimental procedures to study the effect of various treatments on the prevention of disease (in particular, heart failure). Appropriate age or life stage for experimental use such as surgical procedure or tissue collection will be determined by tissue type required or particular experimental use and the optimal sex, size, and weight for specific use. For example, for the surgical induction of a heart attack occluding one of the coronary arteries or for the induction of cardiac disease by restricting blood flow from the aorta artery, animals of a specific sex and body weight range will be used to enable efficient and safe surgery, improved recovery time, intended result, and reduce the risk of post-operative mortality.

The use of mice and rats in these procedures is well-documented, extensively characterised, and an accepted model to study key disease processes. Information on the role of new mechanisms leading to heart failure that is obtained in rodents is generally translatable to the human disease because of conservation of key pathways in mammals. Appropriate genetic alterations are available in both mice and rats. Adult mice and rats, 3-weeks and older, will also be used after humane killing and terminal anaesthesia to obtain primary adult cardiac cells. The recovery of these cells is important to study adult heart specific processes in a more controlled manner compared to in vivo, and in a more physiological setting compared to the use of cell lines. Embryonic and neonatal animals, and cells derived from their hearts after humane killing, will be used to assess the events that occur during cardiac development, in particular cardiac regeneration, as some of these processes are not observed in adult animals.

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

Typically, the majority of animals used for experimental surgical procedures will undergo single or serial administration of substances after receiving one or two surgical procedures. For consistent dosing pellets or minipumps steadily releasing the investigated compound may be implanted under the animal's skin. The substances injected include specific proteins, nucleic acids (DNA or RNA), modified viruses used for gene therapy, chemical drugs or cells. The surgical procedures include the closure of a coronary artery to induce a heart attack, the constriction of the aorta to force the heart to pump against higher pressure than normal, and the insertion of a device monitoring cardiac function. Finally,

all animals will be humanely killed or euthanised during terminal anaesthesia. The number of procedures will be kept to the minimum necessary to pursue the main objectives.

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

The experimental procedures are expected to result in altered cardiovascular function, in particular heart failure, and the expected effect of the experimental therapies tested is to prevent this outcome. Appropriate analgesia will be applied to mitigate post-surgical pain. Adverse effects associated with cardiac disease include changes in blood pressure, respiration, cardiac output, inflammation, and may cause discomfort, pain or distress. In rare cases, animals may experience severe adverse effects associated with procedures performed in the study including abnormal heart rhythms, haemorrhage or infection. A small group of animals with constriction of the aorta will undergo second surgery aiming to de-constrict the aorta, however, the adverse effects of that second treatment are connected only to surgery itself, as de-constriction removes the heart pressure overload. No harmful phenotypes are expected as a result of gene transfer or gene editing, however in rare cases where they may occur, possible adverse effects may include cardiac disease and heart failure. Some animals will have telemeters implanted. These animals need to be separately housed to prevent signal interference. To avoid social separation of the animals, these will be always housed with a buddy without implanted telemeter. Any animal showing adverse effects that cannot be immediately ameliorated by simple methods or does not recover within 24 hours will be culled by Schedule 1 method or under terminal anaesthesia (AC). In some animals experiencing heart damage there will be a risk of a sudden death (as in case of human patients suffering from acute heart damage), however these animals will be closely monitored; the function and state of the heart might be assessed using e.g., ultrasound imaging and/or magnetic resonance imaging and these together with frequent assessment of the animal welfare may significantly reduce the risk of such events.

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)?

Animals in protocols with no surgery can experience a mild severity, in particular associated with tissue or blood sampling methods. The majority of animals in protocols with surgery will have a moderate severity. These animals will be closely monitored and additional provisions such as supplemental heat, access to food and water and painkillers will be provided to minimise adverse effects. Cardiovascular procedures such as the surgical induction of a heart attack and the restriction of blood flow through the aorta, are associated with mortality rates of up to 10 animals per 100 hundred animals. Most animals that will exhibit adverse effects are expected to be identified during the surgical operation, and will therefore not be recovered, but instead culled under anaesthesia. In a small

number of cases, severe adverse effects may occur following surgical cardiovascular procedures, such as the onset of an acute form of heart failure. Any animal showing such signs that do not improve after simple treatments or do not recover within 24 hours will be humanely killed. Some animals may possibly experience severe adverse effects due to combination of surgery, genetic alteration and experimental treatments; these are especially the untreated, positive control animals.

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

- Killed
- Used in other projects
- Kept alive

A retrospective assessment of these predicted harms will be due by 01 February 2028

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 has specific therapeutic goals, namely we aim to develop novel treatments for heart attack and heart insufficiency. Therefore, the main purpose of using animals is to develop reliable models that mimic human cardiac diseases and could thus be used to test novel therapies. Cardiac diseases are complex conditions, involving the interaction of multiple cell types and characterised by changes in blood pressure, metabolism, heart pump function, inflammation and blood perfusion at the organism level. There are no cellular or computational alternatives to animals to recreate such complex conditions.

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

All our treatments are first tested in cultured cells, also including the use of robotic screening tests, and several of them also rely on prior computer-assisted modelling.

The testing is based on well-established assays using primary cardiac cells originating from mice or rats. As our ultimate goal in several of the projects is the development of therapeutics for clinical treatment, we also use cells from human induced pluripotent stem cells (hiPSCs) and human myocardial slices from patients undergoing cardiac surgery.

Why were they not suitable?

Experiments in cultured cells and computational modelling are routinely used in our activity. However, these are eventually not suitable to reproduce the complexity occurring in the heart, and are thus unable to recapitulate fully the events occurring in patients with cardiovascular diseases.

A retrospective assessment of replacement will be due by 01 February 2028

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 experimental design is based on carefully considered analysis to achieve sufficient statistical power, in order to render the experiments meaningful. The individual protocols have undergone stringent review by the granting agencies.

Typically, we compare two groups of animals with an induced cardiac disease (e.g., occlusion of a coronary artery to induce a heart attack), followed by treatment with a substance under investigation or a control substance (placebo). The animals are then followed over time to monitor the effect of treatment. The minimum number of animals sufficient to provide a meaningful result (i.e., understand whether treatment is effective or not) is calculated statistically according to the size of the expected effect. This means that the stronger the therapeutic effect is expected, the lower is the number of animals that need to be tested.

Where possible, historic control group data are used to reduce the number of animals required to be used as controls for the project. However, given the variability intrinsic to experimental cardiovascular research, part of which is operator-dependent, in some experiments we use one group of control animals, often undergoing a sham procedure. As variability in this group of control animals is smaller than in animals undergoing complete procedures, we usually keep the number of these animals the smallest to have statistical significance.

In some of the experiments, we test a large number of genes simultaneously in the same animal. Typically, this is the case when we screen collections of genes to search for genes effective against one specific disease condition. Also in this case, the number of animals is kept to a minimum to ensure that the results provide a meaningful result.

Most experiments with animals undergoing surgical procedures are performed by visualizing the effect of treatment by ultrasound analysis (echocardiography) or magnetic resonance imaging in each individual animal over time (like human patients). For qualitative experiments (e.g., to analyse the heart at the end of the study), the animals used are the same as those used for functional assessment of cardiac function over time. These experimental approaches are in accordance with principles of reduction.

When a substance is tested and we need to determine its pharmacological properties (in particular, its distribution in the body and how long it does persist after administration), we will adopt a micro-sampling technique that allows reducing the number of animals and refines the procedure by reducing volume. In micro-sampling, small amounts of blood are repeatedly sampled from an individual animal (up to 20-30 µl per a sample). This reduces physiological stress by reducing the amount of blood taken (20 µl vs ~70-100 µl normal sampling technique) and a reduction by 75% of animal numbers on studies aimed to assess the properties of the investigated drugs. Micro-sampling also increases the data output from animals used by enabling in life sampling over more time points, minimises variability as the data is sampled from individuals over time and data output is increased using fewer animals overall.

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

Principles of good experimental design are followed when designing the experiments to ensure the minimum number of animals required are used to obtain the expected biological results for adequate analysis. NC3R's and the ARRIVE and PREPARE guidelines are used during experimental design and planning.

The use of non-invasive techniques (in particular, the assessment of cardiac function using echocardiography and magnetic resonance imaging, as in patients) in each individual animal reduces the number of animals that would have been used if a new group of animals were to be used for each timepoint.

At the end of the experimental period, when the animals will be killed, we will maximize the use of the same post-mortem samples for multiple analyses (e.g, the microscopy analysis of cardiac tissue together with the assessment of the levels of expression of cardiac genes). This also significantly reduces the total numbers of animals required.

Wherever possible, laboratory systems will be used to determine cellular responses before moving into animal models to confirm findings. Additional information will be obtained wherever possible and appropriate from studies in cultured cells.

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

1. We will optimise the breeding of animals.

2. Where possible and allowed, tissue may be shared within group members or groups to maximise output per animal.

3. Pilot studies will not be required in most cases as the procedures performed and techniques used have previously been established and well developed prior to undertaking this project.

4. Longitudinal studies will be used where possible in order to minimise the number of animals used for various time points.

5. For genetically altered animals, where suitable lines already exist, animals will be obtained from the relevant supplier.

A retrospective assessment of reduction will be due by 01 February 2028

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.

Small animal models using rats and mice will be used.

Mice and rats as animal models for cardiovascular disease research are well established in the literature, and surgical techniques are well developed in these models, and refined surgical methods have been published. Additionally, high availability of genetic alterations which allow for the study of specific biochemical pathways involved in the disease. All surgical procedures will be performed under aseptic conditions by staff who hold appropriate PIL categories and have been signed off as being fully competent. Animals will be maintained to high husbandry standards as set out in the Home Office and NC3R guidelines, including additional monitoring provided following surgical procedures and experimental use.

The major advantage of using mice is the wide availability of genetically altered mice and the relative ease of their generation. This allows the impact of specific genes to be examined specifically, despite some recognised differences between rodents and humans

as far as the cardiovascular system is concerned (e.g, in mice, higher heart rate and capacity of surviving even in the presence of large regions or the heart with damage). State-of-the-art technology is available to assess rodent cardiovascular structure and function in an analogous manner to humans. Rats will be used less frequently; their larger size may make them a more suitable model for some studies involving gene transfer or in cases where experimental techniques/reagents are better established in this species.

Choice of models and methods

We will consider a series of protocols that mimic different human disease conditions. The blockage of a coronary artery induces acute lack of oxygen and nutrients in the region of the heart downstream the blockage, which is similar to what occurs in patients with a heart attack (myocardial infarction - in this case, blockage of an artery is usually due to abnormal blood clotting). If the blockage is followed by release of the coronary artery tie, reperfusion occurs and blood returns to the cells, which closely mimic the effect of the procedure called balloon angioplasty, often performed in humans after a heart attack.

Aortic constriction (i.e., narrowing of the aorta at either the abdominal or thoracic level) is obtained by placing a restriction in the diameter of the aorta. In both conditions, the heart is forced to pump against a pressure that is higher than normal, which eventually leads to pathological enlargement of the heart (cardiac hypertrophy) and later to cardiac dysfunction (heart failure). For transverse aortic constriction, we will follow a procedure based on minimally invasive surgery without opening the thoracic cavity, which reduces mortality and represents an important refinement. Pressure overload is likely the most important factor that inhibits the capacity of cardiac muscle cells to proliferate and the main reason why cardiac regeneration does not spontaneously occur after injury. In this respect, the specific effect of increased pressure and the mechanism involved (which can later be used for therapeutic purposes) is specifically assessed by aortic de-constriction (release of the narrowing at the abdominal level), which is the only model for reverting pathological heart enlargement that is reasonably well established in mice.

In one of the Protocols, assessment of cardiac function will be performed in animals subjected to non- surgical procedures. Treatments here include administration of drugs affecting the cardiovascular system, for example by inducing high blood pressure or increasing the heart beat rates. Other drugs induce death of cardiac muscle cells, as is the case of doxorubicin, which is commonly used in patients with cancer. Thus, the purpose of these experiments is to mimic the cardiac damage occurring in patients undergoing cancer chemotherapy and to find therapies protecting the heart against this damage.

A class of treatments that will be administered both with and without surgical procedures will be viral vectors. These are viruses that have been disabled of their harmful properties and are used as vehicles for the delivery of genes (the AstraZeneca COVID-19 vaccine is an example of the use of a modified virus as a vector for the spike protein of SARS-CoV-2). The vectors we will use in most of the cases are based on a virus named adeno-associated virus (AAV). This is a small virus, which does not cause any disease and can

enter cardiac muscle cells at high efficiency. Some of our studies will also involve the development of treatments that can improve the efficiency of these vectors themselves.

The surgical procedures under all Protocols will be conducted under aseptic conditions, with appropriate pain relief, the highest levels of post-operative care and appropriate veterinary consultation. In the first 24 hours after surgery, animals will be closely monitored at frequent intervals during this period. Careful attention will be paid to heating, pain relief, body weight, surgical wound- sites, hydration, and signs of pain or distress. During the chronic progression to heart failure in all Protocols, animals will continue to be carefully monitored and any that are in a poor clinical condition will be humanely killed if there is no improvement.

To ensure high standards of the performed works and maximize animal welfare, all the procedures are going to be performed by well-trained and rested operator. To maintain high quality of work, the operators will frequently have breaks to refresh themselves, move and relax the muscles. The workstations will be ergonomically designed to further support good performance of operators. New members of personnel will be extensively trained using dead corpses (eg., ones of humanely killed surplus animals or ex-breeders from breeding protocols or humanely killed mice of unwanted genotype), or using dead objects (eg., silicone pads and latex membranes for suturing practice).

In case of myocardial infarction model, opening the intracoastal space often involves cutting the pertoral muscles located on chest. We will use the refined method developed by our group which does not require cutting of any of these muscles, ending up in cutting only the intracostal muscles to open the thorax. Also, in our practice we have managed to minimise the skin incision. All these refinements lead to less muscle damage associated with the surgery, shorter surgery and anesthesia time, significatnly increasing success rate of the procedue. In case of transverse aortic banding (TAC) we are also going to use less invasive method used by other groups at the establishment, requiring smaller incisions and shorter time compared to the prectices described in literature. Appropriate planning of the surteries will additionally contribute to better post-surgical care. If several animals are going to be operated and the group will include eg., mice with induction of damage and sham operated control mice, we will start with the ones which will have the damaged induced. This will allow for longer recovery time and close observations after the surgery, while sham-operated animals will be operated later, as their recovery time is considerably shorter and survival rate is substantially higher. This way all the animals will receive best possible care. We are going to use standardised scoring system to unequivocally determine the condition of the animal, even if the assessment is done by different operators, for close and frequent monitoring - this will allow to detect any animals requiring additional treatment (heat provision, fluid replacement, additional analgesia, etc.) or if the remedial measures are not sufficient, to identify the animals which have to be humanely killed not to suffer. After surgery the animals will be usually single-housed for efficient monitoring of food and water intake and presence of urine and feces, but as soon as the animals will recover, if possible, they will be housed together with other individuals (whenever possible with ones subjected to the same treatment). Animals will be co-

housed in the morning to observe if there is no cage-aggression. Whenever possible, the animals will be co-housed with the same animals as before the surgery. Additionally, in case of the animals which may develop some adverse phenotypes during the experiment, eg., heart failure after myocardial infarction), frequency of their monitoring will be also increased and these individuals will be additionally assessed (eg., with heart echo) to identify the individuals with serious symptoms and if necessary, identify ones requiring humane killing to prevent their suffering.

In majority of the experimental work we will to use healthy, viable and strong animals (like outbred CD1 mice or Spraque-Dawley rats), so the effect of strain phenotype on severity will be minimal. Such animals are more likely to have less health concerns, go through the procedures with lower severity and have better overall survival. In case of genetically altered animals, if a strain with desired alteration is already developed and readily available, we will use that one after careful evaluation of descriprion of its phenotype. If a generic modification is not available, we will develop a new strain basing on the most widely used inbred strains (eg., C57BL/6). Whenever possible and in line with scientific purpose, we will develop conditionally altered animals, eg., that expression of the transgene requires indiction, either by crossing with driver line (eg., expressing Cre), by pharmacological induction (eg., with tamoxifen), or both. When choosing the strain for particular model we will investigate if there are any reported welfare issues; eg., in studies involving myocardial infarction we will avoid using mice of 129S6 strain, which was reported to be very susceptible to infarct rupture, what greatly increases occurrence of sudden death events.

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

Less sentient animals such as invertebrates or non-protected species are not suitable for this research as they do not exhibit comparable cardiovascular disease effects for the purpose of the aims and objectives of our research. For example, this is the case of fish (eg. zebrafish), in which the heart is a primordial, two-chamber (one atrium and one ventricle) organ and in which regeneration after damage may occur throughout life. In contrast, the mammalian heart has four chambers (2 atria and 2 ventricles) and regeneration does not occur in adulthood – lack of regeneration after myocardial infarction in human patients is one of the main problems we aim to address in our studies. Additionally, immune responses, which take part in cardiac repair after damage, are drastically different in mammals and in fish.

Mice and rats instead reproduce reasonably well the human conditions and are representative of the complex interactions that occur between body systems. The major advantage of using mice is the wide availability of genetically altered mice or the ease of their generation, allowing the impact of specific genes to be examined far more specifically than achievable with most pharmacological tools. Finally, the development of cardiac disease after damage or treatment is a complex and chronic process, which cannot be achieved in the short time span of an animal that is anaesthetised before being killed.

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

Procedures will be refined as much as possible including, but not limited to, increased monitoring, post-operative care, heat supplementation, food and water made more accessible or available, pain management by provision of appropriate analgesia and training of animals by previous handling leading up to experimental use which will reduce stress and intensity of restraint required during procedures. All procedures will be performed by competent, trained and licensed personnel, and procedures regularly reviewed to ensure all techniques are performed at a minimum standard and any further refinements may be implemented where appropriate.

As the major component of mortality or expected side-effects are in the first 24 hours after surgery, animals will be closely monitored at frequent intervals during this period. Animals will be reviewed at the end of the working day on the day of surgery and any considered likely to die overnight will be euthanized. Careful attention will be paid to heating, pain killers, body weight, surgical wound-sites, hydration, and signs of pain or distress. We will also pay particular attention to the development of signs of heart failure (loss of weight, listlessness and rapid breathing). While animals will be rarely allowed to progress to such a stage of clinical disease, they will nevertheless closely and regularly be monitored during the study. Any eventual problem will be solved in consultation with the veterinary surgeon to ensure all experimental animals are humanely killed when appropriate. Animals will be humanely killed at a pre-determined time or at the end of the study, whichever happens first.

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

The NC3R’s, publishes several resources including the ARRIVE guidelines (Animal Research: Reporting In Vivo Experiments), PREPARE guidelines (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) and the EDA (Experimental Design Assistant) which are made available to researchers throughout experimental design processes and reviews, and Tech3R's for those carrying out regulated procedures and animal handling.

The Home Office Animals (Scientific Procedures) Act 1986 (ASPA) and Code of Practice for the Housing and Care of Animals Bred, Supplied of Used for Scientific Purposes (published 2014) are used to ensure legal compliance with the Standard Conditions of all license holders and animal users.

The AWERB "Guiding principles on good practice for Animal Welfare and Ethical Review Bodies". 3rd Edition – September 2015.

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

All researchers and animal users will be kept up to date on advances by journal reviews, reading published information or updates, and by systematic review of processes with implementation of any appropriate refinements or improvements where possible and required. Training and additional resources will be made available to all staff throughout the project. We will also monitor the NC3Rs website and follow guidance from Animal Welfare & Ethical Review Body (AWERB), and seek advice of NIO in terms of 3Rs development, implementation and promulgation. Good internal communication will ensure cascade of information to all researchers involved in the project.

A retrospective assessment of refinement will be due by 01 February 2028

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. Discovery and Development of Anticancer Therapeutics 2022

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 models, Drug resistance, Target validation, Combinations, Therapy

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 aim of this licence is to support the development of novel agents and treatment modalities to combat cancer. This includes establishing the correct model to use and optimising the dosing and scheduling of novel treatments to show proof of target engagement and efficacy.

A retrospective assessment of these aims will be due by 23 June 2028

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 main objective is to develop novel 'personalised' cancer therapeutic agents with good potency, selectivity, pharmacokinetic/pharmacodynamic (PK/PD) properties and evidence of efficacy in appropriate, molecularly characterised tumour models. This increases our confidence that agents entering clinical trial are optimised as far as possible pre-clinically, reducing the attrition rate in cancer drug development, for increased patient (and economic) benefit. Therapies are designed to target specific molecular pathologies of cancers and spare normal proliferating cells, which established cytotoxic therapies do not.

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

We will validate novel targets for anticancer drug development. We will create new molecularly characterised tumour models to be used for testing novel anticancer agents. We will ensure these novel therapeutics are given at tolerable doses and by the most effective routes. We will study the changes effected in the tumour and tissues by these drugs. Finally, our testing of new drugs on tumours grown in mice or rats will benefit the drug discovery and development project by providing direct evidence of the drug activity (and safety) in a setting as close as we can get to that found in a cancer patient.

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

Basic science – The novel information acquired and the model systems developed from this proposal will have a direct benefit for both national and international academic institutions that are carrying out research into cancer

Clinical translation : Our animal models will provide important information to clinicians about how cancers respond to treatment. This will help clinicians in making decisions about how to treat patients more effectively and in the design of new treatment strategies. The development and testing of clinically relevant tumour models for treatment will improve our confidence that agents going forward to clinical trial can successfully reach and shrink tumours. The detection and use of chemical markers in patient blood or biopsy samples to select the most appropriate patients for trial and then to monitor response to treatment will be invaluable to ensure we select the patients for clinical trials who are most likely to benefit from a treatment and can assess treatment response as early as possible.

Patient benefit: This work should directly produce new treatment strategies for clinical trials. We hope to increase the overall survival and quality of life of these patients by providing new drugs or combinations of drugs/treatments that will give better, longer disease free survival times and may overcome acquired or inherent resistance.

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

How information will be disseminated: All information gathered from our studies will be shared and discussed with teams within our unit to maximise the benefit to all our research programmes. We will disseminate findings externally through a wide range of channels including presentations and posters at relevant conferences, investigators brochures for clinical trials, organisation of workshops and publications in relevant journals. In addition we will also announce breakthroughs and updates through social media channels such as Twitter, the institutional website and where appropriate through the news media.

Species and numbers of animals expected to be used

- Mice: 32500
- Rats: 3350

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 mouse is the most appropriate model species for this investigation as they are the lowest animals in the evolutionary tree in which suitable models of cancer treatment can be carried out. The project

requires genetically modified mice strains that are immunocompromised to allow the growth of human cell lines and prevent rejection by the mouse immune system.

Some work (but much less) will be done in rats especially to check the pharmacokinetic and pharmacodynamic properties of novel therapeutics. Sometimes a difference is seen between the tolerability or pharmacokinetic/pharmacodynamic (PK/PD) profile of drugs given to different species so it is important when using novel drugs more than one species is checked.

All experiments will be conducted in adult animals.

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

1) Animals will receive an injection of tumour cells to induce tumour formation, usually subcutaneously. This could be to develop a new tumour model or to test a novel therapeutic

2) Animals will be assessed for tumour initiation by observation or imaging, if appropriate . If a new tumour line is being developed the tumour will be measured as soon as it is palpable and until it approaches licence limits. These 'new' tumour lines are often harvested and analysed to characterise them. If the tumours are to be treated with a therapy the mice will be observed until the tumours reach the correct size for treatment (typically within 2-8 weeks) usually about 5-6mm in diameter. The animals will be randomised into groups for treatment.

3) Some animals will not be given tumours but will just receive therapy to establish the maximum tolerable dose to treat tumour bearing mice with. Tumour-bearing animals will be given either single or more usually multiple doses of therapeutic agents(at the

previously determined tolerable doses) dependant upon the properties of the agent i.e. once or twice daily or the route of administration e.g. intravenous or by mouth. Sometimes more than one therapeutic agent will be given (combination therapy) and sometimes therapeutic agents are given alongside other treatment methods e.g. radiotherapy or CAR T-cells.

4) Not all animals will be treated. Some will be used for tumour characterisation studies. Some will be used to assess the genetic changes within the cancer that have led to its formation or spread around the body. Others may be used to monitor biomarkers of cancer within the body.

5) Animals will be monitored for tumour growth and treatment response by either simply measuring the size of surface tumours or using imaging methods to follow treatment outcomes such as changes in tumour size and tumour cell death. The typical duration of experiments will be 1 to 3 months depending on the growth rate of the tumour.

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

Animals will undergo tumour development and tumour treatment and as a result of this may experience weight loss or a general loss of condition. Animals will be checked daily and weighed at least 2 to 3 times a week if not daily to monitor . The body condition and behaviour of animals will also be assessed at these times.

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 severities are mostly mild to moderate. Some 45% of animals will experience only mild effects with few side effects and with tumour regressions due to therapy. Some 50% will have moderate effects with more loss of condition due to treatment (similar to that seen in humans) and having tumours of larger size e.g. control animals who receive placebo treatment. As we need to establish maximum tolerated doses of unknown therapeutics it is possible some animals may experience severe severities <5% would be expected to come into the severe category.

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

- Killed

A retrospective assessment of these predicted harms will be due by 23 June 2028

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?
Human cancers develop within specific tissues in the body, each providing a unique micro-environment. All the cells within a tissue can contribute to tumour formation and growth - not just the cancer cells we implant but the cells that make up the surrounding 'normal' tissue. These complex conditions and interactions between cells cannot be adequately modelled in the laboratory without the use of animals. What is more the spread of cancer from its primary site to distant secondary sites (the major cause of treatment failure) is a phenomenon only fully seen in whole animals. Since cells need to access the circulation in order to move to and survive at secondary sites this research can only be done in whole live animals. Similarly, the effects of drugs must be tested in animals to determine that adequate drug levels are achieved in tumour tissues, that adverse effects on normal tissues are minimised and that efficacy tracks with measurable chemical markers in the blood or tumour – i.e. evidence that the compound reaches its target and selectively inhibits it.

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

We have carried out extensive studies in the laboratory without the use of animals to mimic as many of the basic cellular processes as possible. We have developed a battery of high-throughput 2D and 3D assays of tumour cell proliferation, migration, invasion, and enzyme activity (processes involved in metastasis) and also use endothelial cells (which make blood vessels) in multiple assays of new blood vessel development. We use permeability assays to predict compound absorption, protein binding assays to study distribution and microsomes and hepatocytes to study metabolism in the laboratory however this still does not match the complexity of the situation in a live animal. We also have in vitro target engagement assays to assess the relationship between target engagement and growth inhibition. A theoretical model of the relationship between compound level and activity can be derived but ultimately, it needs to be validated in an animal .

Why were they not suitable?

None of these assays adequately predicts the complexity of in vivo responses in whole live animals. Before drugs can move forward towards clinical trial we must assess the level of drug both in plasma and in tumour as well as checking for target engagement and therapeutic response. None of this can be completely achieved in non-animal alternatives.

A retrospective assessment of replacement will be due by 23 June 2028

The PPL holder will be required to disclose:

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

Reduction

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

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

We have estimated the number of animals we will use based largely on previous experiments, the literature and the projection of our needs based on current projects. We have extensive prior experience of using these models and therefore have a good idea of how many animals we require to achieve our aims. Based on our previous data we will use the minimum number of animals required to give a statistically meaningful result. We will consult with our bioinformatics team for advice on experimental design to keep numbers used as low as possible and choose the most appropriate statistical methods to analyse our results.

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

All animal studies are designed with assistance from the NC3Rs Experimental Design Assistant and using ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines. We will consult with a statistician with regards to the experimental design to minimise the number of animals used whilst ensuring meaningful data can be collected.

We consistently use in silico (computer modelling) methods to predict pharmacokinetic behaviour of our compounds. We carry out in vitro permeability assays and Metabolism assays to characterise the compounds ahead of in vivo testing thus reducing the number of mice used. The structure activity relationship (SAR) of compounds in vitro and later in vivo helps us establish in silico, which parameters can be optimised to obtain development compounds with suitable pharmaceutical properties. This leads to a reduction in the number of compounds taken into animal testing as some can be excluded by computer driven analysis of their chemical properties. Once again this leads to a reduction in the number of animals used.

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

For tumour studies, we aim to use the minimum number of animals per group that will be informative whilst maximising the information from each study. The animal numbers required to obtain significant results are dependent on the particular tumour model. The most important characteristic of a model is the reproducibility and thus predictability of tumour development. We use inbred animals that exhibit minimal variability and all consistently show the same growth characteristics for a given tumour. Our use of immunocompromised animals ensures we do not experience rejection of tumours by the host immune system. Initial checks on whether a particular tumour grows and experiments to characterise the tumour - i.e.' pilot studies' to find the most reliable cell line or genetically manipulated variant clones - are done on this licence to ensure the correct model is used. These pilot studies also allow us to use statistical tools such as G power calculations that tell us the minimum group sizes needed to show statistically significant results. The dose, route and vehicle to give the best PK profile and biomarker modulation will also have been assessed before we move into full therapeutic testing . We have also pioneered the use of ‘microcapillary bleeds’ (~10µL blood samples) together with highly sensitive mass spectrometric methods to assess circulating drug levels during therapy and to detect the chemical markers of drug action. This once again reduces the need for large groups of animals for repeated sampling, as we can take many very small samples from the same animal over time.

A retrospective assessment of reduction will be due by 23 June 2028

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 drug development studies and are widely used for this purpose. Most of our work is carried out using well-characterised human tumours in mice that are bred to be immunodeficient (nu/nu, SCID or NSG mice) to avoid tissue rejection. This enables us to study human cancers in a mouse host. The animals are maintained in individually ventilated cages using sterile food and bedding and all procedures are carried out in laminar flow cabinets to avoid infections.

The animal models we have chosen are well characterised and well documented to produce reliable results while only having moderate effects on the mice. We use well-established methodologies that we know have little or no adverse effects, by themselves, to activate or inactivate specific genes. We will then monitor mice for signs of tumour development, typically including weight loss, inactivity or sometimes other specific characteristics of a particular tumour type all the while minimising pain, suffering and distress.

Suffering will be minimised by keeping tumour burdens within tolerable and acceptable limits detailed in each protocol as appropriate. This will be achieved by careful monitoring of the animals

Compounds to be evaluated will have been triaged for potency, stability and tolerability and are generally of low toxicity (e.g. agents targeted to molecules selectively overexpressed or mutated in human cancers).

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

Mice are the lowest species that are appropriate for in vivo drug development studies and are widely used for this purpose. For our studies we need to use adult mice as this is more physiologically relevant to man. In addition, we will be studying how cancer grows and responds to treatment over a period of time that could be as long as 2-3 months, so the use of young or terminally anaesthetised mice would be impractical.

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

Our aim is to create new therapeutic regimes for the treatment of human cancer. We will not subject any animal to tumour implantation and treatment until we are sure we have enough in vitro evidence that this could be an effective treatment strategy which it would be possible to transfer to patients in the clinic.
Animals undergoing surgical procedures will receive anaesthesia and pain relief. After surgery, the animals will be intensely monitored until they have recovered from the anaesthesia. If there are no clinical signs and the wound shows no swelling or bleeding, the animals will be monitored at least 2 to 3 times a week and often daily by both researchers and animal care staff. We are continually developing technologies to refine our experiments and to minimise suffering of our research animals
e.g. the use of microbleeds to reduce animal numbers used.

All our staff follow a program of competency assessment that is continually reviewed and updated as required.

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

We will follow the guidance given in the NC3Rs 'Resource Hub' (https://nc3rs.org.uk/resource-hubs) for example on blood sampling (https://www.nc3rs.org.uk/blood-sampling-mouse). We will also refer to the National Cancer Research Institute guidelines on using animals in cancer research published by Workman et al 2010 (British Journal of Cancer 102, 1555 – 1577) and its next version which is currently being written.

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

We will keep up to date with the latest developments on refining animal research methods via the NC3Rs website (https://www.nc3rs.org.uk). Animal house staff will ensure that any advances are fully implemented throughout the facility.

A retrospective assessment of refinement will be due by 23 June 2028

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. Preclinical Development and Assessment of Medical Interventions Against Bacterial Pathogens

Project duration

5 years 0 months

Project purpose

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

Key words

Microbiology, Vaccine, Antibiotic, Intervention, Countermeasure

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?

The overall aim of this project is to develop and/or assess new vaccines, therapies, and treatments (interventions), in animal models of infection, against several infectious diseases which are considered to be a public health threat, in the UK and globally, (e.g.

Anthrax, Plague, Melioidosis, Glanders and Q Fever), and for which current countermeasures are absent, inadequate, or unproven.

A retrospective assessment of these aims will be due by 19 February 2028

The PPL holder will be required to disclose:

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

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

Why is it important to undertake this work?

This project will allow for the use of established animal models as well as furthering their development. This will inform studies into pathogenesis, transmission of bacteria and efficacy of vaccines and treatments that have most clinical relevance.

Data generated will enable the selection of potential vaccines and treatments. This will benefit humans because bacterial pathogen outbreaks (i.e. Anthrax, Melioidosis, Glanders, Plague and Q Fever) can harm or even kill large numbers of people.

We will also improve the data available to scientists about how animal models respond to specific bacterial pathogens and the vaccines and treatments that treat them. This will benefit humans because in the future, vaccines and/or treatments will be selected with a growing likelihood of success.

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

There are several benefits of this project in developing models for and evaluating the efficacy of treatments for bacterial pathogens.

Outputs will include:

- Model development and optimisation in a range of species; new information on the models will lead to publication and dissemination of refinement information to with wider bacterial pathogen and animal community
- Evaluation of novel vaccines and treatments against bacterial pathogens to assist in development and licencing before they are used in humans in clinical trials.
- Capability to assess vaccines and treatments against emerging bacterial pathogens, where human trials are not possible

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

This project will contribute to the understanding and refinement of animal models of bacterial pathogens allowing long term contribution to the field. We anticipate that model

development and optimisation in a range of species can be achieved within this project. Knowledge and findings will be transferred to subsequent studies and disseminated through the scientific community through publications where appropriate. New techniques and findings will be also be disseminated to the wider community.

This project will enable us to select effective vaccines and medicines from a range of candidates. By filtering these candidates through our models of infection, we will reduce the number of candidates required to be tested in humans and advance translational research.

This project will provide the capability to assess new interventions against the most serious forms of infectious disease, where human trials will not be possible due to the severity of the form of disease being investigated. Regulatory bodies will, however, accept preclinical data generated in animals in such circumstances so this project may assist in the licensing of new or improved interventions against serious forms of infectious disease.

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

Our work is highly collaborative, with the assessment of potential new vaccines and medicines being carried out with either commercial partners or through research programmes.

Knowledge and findings will be transferred to subsequent studies and disseminated through the scientific community through publications where appropriate to ensure that our findings are subject to the scrutiny of the scientific community. In addition to peer-reviewed publications, the work performed under this licence will be disseminated widely at international conferences which would provide opportunity for informal feedback and in-depth discussions to disseminate new knowledge. The data provided to collaborators and customers will direct the appropriate generation of products which will have direct benefits to human health.

Species and numbers of animals expected to be used

- Mice: 6000
- Guinea pigs: 875
- Rabbits: 875

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, guinea pigs and rabbits are all recognised animal models for the assessment of the bacterial pathogens mentioned within this project. Mice, Guinea pigs and rabbits will be used to evaluate the effectiveness of vaccines and medicines against bacterial pathogens.

The scientific community working on these bacterial pathogens worldwide use the same animals as these are the most appropriate species in these disease investigations. Using the same animals allows for data that is generated from this project to be compared to other related work that has been published. This is an important and critical step in the development and assessment of various vaccines and medicines.

The use of two or more species is required by licencing authorities to provide reliable data, which fully assess new vaccines and interventions candidates. Adult animals for each of these species are most suitable for these experiments.

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

Typically, the following will occur during and experiment:

Following acclimatisation, animals will be sedated and have a small amount of blood taken, to assess normal immune responses prior to intervention. Animals on this project may also be implanted with a biometric chip or other telemetric device to provide a unique identification code and/or to track body temperature or other data.

Animals may then be vaccinated, by routes similar to those used in people, such as into the muscle or up the nose. Alternatively, medicines may also be administered, most commonly orally.

If a vaccine is being assessed the duration of the experiment can range from one week to several months depending on the length of the vaccination phase.

A bacterial pathogen will be administered to animals, usually via the aerosol route and allowing the animal to breathe in the pathogen. Once administered the experiment is usually completed within 28 days, depending on pathogen.

These animals will undergo regular health monitoring by trained and expert animal care staff at specific timed points. The frequency of these health monitoring checks may be increased as signs of disease present in these animals. If clinical signs approach the severity defined within the project the animals will be humanely killed to relieve their suffering by an approved method.

Animals can be expected to be sedated and sampled (nasal washed/swabbed) daily in order to study the shedding of the bacterial pathogen administered. Animals may be sedated and have a small amount of blood collected over the duration of a study. Ensuring that published recommended guidelines for blood withdrawal are never exceeded.

At the end of all studies, animals will be killed humanely by an approved method. After being humanely killed, samples of organs, tissues or relevant biological material will be collected for analysis.

The majority of animals on this licence will be manually handled, with or without anaesthesia, in order to inject or give them vaccines or therapies against specific bacterial infections; or to check their overall health and weight.

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

Potential adverse effects to pathogen challenge, anaesthetics and administration of substances have been identified.

Animals may be manually handled, with or without anaesthesia, this process may cause short term distress to the animals. Animals may also be implanted with a biometric chip or other telemetric device, typically under their skin. This is expected to cause no more than mild and transient discomfort.

Some of the experimental drugs may cause harm for example by disrupting the normal community of beneficial bacteria in the gut. Respiratory infection in animals is likely to develop very quickly and may overwhelm the animal due to rapid septicaemia. The likely adverse effects will include a period of fever, a combination of clinical signs of infection associated with the infectious agent. If clinical signs include signs of imminent death, the animals will be humanely killed by an approved method.

Regular health monitoring will be conducted by trained and expert staff members to detect clinical signs of disease. If clinical signs are present, the frequency of these health monitoring checks can be increased, but in some cases, animals may die before such signs are evident. Our previous experience indicates that although this can happen, most our procedures will be moderate in severity.

Septicaemia is expected to occur within a small percentage of animals (approximately 10%). The bacterial pathogens named within this licence are expected to have a range of expected mortalities due to the nature of the disease they cause, especially in organisms like B. anthracis which typically causes death within a very short time frame. In such a scenario, it is unlikely that there will have been a significant period of 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)?

Protocol 1- Model development

The expected severity from bacterial pathogen infection is expected to be severe in more than 60% of mice, guinea pigs and rabbits on this project

Protocol 2-Immunogenicity

The expected severity from vaccine administration is expected to be mild in 100% of mice, guinea pigs and rabbits on this project

Protocol 3-Pharmacokinetics

The expected severity from therapeutic/antibiotic administration is expected to be moderate in 20% of mice and guinea pigs on this project

Protocol 4- Protection Testing

The expected severity from protection testing against bacterial infection is expected to be severe in 10% of mice, guinea pigs and rabbits on this project unless protection is observed.

Protocol 5- Refined Model development

The expected severity from bacterial pathogen infection is expected to be moderate in more than 60% of mice, guinea pigs and rabbits on this project

Humane clinical endpoints have been clearly defined therefore unnecessary suffering is avoided. At the end of all studies, animals will be euthanised by a Schedule 1 method or by terminal exsanguination under full anaesthesia

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

- Killed

A retrospective assessment of these predicted harms will be due by 19 February 2028

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 research focuses on assessing the effectiveness of treatments for infections caused by dangerous bacterial pathogens. There is a requirement to develop new treatments for a variety of reasons; either current treatments have adverse effects, a lack of evidence of efficacy or are untested against new or emerging health threats.

Due to the severity of disease caused by the bacterial pathogens involved in this project, it is not possible to carry out classical human clinical trials to determine efficacy against

infection. In addition, as natural disease occurs sporadically and unpredictably, trial recruitment is impractical. In the absence of classical clinical trial data, regulatory bodies, such as the UK Medicines and Healthcare products Regulatory Agency, will accept robust efficacy data derived from relevant animal models of infection in support of license applications for new treatments.

Suitable and appropriate animal models must be utilised to fully assess the immunological response to a treatment or vaccine within a biological system. These animal species are the most appropriate models for the bacterial pathogens assessed within this license, as they have similar respiratory and immunological responses to that of humans. Currently here are no alternative or substitutive methods which could be utilised to fully achieve our aims.

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

As much evaluation of the efficacy of new treatments as possible will be done using in vitro systems, such as Minimum Inhibitory Concentration (MIC) analysis for antibiotics and cell-based assays of biological activity. In addition, insect models of infection have been developed for some infectious organisms and have been used as a first "in-life" model system to help filter through large numbers of potentially useful antibiotics. In certain cases, the number of drugs required to be tested in our mammalian models may be reduced by replacing the initial drug screens required. Drugs that fail in these special cases will not be taken forward.

We will, however, try to use human sera, wherever possible to assess human vaccines for biological activity. In addition, although insect models of infection are available, the relatively simple nature of these models makes them only suitable for early-stage high throughput screening. However, if insect models suggest a therapy or intervention may show promise, mammalian studies of efficacy will need to be conducted to assure regulators that the candidate is effective in more complex animal models.

Why were they not suitable?

To establish reliable evidence of efficacy of new treatments which show promise using in vitro testing or insect models of infection, it is still essential to assess them using the full range of host-pathogen- treatment interactions which mammalian model systems offer. Regulatory bodies will accept robust efficacy data derived from relevant animal models of infection, where classical human clinical trials cannot be performed, to support license applications. At present, there are no alternative in vitro technologies that replace the need to use animals since there is a requirement for all components of an immune response must be present within the systems used to investigate novel new vaccines, therapies and treatments (Interventions), to better predict the effect of that potential new treatment in man.

A retrospective assessment of replacement will be due by 19 February 2028

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 next five years, it is possible that a variety of species are required to develop models of infections and to assess the properties of new interventions. Although it is very difficult to predict how many treatments we will test during the life of this project, our past performance indicates that we will use at least 1000 animals each year. The level of usage may, however, increase in any year due to the possibility that a candidate vaccine or therapy which may suddenly require a lot more testing, as it enters clinical or other critical studies. As a result, we predict to use the following: Mice 6000, Rabbits 875 and Guinea pigs 875

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

The organisation has a vast amount of knowledge and experience when designing studies, with the aim of using the number of animals per group while still generating data that are useful. Statistical advice is available, and this advice will be used to minimise animal usage in studies.

We always use statistical calculations to identify and use the minimum number of animals required on a study, whilst still providing robust scientific data which will be accepted and give statistical relevance and comply with any relevant regulatory requirements.

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 required for a study will be determined by how variable the infection model outcome is. We reduce our model variability by optimising our infection procedures using good microbiology and world class instrumentation.

Studies will be conducted in a step-wise manner so that the number of animals used will be minimised if the vaccine/treatment shows no likelihood of working; for example, if a new vaccine does not elicit an appropriate immune response, then it would not progress to a challenge efficacy study.

Where appropriate, pilot studies with a reduced number of animals or groups, will be performed. This is particularly important where challenge dose must be determined, or to assess vaccines ability to elicit an immune response or to establish a dosing regimen. Although statistical significance would not be determined, in the long term, this avoids a negative outcome involving large number of animals.

Robust scientific quality control of the test materials and methods will ensure studies are carried out successfully the first time, minimising the need to repeat studies and subsequently reduce the number of animals used. All protocol and study designs would be reviewed by the organisations ethical review panel and improvements and suggestions would be implemented where possible.

A retrospective assessment of reduction will be due by 19 February 2028

The PPL holder will be required to disclose:

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

Refinement

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

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

The majority of our work will be conducted in mice. Mouse models are very useful as they are widely used by the scientific community because there is such a large body of information already published about mouse biology. Sometimes, however, mouse models are not adequate or not as closely matched to human disease in which case we need to use other species such as rabbit.

To assist in the prompt recognition and subsequent intervention, critical periods will have been identified (depending upon pathogen) and monitoring frequency increased. Our staff work in shift patterns, to ensure that animals considered to be in the critical phase of an experiment are regularly monitored. This high frequency, hands-on monitoring, has been shown to effectively minimise the welfare costs to animals and hence reduce the severity and quickly identify if an animal is heading towards a humane end point. Humane clinical endpoints have been clearly defined therefore unnecessary suffering is avoided.

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

The majority of animals used within this project will be mice. The mouse model is an established model for vaccine studies. Less sentient species have immune systems which are too different from humans. The use of other species would not fully represent the complex immune system response to these vaccines and therapies, which could provide a poorer indication of the likely responses if this was to progress to human clinical trial. The use of two or more species is required by licencing authorities to provide reliable data, which fully assess new vaccines and interventions candidates

Additionally, there is a large body of published research which enables a direct comparison of the effects of particular vaccines in mice and humans. As immune responses typically can take weeks to develop a response to infection, vaccine or therapies, it is unfeasible to keep animals under anaesthetic for that long.

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

We strive to conduct the minimum number of experimentations as possible, to collect data to exemplify the interventions associated within this licence. We use rigorous monitoring processes to ensure that we minimise the welfare costs to the animals. Our staff work in shift patterns to ensure that animals considered to be in a critical phase of an experiment are regularly monitored. The frequency of which can be increased whenever necessary depending on the severity of the presenting symptoms. The health scoring system we have developed over many years provides a more holistic overview of the potential harms we are causing and when to intervene such that harms are minimised. The high frequency of hands on monitoring has been shown to effectively minimise the welfare costs to the animals.

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

We will follow a variety of published guidelines where appropriate, including: Local AWERB guidelines

ARRIVE guidelines of the NC3Rs

guidance on Animal Testing and Research from the Home Office Good research practice guidelines from the Wellcome Trust LASA and RSPCA guidelines and

Handbook of Laboratory Animal Management and Welfare, Forth Edition Editor(s): Sarah Wolfensohn, Maggie Lloyd. Published Online: 04 Jan 2013, Print ISBN: 9780470655498.

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

All staff working under this project attend relevant training and refresher courses and keep up to date with reduction and refinements by attending NC3R/RSPCA workshops or equivalent as part of our continual development programme. This information is then

shared among the animal care staff. Any changes that will be beneficial to the animals while maintaining the scientific integrity of the protocols will be investigated and implemented where appropriate. Changes will be made to this project, if required, to implement advances in the 3Rs effectively.

A retrospective assessment of refinement will be due by 19 February 2028

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. Platelets as a Therapeutic Tool

Project duration

5 years 0 months

Project purpose

(a) Basic research

Key words

Platelets, Clotting, Bleeding, Transfusion

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

Retrospective assessment

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

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Contains severe procedures

Objectives and benefits

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

What's the aim of this project?

To fully test and understand how good platelets (blood cells that help stop bleeding) made from stem cells are at stopping bleeding. This will inform future studies and provide pre-clinical data for clinical trials.

A retrospective assessment of these aims will be due by 17 April 2028

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?

Platelets are blood cells whose primary function is clotting. There is a huge unmet need for the transfusion of platelets because at the moment we rely solely on blood donors. We are generating platelets from stem cells in the laboratory and need to test how good these platelets are for transfusion and clotting in mouse models. We need mouse models to fully understand how they work in a live mammalian system because testing in a laboratory is not complex enough to completely reproduce what happens at the site of bleeding in a human.

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

We are generating platelets (involved in blood clotting) from stem cells, some which contain additional factors that are involved in blood clotting and others which contain drugs which can be used in patients. In particular, platelets that will not be rejected by the recipient’s immune system. We expect to produce several high impact publications from this project from the basic biological science we need to develop to push this forward.

In addition, these projects will provide experimental data that will be necessary for approval for the first-in-human studies. We also expect that the data will form the basis of further funding that will be necessary for the translation to human studies.

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

The immediate benefit of this project will be to patients with excessive bleeding because their platelet count is low, or their platelet do not function well and are therefore in need of platelet transfusion.

There is no doubt that making blood cells in the laboratory, particularly platelets, will have a huge impact on transfusion medicine. At the moment donor-derived platelets are the only option for transfusion (280,000 units of platelets are used in the UK every year) but this has issues: supply, exposure to donor-derived infections and rejection by the patient of donor-derived platelets.

This project will deliver knowledge and preclinical data that would push forward the opportunity to deliver platelets made from stem cells into patients. The ability to produce platelets that are not rejected by the patient and/or contain specific therapeutics (such as clotting factors) provides us with the opportunity to develop 'personalised' cell therapies. Platelets are particularly attractive as they do not contain a nucleus (they are unable to make their own DNA) and are therefore less of a risk in terms of their ability to form cancer after their administration, when compared to other cell therapies derived from stem cells.

This project will provide evidence that platelets can be used as ‘drug delivery vehicles’ targeted to the site of interest. The technology developed in this project will be also relevant to other researchers aiming to produce either blood cells in the laboratory (such as red blood cells) or other organ cells in the laboratory (liver, pancreas, heart muscle).

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

We have a number of successful collaborations with academic groups in the UK, the UK transfusion service (NHS Blood and Transplant) and academic groups in Europe. We are in active collaboration with groups that can produce stem cell derived therapies at clinical grade for human trials. We will disseminate the output from this work at various conferences, both in the UK (The Platelets Society Conference) and internationally (ISTH, ISBT, EHA, ASH) and in peer-reviewed publications. This group is very active with Public Engagement.

Species and numbers of animals expected to be used

- Mice: 4480

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 order of mammals that we can use to study the platelets in a whole mammalian system. In general we are using adult mice, so that their bodily systems are fully mature.

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

Superovulation (ovulating more eggs than usual) - Female mice will be given agents to increase egg production twice. Embryos and blastocysts will be removed under terminal general anaesthesia.

Embryo recipients - Embryos and blastocysts will be implanted surgically or non-surgically into the reproductive tract of a mouse made pseudo-pregnant by mating with a sterile male. All surgically implanted mice will be killed by a Schedule 1 method. Non-surgically implanted mice will be killed by a Schedule 1 method or kept alive for potential re-use on a breeding protocol.

Vasectomy (cutting and tying of the tubes that carry sperm) - Male mice will undergo a vasectomy under anaesthesia. These males will then be used to induce pseudo-pregnancy in embryo recipients.

Analysis of blood cell function - Mice will be bled from a superficial vessel, administered a cell modulation agent (a maximum of three times per day for a maximum of 15 days) and then bled from a superficial vessel on a number of occasions (several times in one day, or over the period of 28 days). They will then be exsanguinated (terminally bled) under terminal anaesthesia and killed by a schedule 1 method.

Tail vein bleeding model -mice will be injected with a cell modulation agent (an agent that alters cell function) (a maximum of three times per day for a maximum of 15 days). Mice will then be injected once via the tail vein with platelets made in the laboratory. Terminally anaesthetised mice will have the tip of their tail (<5mm) cut off and the amount of blood loss will be measured.

Myeloablation (irradiation) - mice will be irradiated with a low as possible degree of radiation in a split dose. They will then be given blood cells once via a tail vein injection. Mice will then be bled from a superficial vessel on a number of occasions (several times in one day, or over the period of 28 days), before being bled from a large vessel under terminal anaesthesia and killed.

Myeloablation and the tail vein bleeding model - Mice will be irradiated in a split dose and then administered haematopoietic(blood)/terminally differentiated cells once via an intravenous injection. Mice will then be bled from a superficial vessel on a number of occasions (several times in one day, or over the period of 28 days) before undergoing the tail vein bleeding model. Mice will then be exsanguinated (terminally bled) under terminal anaesthesia and killed by a schedule 1 method.

Transfused mice and analysis of blood cell function using pulsed laser microscopy (the use of a laser to induce injury)- Mice will be administered a cell modulating agent (an agent that alters cell function) (a maximum of three times per day for a maximum of 15 days) and bled from a superficial vein on a number of occasions (several times in one day, or over the period of 28 days). They will then be administered samples containing haematopoietic/differentiated cells once or a control substance before being terminally anaesthetised and undergoing pulsed laser microscopy.

To analyse blood cell production and function post-splenectomy (removal of the spleen) - Mice may be administered a cell modulating agent (a maximum of three times per day for a maximum of 15 days), bled from a superficial vessel (several times in one day, or over the period of 28 days) and splenectomised. Mice will then have follow-up bloods taken from a superficial vessel (several times in one day, or over the period of 28 days). Mice will then be exsanguinated under terminal anaesthesia and killed by a schedule 1 method.

Myocardial infarction (MI) (heart attack) - Mice will be subject to general anaesthesia and will be ventilated. The chest will be opened and the major arteries which supply the heart will be blocked to induce an MI. The wound will then be closed, and pain killers will be given. During recovery mice will be housed in a warm cage for close monitoring. During the initial period of recovery from operation the animals will be checked every 10 min, then

every hour until they can move freely when lightly disturbed. The function of the heart may be measured with echocardiography (ultrasound) during this recovery period. Mice will receive a transfusion of cells via the tail vein post MI once. At the end of the protocol, mice will be humanely killed with Schedule 1 method.

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

Animals which have a weakened immune system (immunosuppressed) may display a higher rate of illness. Mice may lose weight for up to four days following irradiation. Injections into the bone marrow (Intraosseous) may rarely result in lameness, which may cause more than transient discomfort. The main adverse effects following removal of the spleen (splenectomy) are pain after surgery, bleeding during surgery, reopening of the surgical site and infection of the surgical site. The main adverse effects of surgery to induce a heart attack (myocardial infarction) are sudden death due to an abnormal heart rhythm (ventricular fibrillation), stoppage of the heart beating (cardiac arrest), heart failure, pain after surgery, bleeding during surgery, reopening of the surgical site, surgical site infection and developing high blood pressure in genetically altered animals. Drugs given to cause a chemical injury to the blood vessels which supply the heart may have undesirable effects. In some circumstances we may not use non-steroid anti-inflammatory drugs to control pain, but other pain relieving drugs, as these may alter platelet function. Mice in which we will cut the tails for the tail vein bleeding model will be anaesthetised and therefore will not feel pain. It is important for us to carry out the tail vein bleeding model so that we can test the cells that we made in the laboratory in a living system, where there are a number of other cells, as we cannot fully mimic this in the laboratory setting. Some mice maybe injected with a drug that alters their cells (cell modulating substance), for example this drug may bind to the mouse’s platelets and reduce them before we give them platelets that were made in the laboratory. This is unlikely to cause harm which is more than minor and transient.

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 severities in this licence are:

- Mild - 79%
- Moderate - 16%
- Severe - 5%

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

- Killed

A retrospective assessment of these predicted harms will be due by 17 April 2028

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?

This work will be supported by studies in the laboratory of human platelets and megakaryocytes (MK) (the cells which platelets are derived from) wherever possible, including the use MKs derived from stem cells. However, human platelet and MK studies cannot fully replace the animal studies in this project for three reasons.

1) Megakaryocytes represent only 0.01% of all bone marrow cells and therefore we cannot get enough from human bone marrow samples. Although MKs can be grown from human stem cells in the laboratory, studying the way they work (particularly how platelets are released) cannot be done entirely in the laboratory due to our inability to reproduce the complex bone marrow environment that provides vital clues to how MK's mature. The variability in different donors (such as genetic variations) can influence results limiting reproducibility. This is not an issue when using mice colonies where genetic variations are minimized.

2) Although our ability to produce platelets in the laboratory from cultured MKs has markedly improved, it remains to be proven that these platelets are truly similar to fresh platelets in terms of the way in which they work and how effective they are at stopping bleeding (the proposed work plan is looking at addressing this issue). Therefore, in order to study how platelet function (how they work) is affected by the different genetic mutations we have to resort to the study of platelets isolated from fresh blood. Platelets do not have a nucleus (where DNA is made), which means that standard methods of altering genes cannot be used. Human blood can potentially, but regular access to specific patient samples would severely limit the work.

3) Recovery, survival and function (clotting/tissue repair) in the mammalian system of transfused cells is regulated by a very complex environment. These can only be reproduced within the blood environment of a living organism. Mouse studies will provide essential proof-of-principle data that will be necessary in order to gain approval for human studies of blood cells produced in the laboratory.

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

We are unable to recreate the complex 3-dimensional bone marrow environment in the laboratory. Although we can mimic some of these characteristics, particularly using

scaffold structures, these experiments are usually 1-dimensional in what they can provide to the cultured cells vs live bone marrow.

Why were they not suitable?

Chambers which enable blood to flow through can be used to test the formation of blood clots; however, they do not mimic the environment found in blood vessels, particularly the way blood flows in a pulsatile manner (with a push and then ebb) and interaction with cells lining the blood vessels. In addition, there are no models that can mimic the interaction of platelets with other organs in the body.

A retrospective assessment of replacement will be due by 17 April 2028

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?

Pilot studies with a minimum number of animals will be carried out in order to assess the validity of each experiment and to refine group size estimates. If the pilot experiment raises unexpected new questions, a subsequent pilot experiment will be performed. The pilot studies and data gained from mouse experiments carried out over the last 5 years have allowed us to accurately carry out sample size calculations.

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

The project workflow has been designed in a stepwise manner to ensure that at each stage that a biologically significant effect is likely. We will use a combination of taking organs or tissues from a mouse and using it in the laboratory (ex vivo) and only performing experiments in genetically altered mice or giving mice drugs, where there is evidence in the previous steps ex vivo that there is an important effect to be investigated. The aim of this is to reduce the number of animals required during the project. Pilot studies with a minimum number of animals will be carried out in order to assess the validity of each experiment and to refine group size estimates. The pilot studies themselves will be based on the size effect obtained in the ex vivo assays.

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

The methods used will generate the greatest amount of data from the minimum number of animals required to achieve our scientific objectives, whilst minimising any pain, suffering, distress or lasting harm. We routinely expect to gain multiple data sets from a single animal, for example conducting multiple platelet functional assays all from a single blood sample from a single mouse. We can do this because of the use of modern methods we use, such as flow cytometry analysis which we use extensively, that allows us to use very small sample sizes. The overlap in expertise between different members of staff who are trained to carry out the mouse experiments as well as the ex vivo experiments and analysis allows us to blind the experiments, whereby the person who carries out the ex vivo experiments and analysis is blinded to the actual identity of mice from which the samples are generated. It also allows several people to work in parallel in different tissues such as bone marrow and blood that need to be handled fresh, thereby maximising the data obtained from single animals.

A retrospective assessment of reduction will be due by 17 April 2028

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 a variety of mouse models:

Analysis of blood cell function - The injections and superficial vein bleeds will only cause minor and transient pain. Mice will be surgically anaesthetised when terminally bled (exsanguinated) and therefore will not feel pain or suffering.

Haemostasis model -The injections and superficial vein bleeds will only cause minor and transient pain. Mice will be terminally surgically anaesthetised when the tail tip is cut and therefore will not feel pain or suffering as they will not regain consciousness.

Myeloablation (irradiation) - The injections and superficial vein bleeds will only cause minor and transient pain. Myeloablation will be administered at the lowest possible split dose. Following myeloablation animals will be allowed to recover for a minimum of four days before any further procedures occur. During this time the animal's behaviour will be closely monitored and weight measured a minimum of daily.

Transfused mice - The injections of terminally differentiated cells will only happen once and will only cause minor and transient pain.

Pulsed laser microscopy (use of a laser to cause injury) - Mice will be terminally surgically anaesthetised when undergoing pulsed laser microscopy and killed by a schedule 1 method and therefore will not feel pain or suffering as they will not regain consciousness.

Splenectomy (removal of the spleen) - Mice will be subject to general anaesthesia during the surgical removal of the spleen. The wound will then be closed, pain relief will be administered, and the animal will then be allowed to recover from the anaesthesia. Mice will be housed in a warm cage for close monitoring during recovery. During the initial period of recovery from operation the animals will be checked every 10 min, then every hour until they can move freely when lightly disturbed. During this period and in the period post-surgery up to 2 months, endpoints will be monitored. At the end of the protocol, mice will be humanely killed with Schedule 1 method.

Myocardial infarction (MI) - Mice will be surgically anaesthetised and will be mechanically ventilated. The thorax (chest) will be opened and the arteries supplying the heart will be tied off to induce an MI. The wound will then be closed, and pain relief will be administered. The mice then be allowed to recover from the anaesthesia. Mice will be housed in a warm cage for close monitoring during recovery. During the initial period of recovery from surgery the animals will be checked every 10 min, then every hour until they can move freely when lightly disturbed. Animal heart function may be measured with echocardiography during this recovery period. Mice will receive a transfusion of cells via the tail vein post MI. During this period and in the period post-surgery up to 2 months, endpoints will be monitored. In terms of general endpoints, animals showing clear signs of social distress will be killed by a Schedule 1 method. Animals that have a loss of body weight of 20% relative to the baseline will be killed by a Schedule 1 method. Animals will be monitored for distress, reduced mobility, breathing abnormality or uncontrollable bleeding. We will kill mice using a humane method if they display any of the following specific endpoints:

(i) Heart Failure: Any animals showing symptoms and/or body weight loss equal to or greater than 20%, or echocardiography readouts showing 50% or greater reduction in cardiac output relative to baseline

(ii) Post-surgery pain: Any animals showing signs of pain that are not controlled by pain relief

(iii) Bleeding during surgery: Any animals in which bleeding cannot be stopped

(iv) Reopening of the surgical site: Animals with a reopening after the first instance

(v) Surgical site infection: If no improvement is observed within two days of treatment
At the end of the protocol, mice will be humanely killed with Schedule 1 method.

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

Mice are the species of choice for the proposed investigations because they are a good mammalian model with well-characterised haematopoietic (blood) system and, in particular, megakaryocytes and platelets that are very similar to humans. There has been very little work done on platelet function in non-mammalian species. The mouse is the species in which reliable transgene technology is best established. Mouse model of transfusion are also well-established and provide safety and functional data recognized by the regulatory bodies for applications for human trials.

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

To generate transgenic mice (mice with altered genes), wherever possible we will use mice where we can turn on the mutation, rather than having the mutation from birth. This means that we should not see the effects of this mutation until we turn it on. We will only use well-established reagents and protocols to turn on or off the candidate gene. The effects of the mutation will also be limited to the cells of interest using, whenever possible, specific deletion of the gene of interest in the blood system or in cells from which megakaryocytes are derived. When exposing mice to irradiation this may be administered as a split dose to give improved recovery rates.

For the myocardial infarction (heart attack) model and for splenectomy (removal of the spleen) we will use a comprehensive set of clinical sign assessments, humane endpoints, for animals that have undergone surgery. In summary, animals showing clear signs of social distress will be killed by a Schedule 1 method. Animals will be monitored for distress, reduced mobility, breathing abnormality or uncontrollable bleeding, and scored as per a tabulated list based on appearance, clinical signs, unprovoked behaviour and behavioural responses to external stimuli. For a total score that indicates moderate changes animals will be monitored daily, and NVS may be consulted. For a total score that indicates significant changes animals will be monitored closely and be prepared to euthanise the animal. NVS will be consulted. Any animal scoring maximum in any given category, or a maximum total score, will be immediately euthanised.

Clinical score sheet

Appearance

Score= 0. Normal, coat is smooth, lies flat and often has a sheen, eyes are clear and bright. Score= 1. Slightly ruffled coat but no other marked changes

Score= 2. Moderate ruffled coat, eyes and nose may have discharges

Score= 3. Very ruffled coat, external orifices ungroomed, abnormal posture, eyes look pale, pupils enlarged.

NB Check for normal or non-normal urine and faeces

Clinical Signs

0. Respiration appears normal, body temperature feels normal on handling, no twitching behaviour, normal bowel movements.

1. Small changes in above parameters. Weight loss <10% of controls. Up to one toe necrosis (B-D).

2. Body temperature above normal, respiration rapid and shallow, twitching behaviour, altered bowel movements. Weight loss 10-20% of control animals.

3. Marked increase in body temperature, respiration noisy, comatose OR Weight loss >20%. Infection at suture site.

Unprovoked behaviour

This behaviour is best observed from a distance and before any handling is attempted.

0. Normal behaviour pattern

1. Minor changes, e.g. slightly altered walking pattern (WP).

2. Abnormal behaviour, decreased mobility and alertness, inactive. Resolvable dragging of hind legs.

3. Unsolicited vocalisation, self-mutilation, expiratory grunts, very restless or does not move at all

Behavioral responses to external stimuli

Often animals will show inquisitiveness with whisker twitching and sniffing or attempts to escape if frightened. Animals can have good body tone on handling. If the abdominal area of the body is painful then gently pressure and observation is a useful measure to pain.

0. Behavioural responses normal for the expected conditions

1. Shows some minor depression or minor exaggeration of responses.

2. Shows moderate signs of abnormal responses, there may be a change of behaviour.

3. Reacts violently to stimuli or muscular responses may be very weak as in a pre-comatose state

Scores are added for each of the categories above (appearance, clinical signs, unprovoked behaviour, behavioural responses to external stimuli).

For Total Scores 0-2, no action need be taken.

A Total Score of 3-5 indicates moderate changes, which will be monitored daily, and NVS may be consulted.

A Total Score of 6 or 7 indicates significant changes, which will be monitored closely and be prepared to euthanise the animal. NVS will be consulted.

Any animal with a score of 3 in any one of the categories in the tabulated list, or a total score of 8 or above, will be euthanised.

Where possible we will use refinements in husbandry, for example animals will be group housed and extra enrichment will be given to those strains exhibiting aggression. Extra enrichment and a mashed diet will also be given to any animals exhibiting signs of inappetence (not having an appetite) based on weight loss. Additionally we will use less scary handling techniques where possible, and always allow acclimatisation of the animals when brought into the facility before starting a study.

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

We will follow the ARRIVE and LASA guidelines.

We will follow the PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) guidelines.

The humane endpoints webinar (https://www.humane-endpoints.info/en).

The NC3rs resources for breeding and colony management in genetically altered mouse colonies (https://www.nc3rs.org.uk/3rs-resources/breeding-and-colony-management).

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

- We will routinely check the NC3R's resource library and keep abreast with, and implement where appropriate, any new advances in refinement, reduction and replacement.
- We will have regular discussions with the Named Persons and animal technicians within the facility to review current approaches and whether there are any new 3Rs opportunities.
- We are subscribed to an internal 3Rs enquiry list in order to keep up to date with 3Rs news and events, as well as opportunities to share tissues and knowledge.

- We will regularly check an internal website which has a wide variety of resources and information including a 3Rs search tool.

We will subscribe to publications such as ATLA (Alternatives to Laboratory Animals) Journal. We will attend NC3Rs events and workshops where appropriate.

A retrospective assessment of refinement will be due by 17 April 2028

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. Molecular Mechanisms of CNS 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

stroke, ischemia, brain, inflammation

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

The aim of this project is to study the ways in which injury to the central nervous system (brain) affect the body, and to use this knowledge to develop new drugs to treat injury and new ways of diagnosing injury.

A retrospective assessment of these aims will be due by 23 June 2028

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?

When the brain or the spinal cord (central nervous system - CNS) are injured, a cascade of events happen within the body. These begin in the CNS but they affect many other systems. For example, after a stroke or traumatic brain injury patients often experience a rapid activation of the immune system. This can make the original injury much worse. Our knowledge of how this happens is still limited and understanding more about what happens in the brain and the body after a CNS injury would give us more opportunity to intervene in order to slow the progression of the injury. We also have a limited knowledge of how to tell whether new treatments work, whether to stop them or whether to change patients onto a different drug. This project will also aim to use different techniques to develop new tools which will help us make these decisions.

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

Our major outputs from this project will be novel information output as publications, made in relevant scientific journals. We also plan to communicate our findings at regular conferences and conference abstracts are also frequently published. Any novel findings likely to translate to the clinic will be registered as intellectual property with the relevant bodies.

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

Short term benefits: the publications produced during this project are likely to be a short term benefit (>5 years) for the researchers involved. They will enable us to apply for continued funding and to develop as scientists. The pre-clinical CNS injury community are also likely beneficiaries in the short term, as our research outputs will contribute to their knowledge of the field and may ultimately influence the way they conduct their own research.

Long term benefits: the knowledge produced during this project is likely to contribute to clinical practice in >10 years time, and therefore be of benefit to stroke patients. By understanding more about the pathways involved in the progression of CNS injuries such as stroke and traumatic brain injury we are more likely to be able to develop new drugs to prevent these injuries from getting worse, and to prevent many of them from progressing to long term diseases such as dementia which is three times more prevalent than the normal population in patients who've had a traumatic brain injury or a stroke.

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

Collaboration: the participants planning to work under this licence are early career researchers aiming to develop new networks and the research proposed here will allow them to carry this work out with new collaborators

Dissemination of new knowledge: we plan to regularly engage with the scientific community by disseminating our findings on relevant platforms including field-specific journals. We also plan to disseminate knowledge via relevant conferences, with an aim to attend and present work at least once a year.

Negative results: we stand firmly behind the need to publish negative results, and to pre-register large pre-clinical studies. We aim to use platforms specifically designed to publish negative findings.

Species and numbers of animals expected to be used

- Mice: 7900
- Rats: 7900

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.

Whilst CNS injuries such as stroke mainly affect the brain, they also have wide ranging consequences affecting the whole body. As such it is most appropriate to use whole animals to study both novel therapy and the mechanisms underlying cell death in CNS injury. Therapies moving from pre-clinical to clinical will be required to have physiological data from whole animals, something unachievable in cell culture models. Models such as fish and birds have CNS architecture and immune systems which are too diverse from our own to be accurately comparable, as such rodents remain the most sensible choice.

We aim to use both young and old animals, both male and female in this project. It is important to use both sexes in our studies because both males and female patients experience CNS injury. Similarly, aged animals are relevant as the majority of, for example strokes, occur in older patients. However, in our initial studies we will aim to use younger animals as these provide a more robust model with a reduced rate of death from injury. Once our results have been effectively demonstrated in younger animals we will confirm them in an effectively powered group of aged animals of both sexes.

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

Animals will undergo surgery to induce a CNS injury, this may involve surgery to the head (cortical and subcortical areas) or neck, depending on the model. All surgeries will be

carried out under general anaesthesia. Animals will be allowed to recover for a set period of time and subsequent tests will be performed. Time periods of recovery will depend on the outcomes we are interested in. For example if we are interested in the processes going on immediately after the injury, the animals may be killed as soon as 2 hours after the injury induction. However, if we are interested in longer-term recovery the animals may not be killed for ~30 days. During this time animals may be injected with novel drugs, to determine their effect on the outcome of the injury, or they may be subjected to non-invasive imaging procedures in order to view the real-time effects of the injury in a living animal.

We will determine the effects of injury outcome by studying the behaviour of the animals at the time periods above using specifically designed behavioural tests. These aim to study the functional recovery of the animals (for example by measuring the strength of the animals grip or how well it balances on a narrow ledge), something which is important if there is an aim to translate any novel findings into the clinic. Finally, all animals will be killed at the end of their designated experiment and tissue will be collected to study the effects of the injury on not only the brain, but also other organs in the body.

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

Animals experiencing a CNS injury to one side of their brain exhibit many of the same symptoms as those experienced by humans. It should be noted that injury to the brain is not inherently painful as there are no sensory nerves in either region. Any pain experienced by the animals will be transient and as a direct result of the surgery required to induce the injury. This pain is managed with analgesia (pain relieving medicine) and does not significantly interfere with the behaviour of the animals, as demonstrated by the minimal effects of sham surgery (surgery where no CNS injury is induced) on animal behaviour.

For example in models of stroke we would induce a stroke by surgical means, either by direct injection of a substance into the brain or by blocking a blood vessel supplying the brain. Animals undergoing this procedure are likely to develop paralysis on one side of their body. They are likely to stop eating for a short period of time because of partial paralysis of the facial muscles. These effects prevent the animals from undertaking normal day-to-day behaviours such as eating and grooming. As such they are likely to lose weight and appear dishevelled. For moderate strokes, such as those caused by the occlusion of a major blood vessel. these symptoms are likely to last around 72 hours, at which point the animal begins to recover some functionality and weight loss ceases. For severe strokes, which can also be caused by the occlusion of a major blood vessel. these symptoms can persist beyond 72 hours. For mild stroke models, which are made by injecting specific substances into the brain. and models studying inflammation in the circulation animals are unlikely to exhibit any major symptoms beyond some slightly altered grooming behaviour in the first 24 hours. Where any of these adverse conditions surpass expected severity limits animals will be 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)?

Mice

Subthreshold/Non-Recovery: 2% Mild: 28% Moderate: 60% Severe: 10%

Rats

Subthreshold/Non-Recovery: 10% Mild: 10% Moderate: 75% Severe: 5%

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

- Killed

A retrospective assessment of these predicted harms will be due by 23 June 2028

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?

This project aims to study the effect of CNS injury on the body, as well as to study the effect of various interventions on the CNS injury. As such, whole animals are the most appropriate model. Rodents have a similar immune system and basic nervous system to humans, and their response to CNS injury is pathologically similar, i.e. it demonstrates broadly the same cellular changes in the brain and body over time. This makes the rodent model the most appropriate to use to study CNS injury. We continue to have ongoing collaborative projects with clinical researchers which aim to validate our pre-clinical findings. For example, the ethics are now in place for hyperpolarised imaging in human stroke patients and therefore one of our aims is to compare these images to the data we get from our rodent stroke models to determine the degree of similarity.

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

We continue to work with static cell cultures and 3D cell cultures in conjunction with our rodent models. These are becoming increasingly popular and with the use of novel techniques involving stem cells we have been able to develop cell culture models of blood

vessels. We will continue to compare these models with our animal models in order to validate the new cell culture models and with the long term aim of being able to replace many of the animal experiments with cells.

Why were they not suitable?

Cell culture models do not reflect the whole body status of the patient in the clinic. Individual brain cells grown in a dish do not represent the complex network of cells which exist in the living brain. However, by combining these techniques with our rodent models we are able to study the effects of interventions, or drugs, on specific cell populations. For example, it is relatively straightforward to culture some immune cells in a dish, and to study the effects of inflammation on them. Inflammation something which happens a lot after CNS injury and so studying how immune cells behave is important and can be done in isolation and compared to whole animal models.

A retrospective assessment of replacement will be due by 23 June 2028

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 outlined in this licence is based on our previous experimental experience from an existing project licence.

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

We use specific tools to calculate the power of our experiments, in order to determine the appropriate number of animals required for each experiment. We have significant previous data from the field demonstrating what specific interventions might do to the outcome of an injury and we use this collective data to plan our experiments. We use power calculation tools such as G*Power and specific

experimental design tools such as the NC3R’s Experimental Design Assistant. We also adhere to the PRERPARE and ARRIVE guidelines and publish these alongside our results where necessary. Finally we also aim to adhere to the recently published STAIR criteria for

pre-clinical stroke work, which encourages optimal experimental design for pre-clinical brain injury work.

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 compounds and interventional strategies, pilot studies will be undertaken with a small number of animals. The specific outcomes for each experiment will determine the numbers required for these pilot studies. For example, our previous experience tells us that if we are looking for an inflammatory response after a CNS injury this is likely to be quite robust. Therefore when testing a new compound designed to interfere with this response the number of animals required would not be large. However, our experience also tells us that if we are looking for subtle behavioural improvements after CNS injury we are likely to require more animals to see an effect of any new intervention.

For experiments using transgenic animals optimal breeding protocols will be put in place to manage the colony. We have access to an in-house digital colony management system which enables us to monitor colonies remotely, including ages, breeding data and litter sizes. For experiments where tissue culture is being used in addition to rodent experiments we plan to utilise surplus breeding animals, rather than to specifically purchase animals for this purpose. However, in the majority of cases transgenic animals will be bought in directly from external breeders, this reduces excess heterozygous animals from being culled through local breeding and reduces the possibility of over-breeding. The only cases where this will not be performed is where lines are not commercially available but rather have been bred and kept in a specific collaborative laboratory.

For experiments where we only require one tissue, such as the brain, we aim to collect all the remaining tissue and advertise it on tissue sharing schemes. The UK pre-clinical stroke forum is a large advocate of this strategy and has a nationwide tissue sharing scheme available to all participants.

A retrospective assessment of reduction will be due by 23 June 2028

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 variability of the human CNS injury population is often cited as a reason for the failure of the translation of pre-clinical research to develop new drugs to treat it, and thus the capacity to test both therapy and to study mechanism in a number of different models is important. It is also important to note that the majority of CNS injuries happen because of some external factor and cannot be induced by genetic mutations. Therefore the only way to produce, for example, ischemic brain injury is to use surgery.

Where possible we aim to use the least severe injury model for each experiment. For example, when studying a novel stroke therapy we may begin with pilot experiments using a brain injection of endothelin-1. This causes local shrinking of the blood vessels and a small stroke on one side of the brain. These animals suffer from few adverse effects, with the exception of some mild paralysis on one side of their body. The surgery is minimally invasive and can be performed in under 15 minutes, the animals recover quickly and exhibit no to minimal weight loss. However, if we wish to translate our findings into the clinic a more relevant model, such as one where we occlude a major blood vessel to the brain, must be used. This model takes up to 3 hours to perform surgically and the animals often suffer severe paralysis and weight loss but it more accurately represents what happens in the clinic to patients suffering from a stroke.

We plan to use both mice and rats for this project. Mice allow us to introduce transgenic lines, where we might genetically label different cell populations specific colours so that they can be monitored, or we might over or under express specific proteins of interest in order to study their role in disease outcomes. Where transgenic animals are used that express a phenotype, we will always choose a background strain which results in the mildest possible expression of that phenotype. We will use rats in some instances because they are more amenable to specific outcomes. For example, the endothelin-1 model of stroke does not work in mice but is extremely reproducible in rats. Rats also provide a good brain volume for in vivo imaging techniques such as hyperpolarised MRI, where the mouse brain is slightly too small to get good image resolution with the current technology available.

Where possible we will aim to confirm our hypotheses in both species, in alignment with national guidelines.

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

We will aim to use terminally anaesthetised animals for some of our imaging experiments, where the animals will undergo the CNS injury whilst being imaged and be killed whilst under anaesthesia.

However, for the majority of our experiments we need to determine the effect of the damage to the CNS beyond the initial injury. This requires the animals to have a fully developed nervous system and a fully developed immune system, something which species such as zebrafish or flatworms do not have.

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

All surgery will be carried out under general anaesthesia. Animals will be given appropriate post- operative analgesia (pain relieving medication). Where possible this will be administered in palatable foodstuffs, for example nutella and gel food treated with sweet liquids, rather than by injection thus reducing the need for restraint and the possibility of multiple injections. The analgesic regime here aims to minimise pain, without having significant effects on outcome measures such as inflammation. Assessing pain in CNS injury animals is extremely difficult because they often exhibit paralysis which prevents normal behaviour. We have noted that in sham injured animals (where no CNS injury occurs but where surgery is carried out) that local pain relief is effective for managing surgical pain and based on basic pain scoring seems not to be required from ~48 hours post-surgery. In all our models close monitoring of animals will take place in the immediate hours post-surgery, especially in those models where significant behavioural changes such as paralysis are likely to occur. More frequent monitoring of these animals will also take place over the following days to ensure there is no new development of pain as a result of their CNS injury.

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

We aim to conduct our experiments in accordance with the published NC3Rs guidelines, as well as LASA guidelines. In addition there are CNS injury specific protocols published (STAIR criteria) which we will also aim to adhere to in the relevant models.

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

Our establishment has regular NC3Rs update meetings throughout the year and regularly sends out information about national webinars and novel discoveries relating to NC3Rs research. We have a local NC3Rs representative who is readily available for both formal and informal meetings. We will also aim to keep ourselves updated by regularly visiting the NC3Rs and science.rspca websites.

A retrospective assessment of refinement will be due by 23 June 2028

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. Chemical Ecotoxicological Testing in Fish and Amphibians

Project duration

5 years 0 months

Project purpose

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

Key words

Fish, Frogs, Ecotoxicology, Regulatory risk assessment

Animal types	Life stages
Xenopus laevis	juvenile
Zebra fish (Danio rerio)	embryo, juvenile, adult
Medaka (Oryzias latipes)	embryo, juvenile, adult
Brown Trout (Salmo Trutta)	juvenile
Rainbow Trout (Oncorhynchus mykiss)	juvenile
Fathead minnow (Pimephales promelas)	adult, embryo, 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 performance of ecotoxicological studies for the provision of toxicity data to produce dossiers used by risk assessors for assessing and managing the risk associated with the production, use and release of chemicals which are used in or may be released into the environment. These chemicals include plant protection products, pharmaceuticals and any chemicals required under REACH regulations.
Studies typically involve investigations into effects of chemicals on the development, growth, behaviour and reproduction of fish and endocrine disruption in amphibians. Baseline toxicity to fish and frogs is also required – this involves investigating the level of

exposure to the particular substance under test that will result in 50% death of a group of fish (acute toxicity tests). In some cases it may be possible to euthanise animals to reduce suffering - we do this when possible.

A retrospective assessment of these aims will be due by 25 April 2028

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 production of data forms part of the chemical risk dossiers which are used by regulators and authorities to formulate risk assessments for production and use of chemicals as well as their release into the natural environment. Environmental exposure may cause harm to the natural biota. Chemicals that accumulate in fish can pose a risk to end consumers or cause chronic damage to the natural environment and biodiversity. Chemicals may also cause endocrine disruption in humans and other wildlife. Providing data which allows authorities to regulate the production and release of these chemicals is vital for environmental protection and human health.

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

The project will produce numerous studies which satisfy the requirements of the regulatory authorities. Toxicology data is generated in accordance with standardised international guidelines. Typically, studies will follow guidelines written by the Organisation for Economic Cooperation and Development (OECD) or by the United States Environmental Protection Agency (US EPA). Following these guidelines ensures that internationally accepted, good quality, standardised and reliable data can be obtained.

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

The work conducted on this project will provide manufacturers and consultants with high quality fish and frog toxicity data produced in accordance with regulatory requirement and to internationally recognised guideline standards. This data forms part of chemical risk dossiers which will in turn be used by sponsors and regulators to formulate risk assessments for the production and use of such chemicals as well as their release into the natural environment. Once in the environment these chemicals will be of potential harm to the natural biota and, in turn, humans. The work of this project will provide information that will be used in safety evaluation assessments with a view to mitigating that risk and minimise as far as possible any contingent harms arising. Chemicals may accumulate in fish and pose a threat to end consumers, or they may cause chronic damage to the natural environment and biodiversity. Additionally, chemicals may also affect the endocrine system in humans and wildlife, therefore fish and amphibian studies are also required to provide this information. Understanding the effects of these chemicals and placing controls on their production and release is vital in protecting the natural environment and human

kind. The benefits arising from the work covered in this project include short-medium term benefits in regard to risk assessment of individual test substances/chemicals but are also beneficial in the long-term care and management of the environment.

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

By performing these studies to a globally accepted standard in terms of GLP accreditation and by following the internationally accepted test guidelines we will produce studies of a high standard which will be used and mutually accepted by regulatory authorities around the world. This means that the same work should not need to be repeated by another facility unnecessarily. We also share our developments and refinements of studies on global platforms such as the European SETAC conference which brings together scientists and academics in similar fields from all over the world.

Species and numbers of animals expected to be used

- Xenopus laevis: 10000
- Zebra fish (Danio rerio): 3850
- Medaka (Oryzias latipes): 1850
- Brown Trout (Salmo Trutta): 500
- Rainbow Trout (Oncorhynchus mykiss): 3250
- 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.

Once a sponsor has conducted appropriate modelling, in vitro and lower organism studies, they may seek to employ our services to conduct fish or amphibian toxicity testing. The specific tests conducted will be dictated by the sponsor requirements, often in consultation with a regulatory body such as the Chemical Regulation Directorate (CRD) on a case by case basis. The sponsor will typically provide information about the physicochemical properties of the test substance and the specific tests they require. Only when we are satisfied with the quality of the information provided, and that fish or amphibian tests are justified and necessary, will we proceed with in vivo fish or amphibian toxicity tests. The variety of test guidelines which are covered under this projects means that we may use a variety of different life stages ranging from freshly fertilised embryos through to adults depending on which test guideline is required.

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

Fish or frogs are typically exposed to one or more concentrations of the test chemical. Depending on the study type, exposures may be very short (less than 24 hours up to 4 days, acute studies), or they may be longer term with exposures in the region of 3 weeks or even several months (chronic studies). Most of the studies require the test chemical to be applied to the water (aqueous exposure) but there are some occasions where the chemical may be applied via the food (dietary exposure). Test chemical groups are compared to a control group(s), containing no chemical to ascertain the effect of the test chemical on the key endpoints of the study type (e.g. survival, growth, development,

endocrine activity or reproductive capacity). In most study types the procedures used are exposure to a potential toxin in the water or food. In some studies another procedure is to withdraw food but this would only be in an acute study type where exposure only via the water is needed and fish eating may result in ingestion. In some studies there is an additional procedure in which a terminally anaesthetised fish has blood collected from it to analyse for the effects of the test chemical on the hormones in the blood.

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

For acute toxicity tests (the most severe of the tests) involving exposure of fish or frogs to the test substance in water the highest concentration(s) will likely cause death – this usually occurs in a very short period of time, usually within hours but typically within 96 hours (4 days). Symptoms may include change of behaviour, respiratory distress, loss of equilibrium and loss of coordination. Effects seen in chronic studies are normally less severe and may be changes in their usual development (i.e. growth defects such as curved spines) or reduced growth rates. If these effects are not affecting the overall

quality of life they may last for the total duration of the study. However, if it is deemed that these effects are causing suffering, the animal will be humanely euthanised prior to the end of the study to limit the 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)?

Fish or frogs exposed to the highest concentrations in acute tests are most likely to experience the acute effects listed above leading to death (this means a ‘severe’ category). Fish or frogs exposed to lower doses of chemical may be less active or exhibit some of the symptoms given above to a lesser extent and over a shorter period of time. In these cases the severity level will be ‘moderate’ or ‘low’ depending on the period of time the symptoms persist. For chronic toxicity studies the severity levels will be lower as the studies are designed to show sublethal effects. As such, effects will typically fall in the ‘low’ category or below (‘sub-threshold’). For the animals covered by this licence, we would estimate that 14% would suffer 'severe' severity, 24% 'moderate', 20% 'mild' and 42% 'sub-threshold'.

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

- Killed

A retrospective assessment of these predicted harms will be due by 25 April 2028

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 regulatory guidelines require the use of key model species in the generation of toxicity data. Fish and amphibians are two of these key model species. When a sponsor requests a test involving the use of fish or amphibians we make sure that there is no alternative test which could be done which does not use these animals. We also make sure that the test requested is the one most suitable to answer the scientific question being asked.

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

There may be non-animal options for sponsors before they need to employ our services such as (Q)SARs, ((quantitative) structure-activity relationship), TTS (total toxicity scores), read across data and alternative in vivo methods with potentially more sensitive species like Daphnids and algae. If the formulation of a sound risk assessment is not possible after these initial steps then the use of in vivo fish or amphibian tests will be implemented. We always ensure that sponsors have exhausted their alternative options before committing to performing fish or frog studies.

The Fish Embryo Toxicity test (FET) is also a useful new tool for screening some chemicals prior to the use of tests involving protected life stages. Ex vivo tissue and cell tests are also being improved. We will explore the use of these tests but ultimately these use animals too.

Why were they not suitable?

There are regulatory requirements to carry out specific fish and frog studies as detailed in this project so non-animal alternatives are simply not accepted by regulators at this stage. If these studies have to be done it is best that they are carried out following the required guidelines, to the highest possible standard, by a suitably experienced and accredited facility like ours.

A retrospective assessment of replacement will be due by 25 April 2028

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?

An estimation of the type of studies we will have for the duration of the 5 year project, combined with the current capacity for studies in the facility. The numbers factor in the possibility that some species will be used because they are listed in the guideline, however there is a high likelihood that the 'preferred' species will be used and as such the variety of species is also likely to be less than given here. It is likely that fewer animals will be used as the type of work we get is variable year-on-year so these values are estimates of the maximum possible usage.

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

Studies follow a standardised design detailed within the international guideline, however all studies undertaken will be refined such that the lowest number of animals possible are used. Limit tests, which reduce the number of fish required, will be carried out if fish are known, or are shown, not to be sensitive to a test substance. It may also be possible to use the threshold approach for fish toxicity testing where the lowest EC50 (Effective Concentration which produces a 50% effect) from the algae and daphnia toxicity tests is used as the limit concentration in the same manner as a limit test. In certain cases (i.e. without sufficient toxicity data available, or with difficult test items) it may be beneficial to perform a preliminary range finding or scoping test with reduced numbers of fish or frogs to allow dose setting for the definitive test. A range finder allows a small number of fish or frogs to be used to determine the correct dose range for a full study - eliminating the risk of performing a full study without finding the desired statistical endpoint which would result in repeating the study with a different dose range.

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 endeavour to ensure that the test is required and suggest a lower severity test or test with fewer numbers of fish or frogs if at all possible. For example, performing a fish acute toxicity test to the OECD guideline typically requires 42 fish, whereas the US EPA guideline requires 120 fish. In such cases we will do everything possible to ensure that the OECD guideline is the one followed. If necessary, (with sound scientific reasoning from the sponsor, with which we concur) or if the minimum number detailed in a guideline is not deemed to provide data of sufficiently statistically robust quality to answer the scientific question posed, then numbers and replicates may be increased using decision processes such as power analyses. In any proposed case where the number of animals required exceeds the numbers in the UK/EU guidelines, we will seek prospective authority from the Home Office before committing to the study.

A retrospective assessment of reduction will be due by 25 April 2028

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 OECD test guidelines (and their US EPA equivalent) we will use include, but are not limited to:

1. TG 203 Fish acute toxicity test (OCSPP 850.1075)

2. TG 210 Fish early-life stage toxicity test (OCSPP 850.1400)

3. TG 215 Fish juvenile growth test

4. TG 229 Fish short term reproduction assay (OCSPP 890.1350)

5. TG 230 21-day fish screening assay

6. TG 234 Fish sexual development test

7. TG 305 Bioaccumulation in fish: Aqueous and Dietary Exposure (OCSPP 850.1730)

8. TG draft Fish life cycle toxicity test (OCSPP 850.1500)

9. TG 231 The Amphibian Metamorphosis Assay (OCSPP 890.1100)

We may also be employed to perform the EC guideline (currently in draft) for the 'Magnitude of pesticides residues in fish'.

The OECD approved test guidelines have been refined such that they yield the best data possible from the lowest number of animals. The TG 203 was revised in 2019 to include the possibility of euthanising fish to limit suffering but still generating usable LC50 moribund data. TG 210 has the potential for some mortality but the guideline does allow for euthanasia of animals to limit suffering. All of the other guidelines listed here are sub-lethal in their design and therefore the likelihood of prolonged pain, suffering, distress or lasting harm is reduced. Animals used in these guidelines can also be euthanised if required to limit suffering. In any proposed case where the number of animals required exceeds the UK/EU guidelines, we will seek prospective authority from the Home Office before committing to the study.

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

The guidelines are designed to look for specific effects in specific species and life stages. As such the refinement process in choosing the most appropriate animals and life stages has already been done. There are some things we have the ability to refine such as the chemical exposure pattern or the specifics of the test tank setup to better replicate the likely exposure profile in the environment. In these cases, the refined higher tier tests mitigate the need for using animals with a more efficient and better quality study design. It would be counter-productive to try to use less sentient animals in some of these studies

because the data obtained might not be sufficient to protect the more sentient animals in the environment.

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

The department has highly trained staff with the ability to dedicate more time to both planning the tests and monitoring the animals during the tests. As such, time and effort is applied to ensure that the studies are performed to the highest standard on the first time of asking. The preliminary work we perform is done to make sure that when we have to use fish or frogs we do so with as much information as possible to minimise errors and maximise quality data output. If necessary a dose range finder test may be performed on a minimal number of animals (a pilot study) to ensure that the dose range for the definitive study is optimised. This reduces the risk of performing a full scale definitive study (many more animals used than a range finder) with the incorrect dose range. We also dedicate more time than is strictly required by the guidelines to ensure that we are checking the animals regularly. This means that if a fish or frog is suffering and we are allowed to euthanise it we will identify the individual quickly and act to reduce that suffering to an absolute minimum. We are also working towards another refinement by recording key clinical signs data to inform us when a fish becomes ill beyond recovery. This will enable us to euthanise fish rather than waiting for mortality.

Handling procedures will be minimised and carried out by trained staff to limit stress where possible. Marking or tagging of fish will only be conducted where necessary and in a manner that causes no lasting pain, distress, or lasting harm. Water quality is kept at a premium (better than the basic levels required in the test guidelines) wherever possible. Good husbandry management and training in animal handling by experienced personnel will ensure the highest standards of care are given to the animals prior to, during and at the end of toxicity tests. Brood stock fish are given environmental enrichment such as live food and internal tank structures wherever possible and test fish can be given mirrors at the end of each row of tanks to minimise stress by mimicking company if possible and allowed in the study design.

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

The OECD has published not only the specific test guidelines which we typically follow, but also guidance on various other factors such as how to deal with difficult test substances and how to perform statistical analyses on study data. There are other guidance documents on things such as practical techniques for fish blood collection and histological sampling and processing techniques for fish and frogs.

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

We have a Named Information Officer who will be monitoring improvements in the field of animal care and best practises for husbandry techniques. We have a Named Animal Care and Welfare Officer who is independent from the directorship of studies to monitor the stock and study fish and frogs we have on site. They make sure that we perform studies to the highest standard. We are on various publication notice services with several external organisations such as the NC3Rs, the RSPCA and the Home Office Liaison, Training and Information Forum. All of these organisations are excellent sources of information and

updates on developments in the field of the 3Rs. The team has experienced and interested staff so it is easy to communicate any changes or ideas effectively.

A retrospective assessment of refinement will be due by 25 April 2028

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. Fish Telemetry Investigations to Inform Effective Management Actions

Project duration

5 years 0 months

Project purpose

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

Key words

Fish, telemetry, migration, movement, behaviour

Animal types	Life stages
Rainbow Trout (Oncorhynchus mykiss)	juvenile, adult
Brown Trout (Salmo Trutta)	juvenile, adult
Salmon (Salmo salar)	juvenile, adult
Allis shad (Alosa alosa)	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 overall aim of this project is to implant fish with marks or tags, using well-established methods and techniques, during targeted investigations to monitor their movements, behaviour and habitat use in order to assess the impact of anthropogenic pressures and the effectiveness management actions, often driven by legislation.

A retrospective assessment of these aims will be due by 15 February 2028

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?

Fish populations in the UK, including salmonids, coarse fish and conservation species (e.g. lamprey, eel and shad), face many pressures in the freshwater environment, including exploitation, fish stocking, pollution, abstraction, navigation, power generation, flow regulation and habitat modification.
Understanding these pressures, which will be locally specific, is crucial to the implementation of effective management actions and targeted rehabilitation to maintain or restore fish populations.

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

The research will be written into technical reports (for funders), PhD theses and published in international peer-reviewed scientific journals. The outputs will be discussed during project specific stakeholder meetings and disseminated in presentations at national and international stakeholder meetings, technical workshops and academic conferences.

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

The main beneficiaries of knowledge arising from this research is anticipated to be governing bodies (e.g. the Environment Agency in England and their equivalents across Europe), who will be able to use outputs to inform and revise policy, regulation or operational best practice. Implementation of policy, regulatory or operational advancements will ensure freshwater environments worldwide are sustainably managed within legislative frameworks to deliver positive environmental outcomes.

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

The outputs from this research will be maximised by working closely with project specific stakeholder networks and disseminated using appropriate written (e.g. peer-reviewed scientific journals) and oral communication (e.g. international conferences) routes.

Species and numbers of animals expected to be used

- Rainbow Trout (Oncorhynchus mykiss): <1,000
- Brown Trout (Salmo Trutta): <1,000
- Salmon (Salmo salar): <1,000
- 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.
This study is focused on wild fish in a locally specific situation.

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

Fish will be implanted with marks and tags, using well-established methods and techniques, sometimes under general anaesthesia, to monitor their movements, behaviour and habitat use.

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

The procedures in Protocol 1 will either be very brief (subcutaneous and fin marking and tagging) or carried out under general anaesthesia but fish will be subjected to no more than mild and transient stress arising from capture and handling and may experience mild post-operative discomfort. Indeed, the fish are expected to make a rapid and unremarkable recovery and are not expected to experience any lasting harm.

During Protocol 2, I expect the fish to make a rapid and unremarkable recovery from being captured and tagged, and are not expected to experience any lasting harm as a result. The tagging procedures in this protocol will be carried out under general anaesthesia and fish will therefore be subjected to no more than mild and transient stress arising from capture and handling and may experience mild post-operative discomfort. After release, tagged fish may pass through a pumping station / hydropower development and could incur serious injury and / or die. However, the pumps and turbines under investigation are marketed as ‘fish-friendly’, and thus should, in theory, have the potential to reduce or eliminate fish and eel injury and mortality during passage. Fish considered unlikely to recover from injuries will be humanely killed. Recaptured fish may be held in covered keep-cages to quantify and fully understand the processes that lead to delayed mortality or recovery from slight abnormal behaviour and/or moderate injury.

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)?

- Minor = 80%
- Moderate = 18%
- Severe = 2%

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

- Set free Killed

A retrospective assessment of these predicted harms will be due by 15 February 2028

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?
This study is focused on wild fish in a locally specific situation; therefore a non-animal alternative approach cannot be used and all non-tagging alternatives are technically inadequate.

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

Underwater cameras and sonar are non-animal alternatives, while farmed fish could be an alternative approach to using wild fish.

Why were they not suitable?

Underwater cameras and sonar can and will be applied where relevant, but they typically have short range, cannot identify individual fish and have technical limitations, such as do not work well in bubbly environments and deep water, and require secure sites with power supply.

Farmed fish may not behave naturally in locally specific situations. Farmed fish have not developed with the same pressures as wild fish and for this reason they are often ill suited to release with high mortality rates, predation and relocation making their use unpractical in some instances.

A retrospective assessment of replacement will be due by 15 February 2028

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 fish to be tagged during this PPL has been informed by experiences gathered during two previous PPLs and the requirement for the total number does not exceed the cumulative number during multiple specific investigations.

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

The number of fish to be tagged will vary between individual investigations and is typically informed by similar investigations performed by myself and/or published in peer reviewed literature.

Thought will be given to the study objectives, location and species to determine the most appropriate capture method and likely capture rate, and the likely number that will be tagged; only realistic and affordable investigations that use the lowest number of animals for scientific relevance will be planned.

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

A pilot study may be performed to inform full study design. In some instances, investigations may incorporate plans to tag smaller numbers of fish in numerous tranches, enabling an opportunity for

analysis of detection/recapture rate and, conversely, loss rate to be determined and influence whether further tranches of fish need to be tagged and/or the amount of fish in each tranche; tagging will stop when robust findings are gathered. Further, most investigations are conducted over prolonged periods of time and thus while not planned, per se, enable a similar opportunity to review findings and inform whether more fish need to be tagged to achieve study objectives. In all instances, the fewest number of fish will be tagged to achieve the study objectives.

A retrospective assessment of reduction will be due by 15 February 2028

The PPL holder will be required to disclose:

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

Refinement

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

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

Wild fish will be tagged under anaesthetic to ensure no pain is experienced, except during minor procedures when holding fish during and after anaesthesia and/or clearing the anaesthetic after sedation are considered more stressful to the fish. In some instances the procedure will involve a small incision on the belly of the fish, an inert and sterile transmitter inserted and the incision closed with a suture to ensure full recovery and minimal suffering.

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

This PPL will be focused on wild fish in a locally specific situation; therefore, a non-animal alternative approach cannot be used and all non-tagging alternatives are technically inadequate.

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

In all instances, fish capture will only be performed by a competent person(s) using the least harmful and most reliable technique for the study location and species, and will only use well-maintained equipment. Fish will always have the most appropriate mark or tag will always implanted in the most appropriate location to minimise adverse effects. The smallest transmitter to achieve the study objectives will always be employed. Tags will only be implanted by suitably trained and qualitied individuals and aseptic conditions will be maintained during the surgery. In some instances, the adverse effects from capture, tagging or being held tanks will be reduced by adding Protex hydro,

Virkon S and Vidalife to holding tank after capture. These water treatments stimulate the production of heat-shock proteins and enhance the fish's ability to deal with stress, reduce the amount of bacteria in the water to reduce the likelihood of infection and preserves the fish's natural mucous layer to minimise vulnerability to pathogens, respectively. Only once fish have fully recovered from procedures in this protocol will they be released at the study site; assessment performed by a competent person(s)

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

To my knowledge, best practice guidance for fish telemetry techniques has not been published, per se, although the findings from published empirical investigations and literature reviews have been incorporated into this PPL. Where necessary, I may introduce refinements based on personal experiences.

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

As PPL holder, I will keep myself informed of advances in 3Rs through the NC3Rs website, reading published literature, attending fish telemetry specific conferences, routine dialogue with fellow fish telemetrists, seeking advice from the NACWO and NVS, consulting regulatory organisations and through betterment of my own personal practices. If / when necessary, PPL amendments will be applied for.

A retrospective assessment of refinement will be due by 15 February 2028

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. Understanding Mechanisms of Endogenous Regeneration in the Adult Heart

Project duration

5 years 0 months

Project purpose

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

Key words

MYOCARDIAL INFARCTION, NEOVASCULARISATION, THERAPY, ANGIOGENESIS, REGENERATION

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 define the mechanisms through which cells in the adult heart divide and communicate to maintain structural integrity in healthy conditions and contribute to repair, regeneration and remodelling of the heart, in response to a heart attack (known as a myocardial infarction). This work will identify and test new targets that may control regenerative responses in the adult heart following injury, with a focus on blood vessel regeneration and finding new information regarding how different cells in the heart communicate and interact.

A retrospective assessment of these aims will be due by 28 May 2028

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?

Heart failure following acute myocardial infarction (MI) affects over 26 million patients worldwide and is now widely considered a global epidemic. There is no current cure for heart failure and patients have a poor prognosis with less than 50% survival rate after 5 years. The Transnational Alliance for Regenerative Therapies in Cardiovascular Syndromes (TACTICS) have recently published a consensus statement outlining 7 key mechanisms for heart repair and regeneration (Circulation Research. 2018;122:199–201). These are:

1. Survival and Protection

2. Inflammation reduction (neutrophil-endothelial interactions)

3. Cell-cell communication (EndMT: EC-derived fibroblasts)

4. Angiogenesis/ vascularisation

5. Cardiomyogenesis

6. Molecular regulation of proliferation and cell cycle

7. Ageing

It is well-documented that each of these mechanisms can act both independently but also collectively. However, little is understood about the innate regenerative response of cardiac and vascular tissues, which forms the focus of this project, with a long-term aim to identify new therapeutic approaches to modulate these regenerative and reparative processes.

Autologous bone marrow cells have been delivered to thousands of patients with heart disease, on the premise that they contain so-called endothelial progenitor cells (EPC)-thought to be capable of forming new blood vessel networks. However, the outcomes of these studies have proven disappointing showing very little in the way of any direct patient benefit. One potential explanation for this lies in our recent finding that EPC do not originate from the bone marrow in humans, as widely believed. This represents a paradigm shift that mandates a reevaluation of our approach to harness EPC for clinical regeneration and highlights the necessity for new strategies to prevent or delay progression to heart failure after MI. One approach will be to interrogate existing regenerative pathways in the adult heart. However, very little is understood about the mechanisms that regulate endogenous myocardial regeneration.

We have recently shown that blood vessel regeneration in the adult mouse heart following acute MI is primarily driven by a population of cells, known as endothelial cells, which form the lining of our blood vessels- and not from the bone marrow . In the same study, we mapped the expression of genes in the endothelial cells in the heart from the healthy and injured adult mouse at 7 days post-MI. This generated an in-depth atlas of novel targets, which will be studied in the project proposed herein to evaluate their role in blood vessel regeneration and improving overall heart function.

In summary, a clearer understanding of the cellular and molecular mechanisms of endogenous cardiac repair, including with morphological context, will provide valuable information to assist the future development of new clinical treatments.

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

1. New information to address fundamental unknowns associated with endogenous mechanisms of myocardial regeneration including a spatiotemporal analysis of tissue regeneration in the infarcted adult heart, cross-talk between vascular lineages and other cardiac, lymphatic and inflammatory cells, and insight into the key regulatory pathways of myocardial neovasculogenesis post-MI. We will identify novel targets associated with myocardial neovasculogenesis and validate these targets in vitro and in vivo, including in human tissues and cell lines. This may lead to the generation of new products in the form of transgenic mice.

2. We aim to publish this research in high-impact factor journals and to apply for large funding applications to support future studies leading from this work.

3. We will disseminate data from these studies at UK and International conferences. We will also participate in public engagement to communicate our research to the public and increase awareness.

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

We believe that these studies will benefit:

1. Numerous academic communities e.g. vascular biologists, cardiovascular scientists, single cell/ spatial transcriptomics researchers in the short-term.

2. The University, College and Centre will benefit from any significant publications or funding awarded as a result of these studies in the short term

3. In the longer-term, we predict that these studies may have impact at the clinical level e.g. (i) an in- depth understanding of the cellular mechanisms associated with endogenous repair in the infarcted adult heart will provide critical insight into future clinical therapeutic strategies for patients with heart disease (ii) the identification and validation of novel targets shown to provide significant benefit in pre- clinical models.

4. Novel therapeutic targets identified through these studies may be of interest/ benefit to the pharmaceutical industry or companies with an interest in therapeutic angiogenesis (mid-long term).

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

The outputs of this work will be used to strengthen existing collaborations and to establish new collaborations with UK and Internationally-based academics, with Industrial/pharmaceutical companies interested in angiogenesis, and with the University clinical and surgical community to ensure rapid clinical translation of findings.

We aim to disseminate new knowledge through publication in 4* journals, and will use preprint repositories such as bioRxiv. This will include negative findings and unsuccessful approaches, to prevent unnessary replication of experiments. We will also aim to present data at internal and invited seminars, and at UK and International academic meetings.

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.

We study mice because their genome, cardiovascular system and immune responses are very similar to those of humans. The mouse is an established model system to test gene function including alterations in genes that may affect tissue regeneration after a heart attack. Moreover, mouse models also permit precise study of potential therapeutic agents. We will study adult mice and many of our models are multispectral lineage-tracing models that will allow us to visualise cell fate and intracellular relationships in a spatiotemporal fashion. We will study phenotype in new genetically altered mice during different life stages in order to gain a comprehensive understanding of how the genetic alteration affects development and tissue function. The coronary artery ligation model will only be undertaken on young adult mice in order to reduce mortality.

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

Most experiments will involve the switching on of a fluorescent label in a specific cell type in adult mice (wild type or genetically altered models). This will allow us to visualise the fate of that cell during normal healthy circumstances in the adult heart, and the short- and long-term responses of that cell type (changes in behaviour, division, and gene expression) to injury similar to that caused by a heart attack (myocardial infarction), including interactions with other cell types. Mice will then undergo myocardial infarction by permanent ligation of the LAD coronary artery followed by imaging (e.g. by echocardiography) at key timepoints. Mice undergoing coronary artery ligation will be anaethetised, intubated and ventilated. A left thoracotomy will be performed as a small incision between two ribs, the pericardium opened and a suture placed around the proximal left anterior descending artery. The thorax will then be closed and intubation maintained until the animal is able to breathe spontaneously. Mice will be provided homeothermic support until able to independently regulate body temperature.
Mice will be housed singly (males) or in pairs and monitored rigourously to detect any signs of pain, distress or ill health. Anaesthetics will be given before surgery and analgesics given. Mice may be administered a substance thought to influence tissue regeneration at key timepoints by injection. At one - two hours prior to cull mice may be injected intravenously with isolectin to label perfused vasculature and/or a reagent used to

label proliferating cells (e.g. EdU) will be administered subcutaneously. Mice will be humanely killed and tissues collected for analysis.

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

The animals subject to the coronary artery ligation may experience a range of effects such as pain, lethargy and weight loss, which will be managed with approriate analgesics, good surgical technique and post-surgical care and close monitoring. The estimated duration of these effects are expected to be less than 24 hours post-surgery.

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 50% of mice will be used for establishment and maintenance of genetically-altered mice through breeding, or for the production of genetically-altered mice that will be used to harvest and study tissues. These mice will fall into a subthreshold limit of severity.

Approximately 10% will be in the moderate severity category, including breeding of genetically altered mice.

The remaining 40% of mice will be in the severe category and undergo permanent coronary artery ligation to replicate a heart attack using an established model. This model involves relatively invasive surgery and a risk of mortality.

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

- Killed

A retrospective assessment of these predicted harms will be due by 28 May 2028

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 cellular and molecular mechanisms that regulate the endogenous regenerative responses of the infarcted adult heart are poorly understood. However, it is clear that these mechanisms are highly complex with distinct spatial and temporal variances. Moreover, these mechanisms involve multiple different cell types, including inflammatory cell infiltration and the extent of functional and transcriptional heterogeneity of cells in the heart is only just becoming apparent. The latter has been realised through advances in

single cell technologies, and our group has recently contributed to this field through publishing an in-depth single cell atlas of gene expression by resident cardiac endothelial cells from the healthy adult mouse heart and at 7 days following acute myocardial infarction. It is not possible to accurately recapitulate the complex, dynamic multicellular environment of the injured adult heart using cell culture models. Mouse models will also permit detailed study of the potential regenerative role of genes and their regulatory networks using established and reliable models of myocardial ischaemia, which can not be assessed using cell culture or human tissues.

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

Post-mortem cardiac tissues from patients with ischaemic heart disease and acute myocardial infarction

Human cell lines such as coronary artery endothelial cells.

Why were they not suitable?

Our group studies fixed frozen or paraffin-embedded human cardiac tissues from the post-mortem of patients with ischaemic heart disease or acute myocardial infarction to validate the expression of targets at the protein level, that have been selected from our single cell transcriptional analysis of

mouse cardiac endothelial cells following myocardial infarction. This has proven useful, but is limited for several reasons (i) these tissues are scarce (ii) it is not possible to gain detailed information about the patients (iii) these tissues are often in poor condition e.g. have been over-fixed (iv) human tissues do not permit study of the effects of gene knockdown/ over-expression (v) it is not possible to undertake lineage-tracing studies in these tissues (vi) it is not possible to determine the effects of therapeutic interventions in these tissues or to assess dynamic cardiac and vascular function. All of these issues are overcome with the mouse models proposed in this project.

Cell lines can be used for genetic manipulation and for functional assessment of an intervention on a single cell type. However, it is not possible to recreate ischaemic disease in a single cell type in culture. Moreover, cultured cells do not provide an accurate representation of the complex, dynamic multicellular environment of the injured adult heart.

A retrospective assessment of replacement will be due by 28 May 2028

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 have been estimated based on previous experience and annual returns for the past 4 years, where we have carried out very similar protocols on mice using the PPL of a colleague. The numbers are also based on current funding and the number of individuals working on projects in my group, and also the projected funding for these studies 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?

The Animal Research: Reporting In Vivo Experiments (ARRIVE) guidelines and principles guiding the Replacement, Refinement and Reduction of Animals inResearch (www.nc3rs.org.uk/ARRIVE/) were consulted during the experimental design phase. All experiments will be executed adhering to the ARRIVE guidelines and the principles governing the NC3Rs to the best of our ability.

We will use a randomized block design for our experiments to reduce factors known to cause differences between animals that are unrelated to those factors being tested in experiments (e.g. cage to cage variation). By reducing natural differences between animals in this way, we reduce the number

of animals needed to identify differences caused our experimental test. In cases where we cannot use randomized blocking to reduce cage effects, we will still ensure that animals are littermate-controlled to reduce variations that can arise between litters. Our experiments are also designed to reduce the number of variables to as few as possible and thereby reduce the number of control groups required.
In this respect, we will always consider carefully whether it is important to include ‘naïve’ as well as ‘vehicle’ or 'sham' control groups in experiments, or if the latter alone is sufficient for interpretation of results.

Planned experiments are discussed regularly within group meetings and also with other experienced internal collaborators and with the vets and technicians in the facility. This ensures that all potential variables are considered and incorporated where possible. Moreover, we ensure that as many tissues are collected from each mouse as possible at the end of the study, to facilitate sharing of tissues/data for the most effective 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?

Breeding will be optimised, where possible, to produce only the genotype required e.g. homozygous breeding pairs. For transgenic animal lines, wild-type litter mates (approx. 50%) generated throughout the breeding phase will be used as age-matched controls, thus eliminating the need to obtain wild-type animals for this purpose and reducing unnecessary animal use.

Studies have been designed to gain as much information as possible from each animal without compromising animal welfare, thus reducing animal numbers. The experimental protocols included in this project have well-validated and robust end-points and are well-established. Local expertise and experience is available for each of the experimental protocols. Multiple tissues will be collected from mice at the end of each study to share with other groups and to promote new collaborations where possible.

Power calculations and statistical analysis (GraphPad Statmate software) have been performed based on previous publications and pilot experiments performed by my group and in our Centre. For example,
(i) 15 mice in each MI group is required to achieve at least 10 with a good infarct, allowing for occasional surgical mortality, missed ligation or cardiac rupture; (ii) for infarct size assessed by Masson's Trichrome staining, n=10 will detect a 10% change in infarct size with a power of 0.83 (control expected to be 35±3.0). (iii) for ejection fraction assessment by echocardiography, control mice have an EF of 61±8% (SD). n=10 will detect a 20% change in EF with a power of 0.80.

A retrospective assessment of reduction will be due by 28 May 2028

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 represent the most tractable model for studies of myocardial ischaemia. Permanent coronary artery ligation is a well-established, consistent and reproducible model. Many of the mouse strains that we intend to use in these studies are also already available in house, and have well-established protocols for their use. Mice are an appropriate model since they reproduce many features of the human cardiovascular system and inflammatory response relevant to this project.

For Cre-inducible models currently in use in our group, such as the Brainbow2.1 and Dual ifgMosaic reporter mice, we have recently switched to administration of a single dose of tamoxifen by oral gavage, rather than an intraperitoneal injection. We will aim to use this route of administration where possible in other models.

For the coronary artery ligation model we have made the following refinements to reduce pain, suffering, distress or lasting harm:

1. Young adult mice will be used only to reduce mortality (compared to aged mice, older adult mice and neonatal mice)

2. Early timepoints will be taken where possible

3. Coronary artery ligation will be made at the point immediately superior to the coronary vein to standardise infarct size

4. Post-surgical animal husbandry to minimise suffering will include homeothermic support, analgaesia, fluid admnistration, DietGel, and empathetic handling.

Post-operative pain will be monitored and managed with analgaesia. Mice will be closely monitored in the hours following surgery to ensure they are recovering normally, and those displaying impaired recovery will be culled to reduce the likelihood that they will die after developing significant physiological impairment. Mice will be observed post-operatively until moving about cage, and thereafter will be checked at regular intervals for signs of acute distress. Sham surgery will be undertaken, as this is an essential control for the method i.e. to determine levels of inflammation and endothelial activation that are brought on by the minor tissue trauma induced by thoracotomy and suture placement. Sham surgery has no predicted mortality and is an established method in our group.

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

A living adult mouse model is required for these studies. A less sentient or mature animal would not permit our proposed temporal assessments of the dynamic endogenous regenerative and inflammatory responses in the injured heart, which includes live assessment of cardiac and vascular function.

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

In line with establishment policy, we will adopt the latest techniques in animal handling (e.g. cupping) to significantly reduce stress. Where possible, the least invasive methods for dosing and sampling will be applied.

The surgical model of myocardial infarction by ligation of the LAD is a long-established technique which has been subject to continuous refinement. The method we will employ is designed to minimise tissue trauma and blood loss compared to older CAL techniques. Intubation of animals is performed under a stereomicroscope with illumination of the throat. Successful placement of the catheter is confirmed with the observation of airflow through a water column, avoiding the need to make an incision to expose the trachea. The appropriate region of the mouse heart is then exposed via a small opening made in the intercostal muscle between two ribs, which is gently opened with blunt-ended forceps. The ribs, sternum and pectoral muscle are not cut or damaged. The use of scalpels and sharp-ended forceps, which cause more damage to soft tissue and blood vessels, will be avoided. The LAD ligation will use monofilament microsuture with a round tapered needle in order to minimise damage to the surrounding cardiac tissue.

Anaesthesia and analgesia will be provided where possible (e.g. during and recovery from surgery). To reduce infection risk, the best aseptic technique will be used during surgery (eg sterilisation of instruments between animals, full surgical drapes), mice will be housed in IVC cages where required. Mice will receive rigorous monitoring after surgery to observe any signs of distress.

We will use ultrasound imaging and echocardiography to assess the presence and size of an infarct in the CAL model, and cardiac function. If mice are found to have no infarct or an insufficient infarct, they will be culled at an earlier stage to prevent their continued use in further steps of the protocol.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?
We will follow the NC3R's ARRIVE guidelines to ensure experiments are conducted in the most refined way. We will consult with the named veterinary surgeons, and adopt any refinements to protocols over the duration of the project.

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

Our institute employs a team of dedicated veterinarians that are continually seeking to improve animal welfare and refine animal use. We will consult closely with this team and take full advantage of the extensive resources provided on their website to ensure we are following current best practices. These resources include comprehensive guidelines and standard operating procedures for most common rodent procedures that are continually being updated. Our establishment is also in the process of adopting improved rodent handling methods that reduce animal stress (detailed by Hurst et al. Nat

Methods 2010) and our animal facilities now provide environmental enrichmentment as standard. My group will adopt these methods alongside the staff in our animal facilities. We will also take full advantage of the annual 3R’s seminar day organized by the University’s 3Rs Committee to find out about pioneering developments in best practice.

A retrospective assessment of refinement will be due by 28 May 2028

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. Myocardial Injection Training in Terminally Anaesthetized Pigs

Project duration

5 years 0 months

Project purpose

- Higher education and training

Key words

cardiac arrest, stem cells, therapy

Animal types	Life stages
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.

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?

The purpose of this application is to allow medical cardiologists to train on anaesthetized pigs to develop the skills required to safely inject stem cells into the inside of the heart. Stem cells are undifferentiated cells that develop into specialized tissue when implanted in damaged organs. The potential growth of specialized heart tissue after stem cell injection into hearts damaged by infarction (heart attacks) is exciting because normally, the heart tissue damaged during heart attacks heals imperfectly, and so fails to function effectively. Effective training will allow human cardiologists to apply stem cells more safely to humans and permit a more efficient appraisal of this new therapy. This therapy will involve the use of a new, helical shaped catheter designed specifically for the injection of stem cells into the beating heart.

A retrospective assessment of these aims will be due by 05 January 2028

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?

Effectively training cardiologists to accurately and safely inject stem cells into the hearts of pigs will allow them to go on to study the full benefits of stem cell treatments in humans in this project. Using animal-trained cardiologists in this study will make the latter more valuable (meaningful) because differences in operator skills will be reduced.

Evidence is accumulating from our studies that stem cell injection in humans after heart attacks is having a highly beneficial effect and reducing the likelihood that heart transplantation will be required in such patients.

How will course attendees use their knowledge or skills in their future careers?

Providing course attendees (who will be consultant-grade medical cardiologists who have undergone an established course (PIL) in animal research) "pass" the pig-injection test, i.e., the ability to accurately inject dye into the inside of the beating heart of a terminally-anaesthetized pig, they will be allowed to participate in this study, which will compare the new treatment with current approved therapies in human patients. The skills acquired in "pig training" will conceivably benefit the application of the new treatment in human patients - should it be legally approved upon demonstration of its safety and efficacy this study.

What are the principal learning outcomes from the course?

To be able to: 1) navigate a special injection system through the blood vessels of a terminally anaesthetized pig into the beating heart (after preliminary training in a plastic simulator); and 2) to make dye injections with prescribed accuracy into the lining of the heart.

How are these learning outcomes important to the people on the course?

It will allow them to participate in this study which will examine whether a new method of treating "heart attacks" is better or worse than current treatments. It will also allow them to perform the procedure more capably in human patients should this study reveal the new methods is beneficial.

Who or what will benefit from the transfer of knowledge, or acquisition of skills that this course will deliver?

The trainees will: i) acquire skills allowing them to participate in an approved multi-centre, multi- national randomized, controlled clinical trial study and ii) be better able to use the treatment should this study demonstrate its usefulness.

Human heart attack patients will benefit from more rapid and complete recoveries should this training accelerate completion of the study with anticipated (positive) results. (It is believed - and there is animal and human evidence for this - that the accurate injection of stem cells into the damaged sections of a heart after a "heart attack" can improve the contracting power of the recovering heart, and reduce the likelihood of a heart transplant being required later).

The NHS will benefit because trainees currently have to travel to France and, or Germany to acquire such training.

Stem cell biology and interventional cardiology may benefit from the advances this new treatment may come to represent in clinical practice.

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

The proposed training will increase the number of participants who will contribute to this study and thereby accelerate its completion. Intellectual property and the dissemination of academic material will remain under the control of the two principle commercial collaborators and project drivers.

Species and numbers of animals expected to be used

- Pigs: 30

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 pigs weighing 65 - 75 kg are widely regarded as suitable subjects for the development of interventional vascular and intra-cardiac techniques because of their similarity in size and form to human beings. Compared with small laboratory animal species, surgery and physiological instrumentation is technically easier and medical devices designed for use in human anaesthesia and intensive care can be used in pigs. This facilitates the meticulous management of anaesthetics and improves the quality of the pig model for human interventions.

The size of the porcine heart allows more interventions, e.g. attempted injections, to be taken than is safe in smaller species. Furthermore, porcine and human hearts are histologically and functionally similar in terms of haemodynamic, metabolic and immune function and arrhythmias. Consequently, pigs have become an important model for evaluating interventional cardiac procedures.

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

At approximately 07:15 on the day of study, at least two* pre-selected and examined pigs will be loaded onto an approved animal trailer and transported by road (approximately 10 - 30 minutes) to the facility where they will be unloaded into pens equipped with drinkers and rubber matting. Within an hour of arrival, at least one pig will receive pre-anaesthetic medication (to which hyaluronidase may have been added) by intramuscular injection. This

will typically produce sufficient central nervous depression, anxiolysis and muscle relaxation for the animal to be lifted onto, and moved by trolley - without resistance - to the anaesthesia induction area. Here anaesthesia may be deepened (initially) to allow the painless insertion of an IV cannula, after which anaesthesia will be induced, usually by a combined IV and, or inhalation technique. Thereafter, continuous and close monitoring of autonomic nervous and nociceptive reflexes, combined with alerting signals generated from quantitative EEG- based monitoring technologies will ensure the attending anaesthetist is always aware of the animal's anaesthetic depth, whilst the immediate availability of additional doses of rapidly-acting anaesthetics pre-located in a venous cannula will ensure the rapid restoration of oblivion if required. The anaesthetized animals will then have arterial and venous access lines placed in order to monitor and support it during anaesthesia. The urinary bladder will also be catheterized. Once "instrumentation" is complete the intracardiac cannula will be introduced via an arterial cannula placed in an artery in the leg, and the dye-injecting catheter moved up and into the heart. Under the guidance of fluoroscopy (which produces instantaneous x-ray images of moving organs) the catheter's correct location will be identified and attempts made to inject the dye. The study will end once all objectives have been achieved and within the permitted time limit. The animal will then be terminated whilst anaesthetized at the end of the study and not released for post mortem examination, or disposal, until physical examination confirms the animal is dead.

* The number of pigs required per day will depend on the number of trainees involved, as only 2 trainees will be allowed per pig. A reserve animal will be made available each day of training to cover any losses that may arise. It is this laboratory's policy to minimize - preferably obviate - the time any pig spends alone.

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

In the broadest sense, potential effects adversely affecting the animals could arise from from: anaesthesia, instrumentation and the catheterization of the heart itself. As the training is conducted under terminal anaesthesia, i.e., the animal will not recover once it is rendered unconscious, adverse effects causing the animal to suffer are only possible if the anaesthetic is insufficient. This is very unlikely to occur and if it did, would only be for a very brief period (as measures are continuously taken to ensure this does not happen). During instrumentation and cardiac catheterization, there is a risk of blood loss but this does not cause pain. Disturbances of heart rhythm may occur during cardiac catheterization and on rare occasions, these may result in cardiac arrest. However, this, like haemorrhage would not be painful.

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 band for all pigs in this work is "non-recovery". The most noxious procedure these animals will experience will be the single intramuscular injection made when pre-anaesthetic medication is given.

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

- Killed

A retrospective assessment of these predicted harms will be due by 05 January 2028

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?

Cardiac catheterization and subendocardial injection is not possible at an in vitro level: whole bodies, or at least whole organs, either in or ex vivo are required. In the proposed study, inanimate-models will be used for initial training in manipulation of the catheter tip. After this, an appropriately-sized isolated pig's heart obtained from an animal killed for unrelated purposes, will be used. However, to achieve a clinically relevant level of competence, it is important to be trained in catheter placement in a beating heart.

Training and certification on pigs is necessary to maintain competence until skill acquisition permits the technique’s application on sufficient numbers of people to permit "on the job" retention of competence on humans as is usual with other techniques.

Why can’t your aim be met by observing or by participating in ongoing research or clinical procedures?

Endocardial stem cell injection has been ethically approved as a treatment arm in this study, but the study is dependent on participants being competent in accurate endocardial injection. Achieving this competence in pilot studies involving human beings would run counter to the Helsinki Declaration.

A retrospective assessment of replacement will be due by 05 January 2028

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 pigs required will depend on: 1) the number of individuals requiring training; 2) the quality of the training; 3) the quality of the training medium; 4) pass/fail criteria and hence failure rate;
5) the number of humans presenting for treatment, and therefore whether “re-training” on pigs is required or not (see below).

Three interventional cardiologist surgeons from throughout the UK have been trained under a previous license, but may require re-training (see below). Two new trainees have been identified for initial training under the proposed (new) license. i

A stringent training structure will be enforced so that the requirement for training (and further use of pigs) trends to the minimum. It is currently believed that one pig will provide sufficient training for two interventional cardiologists to be trained and certified. .

The certification of a given trainee will depend on them passing "accuracy criteria" being met on two separate training sessions. Consequently, a maximum of one pig will be required per trainee passing without fault. A maximum of three pigs will be required per trainee who fails either of the 2 stages. As two trainees will use one pig each, the number of pigs required per trainee will lie somewhere between this range.

Re-training, i.e., further pigs will be required in the event that an inadequate number of humans are treated in each 3 – 6 month cycle. The length of time between training sessions will be dictated by BioCardia.

What in silico or ex vivo techniques will you use during training?

A plastic "phantom" model of the heart and descending arterial tree to the femoro-iliac arteries will be used to train individuals on catheter manipulation.

An ex-situ pig heart will then be used to familiarize trainees with catheter tip orientation / manipulation in an immobile but anatomically equivalent structure.

Will these techniques reduce animal numbers? If so, how?

The two ex vivo techniques will improve the likelihood of successful training in the terminally anaesthetized pig so reduce the numbers of these required to confirm competency.

What other measures will you use to minimise the number of animals you plan to use in your project?

In addition to the stringent training program, training will be constantly an closely supervised by the performance evaluator..

Pigs will be anaesthetized by an RCVS-recognized veterinary anaesthetist who, through the use of permissible measures, e.g., extensive and continuous physiological monitoring and anti-arrhythmic drugs, will ensure animals remain safely anaesthetized until the training objectives are achieved or the experimental time-limit of 12 hours is reached, whichever is the sooner.

A retrospective assessment of reduction will be due by 05 January 2028

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 the pigs will be anaesthetised only once and will be killed under anaesthesia without being allowed to recover. They will not experience anything more unpleasant than a single injection in their muscles to sedate them in preparation for general anaesthesia.

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

An animal whose heart is of similar size and structure to that of an adult human, and is beating normally, is required, which indicates the usefulness of terminally-anaesthetized pigs.

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

Training days are organized so that pigs are minimally stressed by transport and isolation, i.e., they are anaesthetized as soon as possible upon reaching the facility using stress-free techniques. The depth of the (terminal) anaesthetic is monitored using the bispectral index.

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

The PREPARE Guidelines* and checklist will be reviewed and completed by the project manager (the applicant) and representatives from the two commercial elements in planning each training day.

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

I am an active member of a number of ethics committees including 1) A local AWERB; 2) EthicsFirst, an online forum promoting animal ethics; 3) The Animal Welfare, Science, Ethics and the Law Veterinary Association; 4) The Laboratory Animal Veterinary Association; 5) The Laboratory Animal Science Association.

I subscribe to the online newsletters of: 1) the NC3Rs; 2) The Nuffield Council of Bioethics; 3) Understanding Animal Research;

A retrospective assessment of refinement will be due by 05 January 2028
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 and Inflammatory Mechanisms 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

stroke, vascular dementia, inflammation, small vessel disease, brain

Animal types	Life stages
Mice	embryo, neonate, pregnant, adult, juvenile, aged
Rats	embryo, neonate, juvenile, pregnant, 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 understand how inflammation contributes to cerebrovascular disease (stroke and cerebral small vessel disease) and to develop new treatments to reduce the impact of these conditions.

A retrospective assessment of these aims will be due by 05 January 2028

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?

Stroke, caused by a reduction in blood supply to the brain, is one of the leading global causes of death and disability, survivors being frequently left with significant complications such as vascular dementia, depression, anxiety as well as physical impairments. Cerebral small vessel disease is a term which is used to describe a range of conditions that affect the functioning of the small arteries in the brain, making them not work as well as they should, thereby affecting brain function. Cerebral small vessel disease is a very common condition particularly in older adults, causing up to 45% of dementia cases worldwide and accounting for approximately one quarter of all strokes.

At present there are no widely effective treatments for stroke and small vessel disease. Therefore, it is important that more research is carried out to develop these much needed therapies to improve survival and quality of life for people with cerebrovascular disease.

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

On completing this project there will be several outputs. These outputs include new knowledge about exactly how inflammation contributes to stroke and small vessel disease. Such knowledge will be shared beyond the research group through various means, including scientific publications, presentations at meetings, social media and dedicated websites. Importantly, our research will have a

strong Patient, Carer, Public, Involvement and Engagement (PCPIE) aspect. We also expect to have developed new drugs that could be potential stroke treatments or to have provided evidence to support the re-purposing of existing drugs for the treatment of cerebrovascular disease.

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

Ultimately the hope is that stroke and small vessel disease patients benefit from this research, due to the availability of new treatments. We have a proven track record in this regard, our previous similar research having led to current Phase 3 clinical trials of an anti-inflammatory drug in a subtype of stroke. Some of the current work we expect to similarly move to clinical trial, possibly within three years. The timescale will be dependent on whether new drugs are identified, or repurposed drugs proven effective, the timescale for developing the latter for use in patients being quicker.

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

We have a proven track record of progressing treatments along the translational pipeline, that is from studies in the laboratory through to testing drugs in stroke patients. To maximise the chances of doing this again from our current work we will work with relevant stakeholders. This will include patients and carers to ensure our research is relevant. We

will also work with experts in our organisation with experience of developing new drugs, providing links with industry and investors, which is important in providing the support needed to progress the work towards use in patients. At all stages in the work, we will disseminate the findings, positive or unsuccessful, in relevant scientific journals that are freely available to everyone. We will also share our findings through collaborative networks and other appropriate routes, ensuring the widest possible reach.

Species and numbers of animals expected to be used

- Mice: 4620
- Rats: 1480

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 work is focussed on deficits in the function of blood vessels in the brain and the reduced supply of blood that result in cerebrovascular disease (stroke and small vessel disease). The correct supply of blood to the brain to allow brain cells to work properly is referred to as neurovascular coupling.
Importantly, neurovascular coupling in rodents is comparable to humans. Rats and mice also show similar damage to the brain when blood flow is reduced and animals develop complications like those seen in humans, including movement problems, cognitive decline, depression and other symptoms. In addition, mice and rats with risk factors for small vessel disease such as hypertension show clinical features of the human condition. The proposed studies could not be undertaken entirely in less complex animals such as flies and worms because they do not show such similarities to humans.

We will mainly use adult animals, though some of our studies in small vessel disease will use older (12-18 months of age) animals. The use of these older animals is important given the strong association between age and cerebrovascular disease in humans.

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

In order to mimic human stroke, we will use experimental procedures to modify blood supply to the brain in mice or rats. To modify the blood supply and mimic stroke the neck of animals will be opened through a small incision in the skin/muscle to reveal the carotid artery. The carotid artery is one of the main ways that blood gets from the heart to the brain. Then, using one of a few different approaches, we will stop or reduce the blood flow in the artery. Alternatively, we will make a small hole in the side of the skull to directly expose the middle cerebral artery. This artery is the most affected in human stroke and is therefore clinically relevant. We will reduce the blood flowing through the middle cerebral artery, using one of a number of different methods e.g. applying a small clip. We may also affect blood supply to part of the brain by injecting a dye that, when exposed to light, causes damage to the walls of the artery. This damage leads to a blood clot forming and a reduction in blood supply. This approach to induce stroke ('photothrombotic') can require the surface of the skull to be exposed. As well as stroke that results from blood clots (ischaemic) we are also interested one of the other types of stroke, intracerebal

haemorrhage. To mimic brain haemorrhage in rats and mice we will directly inject into the brain very small amounts of substances that cause blood vessels to burst.

To model human small vessel disease, we will use mice and rats with relevant risk factors/genes associated with SVD, such as hypertension.

Stroke and small vessel disease are interlinked and strongly associated with different risk factors or co- morbidities. These risk factors/co-morbidities include hypertension, obesity, diabetes, atherosclerosis, and infection. To best mimic the clinical situation, it is very important that our experimental studies include these risk factors. Hence, in some of our studies we will use hypertensive, obese or diabetic mice or rats, and/or animals where infection is induced.

For all the surgical techniques described animals will be fully anaesthetised and will receive drugs (analgesics) to minimise any pain due to the surgery, as well as local anaesthetics at the wound site. We expect most of the animals to fully recover from surgery within an hour or two. The actual surgical procedures will typically last less than an hour, though this is dependent on how long the artery is occluded for, and the experience of the surgeon.

In some of our studies animals will undergo tests of behaviour. These behavioural tests are designed to assess any problems with movement or sensation as would be seen in stroke patients, or thinking problems as seen in small vessel disease, as well as other complications commonly reported by patients, including fatigue and depression. None of the behavioural tests are harmful to the animals and often just require observation for a short period (5-10 minutes) in specialised apparatus. Baseline tests before surgery will often be performed, with repeat testing at different times after surgery (up to 6 months). We will also assess the behaviour of animals with risk factors for small vessel disease as they develop symptoms over time, like the situation in humans.

Occasionally we will re-anaesthetise animals and use specialised imaging techniques to monitor changes in the brain that are important in cerebrovascular disease e.g. blood-brain barrier breakdown, cerebral blood flow, inflammation etc. Such imaging may be repeated several times and be used to see if any drug treatments are working or not. These drug treatments will typically be designed to modify the effects of the stroke and/or reduce small vessel disease. These drugs can be given via various roues, for example a simple injection under the skin, or directly into the blood. On occasion we may want to give repeated drug treatments, thus animals will receive regular injections. Wherever possible we will try and reduce the need for such repeated injections, by giving the drug in the drinking water or food.

At the end of experiments animals will usually be killed by overdose of anaesthetic and we will take blood, brains, and other organs/tissues to investigate various measures that will help us meet our overall aims.

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

Clinically, stroke and small vessel disease are devastating conditions, resulting in significant mortality and morbidity in patients. In trying to model these diseases in animals a balance must be struck therefore between establishing a valid model and in minimising pain, suffering, distress, or lasting harm to the animal.

Stroke leads to brain injury and therefore there are likely to be behavioural effects on the animals similar to those seen clinically i.e. hemiplegia and muscle weakness, reduction in sensory or vibratory sensation, contralateral paralysis or weakness, listlessness, loss of appetite and loss of balance or orientation. However, these well-described behavioural changes are generally limited to the first day or two after the stroke. The impact of stroke, both in humans and in experimental models, is determined by the amount of brain damage. This can vary clinically but can be controlled in animals depending on the method used to induce the stroke. In many of our studies we will use approaches that cause only modest damage to the brain and therefore only modest behavioural changes. At all times it will be our aim to reduce any excessive suffering or pain experienced by the animals and to apply appropriate humane endpoints if an animal shows persistent adverse events.

Symptoms of small vessel disease are not as severe as acute stroke and therefore the models of small vessel disease that will be used do not show the same impact on behaviour. Small vessel disease animals will show modest changes in cognition, affecting how they perform in certain behavioural tasks. Such impairments should not affect their everyday ability to groom, socialise, feed and drink.

During a study animals can be exposed to several different interventions which may affect their behaviour and result in adverse effects. Drug treatment will regularly be used which will require injections or oral dosing. This may involve mild discomfort while animals are restrained and transient brief pain on needle insertion. Similarly, blood samples may be taken while animals are conscious, requiring brief restraint and the use of fine gauge needles/cannula to sample blood. There will be transient pain associated with the insertion of the needle. Ear clipping is performed only on genetically modified animals and on one occasion, therefore a small percentage of animals will be affected, with transient mild pain. Mouth swabbing and hair sampling are used rarely with only mild transient discomfort due to restraint and the sampling process.

In some studies, we will use animals with stroke-related comorbid disease, including hypertension, infection, and metabolic syndrome (obesity/diabetes). The hypertension is only mild and does not impact on animal welfare. Induced infections are mostly sub-clinical and therefore no obvious adverse effects are observed, aside from subdued behaviour for a brief period. Diabetic animals may urinate more frequently and require additional fluid and more regular cage cleaning and/or provision of more absorbent bedding material. Over time, obesity may lead to insulin resistance and low-grade inflammation, like that observed in individuals with type 2 diabetes. These effects of obesity may cause subtle changes in behaviour (e.g. reduced activity), but are not associated with any lasting suffering or distress.

To account for the effects of surgery and other experimental interventions we will often include a sham group in our studies, which is normal practice. These sham animals will experience the process of surgery and as many of the same interventions as the experimental group as possible, short of the actual process being studied i.e. induction of stroke. Where we require accurate administration of substances into the brain animals will be secured in a special (sterotaxic) frame. They will be anaesthetised and no adverse effects of being placed in the frame are expected.

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)?

Although we will always adopt the least severe approach necessary to meet the objectives, the devastating consequences of stroke on a patient could be mirrored in any model designed to closely mimic the clinical condition. Therefore, adverse events are on occasion possible, including death. The latter though is not expected to occur in any >1% of animals and only with certain stroke models. We will use our extensive experience of the models to be used to identify any animals at risk of showing a decline in condition that might lead to death and intervene before this occurs. Many of our studies will be designed with treatments that aim to reduce the amount of damage and therefore any adverse effects will be lessened in such experiments.

The greatest severity level experienced by animals will normally be due to the amount of brain damage. Hence, where stroke is caused by blocking one of the larger vessels to the brain or by causing the rupture of blood vessels then there is the possibility of severe suffering. However, we will most often occlude the artery at a higher point in the brain, leading to less damage which usually means the animals show moderate changes in behaviour, or even mild. In models of small vessel disease there is no injury directly induced in the brain, it occurs spontaneously. Published findings and our own experience indicates that any such brain damage is relatively minor compared to acute stroke and therefore has less of an effect on the animals, most being in the mild or moderate category. As animals age however effects can be more pronounced, though not in the severe category.

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

- Killed

A retrospective assessment of these predicted harms will be due by 05 January 2028

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?

Studying mechanisms involved in brain diseases such as stroke and small vessel disease is extremely complex. Alongside the death of cells in the brains of stroke and small vessel disease patients, these conditions are characterised by changes in behaviour. Such behavioural changes cannot be studied in cell culture or other non-whole-animal alternatives, such as tissue slices or organoids. In addition, there are complex multi-system effects taking place in cerebrovascular disease, which are important in determining the outcome for patients. Such multi-system effects can only be studied in a whole living organism.

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

Non-animal alternatives are used wherever possible to address our aims, with the proposed animal studies complementary to a broad programme of work on stroke/small vessel disease using human samples, isolated cell systems and non-protected model organisms such as zebrafish embryos. The latter are used extensively in our programme of work on intracerebral haemorrhage and small vessel disease. This includes the ability to screen large numbers of drugs to find the most effective, before moving to studies in the mice and rats.

Why were they not suitable?

Non-animal alternatives are used wherever possible. However, these are not suitable to entirely replace the use of animals due to the complex nature of cerebrovascular disease and need to study processes under physiological conditions with all body systems intact.

A retrospective assessment of replacement will be due by 05 January 2028

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?

Pathological and behavioural end points proposed in this project are well established from published studies of stroke and small vessel disease and experiments will be planned based on our own extensive experience alongside previously published data. We will use the minimum number of animals that can answer the desired scientific objectives and will extract all relevant information in the data by using appropriate statistical analyses.

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

Several factors lead to a reduction of animal numbers, including reducing variation (e.g. keeping the environment consistent), good experimental design (including the use of the NC3R's Experimental Design Assistant) and the use of appropriate statistics. Statistical tests will be used to ensure that we use the minimum number of animals possible to reliably interpret our data and so we can refine our questions to then design the most informative experiments. Whenever we get new data, we will always re-do our calculations to make sure we are still using an appropriate animal number to achieve our aims. We will also consult regularly with qualified statisticians about experimental design and statistical analyses.

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

To avoid unnecessary breeding, we will try wherever possible to obtain experimental animals from recognised suppliers and/or collaborators. Where in-house breeding is necessary, we will optimise the breeding programmes to obtain the required number of experimental animals as quickly as possible, using all offspring if we can. As soon as we have bred sufficient experimental animals the breeding will be stopped and animals maintained on minimum tick over, or the colony stopped if no further animals are required.

We will make optimal use of all animals e.g. harvesting multiple tissue samples for possible future use or for sharing with collaborators. For some work (e.g. in hypertensive animals) we will liaise closely with colleagues who will be able to use isolated blood vessels from the animals, leading to important additional information in addition to the biochemical/histological/functional data gathered in the primary experiment.

For many of our studies prior data is available for determining sample sizes. Where this is not the case, we will consult published literature and contact colleagues to see if appropriate data on variability and effect size can be obtained. In situations where such information is not accessible, we will perform

pilot studies in small cohorts of animals. It is hard to be precise on exactly what number of animals will be used in such pilot studies, but we would typically expect n<6.

We will make our data freely available to other groups through appropriate platforms so they can analyse it to answer their own research questions.

Critically, all our studies will be directly informed by the clinical situation, with back translation from observations in patients. This will be achieved through long standing and successful collaboration with clinical colleagues. We are also in the process of implementing a Patient, Carer, Public, Involvement and Engagement (PCPIE) strategy to ensure our research is relevant.

A retrospective assessment of reduction will be due by 05 January 2028

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 previously published methods to induce cerebrovascular disease, the choice of model being dependent on the hypothesis being tested. Models of stroke (both ischaemic and haemorrhagic) and small vessel disease are extremely well established in many laboratories across the world. Though there is no ‘perfect’ stroke or small vessel disease model, those to be used in this project are chosen based on their pathological and behavioural similarities to cerebrovascular disease in humans, which itself is extremely heterogeneous.

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

Our objectives cannot be fully achieved using less sentient animals (such as fish/insects) or with very young (neonate) animals due to differences in their nervous and immune systems, and the fact that cerebrovascular disease is largely associated with older age. We do make use of zebrafish embryos within our research on intracerebral haemorrhage and small vessel disease, for example to screen possible drugs before they are used in mice and rats.

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

All animals will be closely monitored for adverse effects and procedures put in place to minimise these, using the IMPROVE guidelines, and any other guidelines that are relevant.

Optimal post-operative pain management will be used, guided by advice from the NVS.

For all studies and at all times, animals will be handled appropriately by trained researchers (e.g. using tube handling for movement in and out of cages) and the use of suitable home cage enrichment.

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

Throughout the project we will continually review the literature and engage with colleagues/collaborators to learn of any new refinements to the protocols that could be implemented. In this respect the applicant is a co-author on the publication ‘The IMPROVE Guidelines (Ischaemia Models: Procedural Refinements Of in Vivo Experiments), published in the Journal of Cerebral Blood Flow and Metabolism, a leading peer-reviewed stroke journal. These guidelines draw on a wealth of experience in modelling stroke in rodents and were produced through an NC3Rs working group that included veterinary surgeons and other experts in animal welfare.

We will also consult other relevant literature, publications, and recommendations from the most appropriate bodies such as the NC3Rs and LASA, as well being informed from communication with the NVS and NIO and developments within the scientific community in general. For example, for refinements involving injections we refer to Morton et al 2001 and https://researchanimaltraining.com/articles/an-introduction-to-the-administration-of-substances/

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

We will always aim to implement any advances in techniques that adhere to the 3Rs and improve the welfare of the animals. We will stay up to date with the NC3Rs literature and recommendations, through the NC3Rs newsletter and communications with the Regional Programme Manager. Will also be informed from regular communication with the named veterinary surgeon (NVS), named information officer (NIO) and the scientific literature in general.

A retrospective assessment of refinement will be due by 05 January 2028

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. The Link Between Diabetes, Leaky Gut and Severity of Acute Pancreatitis

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

Acute Pancreatitis, Diabetes, Insulin, Gut barrier function, Antibacterial

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

Retrospective assessment

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

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Contains severe procedures

Objectives and benefits

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

What's the aim of this project?

The over-arching aim of this project is to investigate the link between diabetes, leaky gut and the severity of acute pancreatitis. Diabetes is due to a loss of insulin secretion from pancreatic beta-cells leading to high blood sugar. Leaky gut is when bacteria leak from the gut into the blood due to a loss of the protective barrier function of the gut. Acute pancreatitis is an inflammatory disease in which the pancreas digests itself and originates within pancreatic acinar cells that secrete digestive enzymes into the gut.

This project stems from our recent discoveries that insulin has a direct action on pancreatic acinar cells, resulting in:

1-A boost in cellular energy, normally depleted during pancreatitis. This prevents cellular injury and death associated with pancreatitis.

2-Secretion of antimicrobial agents from pancreatic acinar cells into the gut where they maintain a healthy balance of good vs bad bacteria and protect the gut lining that prevent bacteria from “leaking” into the blood.

This means that loss of insulin secretion (diabetes) or loss of insulin action on pancreatic acinar cells leads to worse pancreatic injury and a leaky gut which make pancreatitis more severe.

We aim to compare the severity of experimentally-induced acute pancreatitis in normal mice vs genetically altered mice. These include mice lacking insulin secretion (diabetic mice) and mice lacking insulin receptors that respond to insulin specifically in pancreatic acinar cells (PACIRKO mice).

We also aim to test the effects of therapeutic substances in reducing the severity of acute pancreatitis which includes drugs that mimic the effect of insulin on pancreatic acinar cells and antibacterial substances that repair the leaky gut.

A retrospective assessment of these aims will be due by 02 February 2028

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?

Unmet clinical need—Acute pancreatitis (AP) is a serious and sometimes fatal inflammatory disease affecting 45 per 100,000 with a 30-day case-fatality rate of 8 %. In England alone more than 1000 people die each year. One-third of episodes are severe, characterised by pancreatic injury, sepsis and multiple organ failure. This increases death rate and patients spend prolonged periods in critical care, at an annual cost of £200 million in the UK and $2.6 billion in the USA. Treatment is supportive and restricted to pain control and organ support. There is a pressing need for an effective therapy to reduce disease severity, deaths and critical care occupancy.

Potential solution—We aim to test new therapies that will “heal” the underlying pancreatic injury and “repair” the leaky gut. This will halt the escalation from mild to severe acute pancreatitis, responsible for prolonged critical care and deaths.

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

Short-term Outputs□Within the lifetime of this project, we aim to determine:
1- The relative contribution of direct insulin-mediated protection of pancreatic injury and secretion of antibacterial agents, that prevent “leaky” gut, during pancreatitis.

2- The therapeutic benefits of “insulin-mimetics” and “agents that repair the leaky gut” on the severity of pancreatitis in diabetic and PACIRKO mice.

Longer-term Outputs□Following the success of the above outputs, we aim to immediately progress the most promising therapies to the treatment of severe acute pancreatitis patients. These treatments are likely safer and more amenable to use in patients and will not require the usual and prolonged clinical approvals that new drugs require.

For example, the insulin-mimetic, metformin, is currently licensed and in widespread use for the treatment of type-2 diabetes and if proven to be effective would simply require "repurposing" for use as a treatment of acute pancreatitis and thus would not require the lengthy toxicity studies and approvals of a new drug.

The administration of depleted antimicrobial peptides via a nasojejunal feeding tube to acute pancreatitis patients designed to "repair the leaky gut" would be classed as a "novel treatment" and would thus require clinical approval. However, these are essentially natural products, normally secreted by the pancreas, that would be supplementing the nutritional support that is already being administered to these patients through a feeding tube into the gut and would not enter the general circulation. Therefore, clinical approval for such a treatment would likely to be much shorter than a novel synthetic drug that enters the blood stream with potentially unknown toxicity to other organs.

Similarly, the use of the natural plant-derived sprouted multigrain nutraceutical, SPROTONE, may have "insulin mimetic" activity and/or may contain "agents that repair the leaky gut", however, we do not yet

know the identity of any active ingredients, which will be the focus of parallel non-animal laboratory studies. Nevertheless, the successful outcome of these studies would add SPROTONE, or its active ingredients to the pipeline of drug discovery for the treatment of acute pancreatitis.

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

Short term (scientific)—These include the scientific and clinical research communities in the field of Pancreatology. We aim to publish our findings, both positive and negative in high impact journals that will reach as wide an audience as possible. This means that other scientists will benefit from any positive findings that will help to move the field forward, but also learn from any negative findings, thereby avoiding any unavoidable repetition and thus animal suffering, with the benefit of taking the field into new directions.

Longer-term (Clinical)—Although any successful treatments will be in animals, these can be easily progressed to clinical studies and are within touching distance of reaching patient benefit with genuine potential to make a real-world clinical impact.

Therefore, the major beneficiaries are the patients that suffer from acute pancreatitis and the healthcare professionals that care for these patients. Specifically, severe acute pancreatitis patients who are critically ill and at very high risk of death will benefit the most. The NHS and UK tax payers, and other national healthcare systems across the world, will also benefit from these discoveries. Acute pancreatitis represents the majority of gastrointestinal-related emergency hospital admissions, putting a huge burden on health

care systems. If these treatments reduce the time patients spend in critical care by half, this will save the NHS £100M.

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

All outputs from this project (both positive and negative observations) will be published in high impact scientific journals and communicated at major international conferences. These include the annual American Pancreatic Association (APA), European Pancreatic Club (EPC) and Union of European Gastroenterologist (UEG) conferences, which are the major international fora for pancreatitis research.

We have established strong collaborations with key clinical researchers within critical care and Hepato- Pancreatico-Biliary (HPB) surgery who jointly manage acute pancreatitis patients admitted to hospital. This will facilitate the seamless clinical translation of any positive outputs of this project to early phase clinical studies. Any commercial exploitation from our discoveries will be managed by the Establishment's Innovation Factory. This will help to speed up the clinical translation and ensure therapies reach patient benefit without unnecessary bottlenecks.

There is also a strong culture throughout the Establishment that promotes public and patient involvement in research. Our clinical collaborators regularly liaise with patient groups and both clinicians and basic scientists from within the research group regularly present our research to patients and family members at annual Supporters Conferences.

Species and numbers of animals expected to be used

Mice: Total = 11,616 (2904 each for Ins2Akita, WT, PACIRKO and IRlox/lox)

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.

Why choose mice?—Mice represent important models for the characterization of complex cellular processes underlying disease, how these cellular processes interact within a whole organ context and how multiple organs interact with each other to produce a “whole body” response to disease. One advantage relies on the targeted deletion of multiple genes to identify the precise mechanisms underlying disease, which has been particularly successful in mice.

Why choose Diabetic mice?— These mice carry a mutation in the insulin gene that leads to the accumulation of faulty insulin protein within the pancreatic beta-cell. This leads to the selective loss of these cells and the gradual loss of insulin secretion and therefore the mice develop diabetes. This makes this mouse strain a good model for studying the effects of impaired insulin secretion during acute pancreatitis.

Why choose PACIRKO mice?— The PACIRIKO mice represent a drug-inducible Pancreatic Acinar Cell-specific Insulin Receptor Knock Out (PACIRKO) mouse. This means that the receptor that normally responds to this drug has been genetically engineered into the mice so that administering the drug over 5 days causes the insulin receptor gene to be permanently cut out of the DNA of the pancreatic acinar cells (gene

deletion). Permanent deletion of the insulin receptor in pancreatic acinar cells means that these cells no longer respond to circulating insulin, whereas all other tissues around the body respond to insulin normally.

PACIRKO mice are essential because mice lacking insulin receptors in every tissue throughout the body develop diabetes as soon as they are born and die young due to severe ketoacidosis (a dangerous condition caused by a toxic build-up of ketones and acid). Furthermore, insulin is required for normal development of the pancreas which is why it is necessary to use a drug inducible insulin receptor deletion in adult mice (when they reach 6-9 weeks old) to allow the pancreas to fully develop.

Choice of life stages—The use of diabetic mice at 6-9 weeks old was chosen as this is the youngest age in which consistent high blood glucose and thus loss of insulin secretion is achieved. This prevents any long-term adverse effects of chronic high blood glucose produced in older mice. Although there are no adverse effects the use of PACIRKO mice at a comparable age removes any confounding effects of age.

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

1- Breeding of genetically altered mice (diabetic and PACIRKO mice) by conventional methods and the administration of drugs to induce gene deletion (PACIRKO mice), either using an oral feeding tube or using food containing the inducing drug.

2- Acute pancreatitis is induced by repeated abdominal injections of caerulein for up to 2 days. Caerulein is an analogue of the naturally occurring hormone, cholecystokinin, which normally stimulates the pancreas to secrete digestive enzymes into the gut. However, at high doses caerulein over-stimulates the pancreas and mimics pancreatic inflammation similar to the disease in humans. At the end of the experiment mice are killed painlessly and humanely and the pancreas, gut, faeces and blood collected at various time points after the last injection (2 hours to 14 days) to determine severity and recovery of pancreatitis. The extent of pancreatic injury, impaired gut function, altered gut bacteria in the faeces and the infection of gut bacteria in the blood and pancreas will all be used to determine disease severity and recovery.

3- Administration of therapeutic substances, either before or during pancreatitis. These include substances that either mimic the protective effects of insulin on pancreatic acinar cells or antibacterial substances that repair the leaky gut. These will be administered either using oral dosing, added to drinking water or by abdominal injection.

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

Breeding and maintenance of genetically altered mice—The breeding of diabetic mice can produce harm in some of the offspring. Male mice gradually develop high blood sugar causing excessive drinking and urination at 6-9 weeks old. The breeding of PACIRKO mice produces no harm to the offspring, until the mice are administered the drug that induces gene deletion.

Experimentally-induced acute pancreatitis— Mice receiving repeated injections of caerulein experience abdominal pain associated with acute pancreatitis. This pain gradually accumulates due to inflammation of the pancreas and persists for up to 48 hours after which the pain progressively declines as the pancreas recovers. In normal mice the

pancreas completely recovers to normal within 5-7 days although the pain may be worse and persist longer in diabetic and PACIRKO mice.

Administration of substances—Whether inducing pancreatitis or administering therapeutic substances to reduce the severity of pancreatitis the route of administration of substances (abdominal injections or oral dosing) may cause some discomfort or pain. There may also be a small chance that the oral dosing of therapeutic substances may cause diarrhoea.

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

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

Breeding and maintenance of diabetic mice – Moderate in 25% of offspring

Breeding and maintenance of PACIRKO mice – Mild in all mice

Treatment with tamoxifen to induce gene deletion – Moderate in all mice treated

Insulin receptor deletion in PACIRKO mice – Moderate in all PACIRKO mice

Experimental induction of acute pancreatitis – Severe in 50 % of the total number of mice used in this study, which represents those mice that receive caerulein. The remaining 50 % of mice will receive innocuous salt solution

Administration of therapeutic substances – Moderate severity in all mice treated

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 02 February 2028

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?

Mice represent the least sentient species that have been used extensively for genetic deletion and manipulation and in the study of pancreatitis. This is because they have a well characterized biology that allows sensible comparison to human biology and disease.

One of the major strengths of this project is the use of PACIRKO mice, in which the insulin receptors are specifically deleted in acinar cells of adult mice. The major benefit of using both PACIRKO and diabetic mice is that we can distinguish between effects of reduced insulin secretion and high blood glucose (which occurs in diabetic mice) from loss of direct action of insulin on pancreatic acinar cells (which occurs in PACIRKO mice). This will reduce any ambiguity when interpreting our results and provide a very precise and holistic understanding of severity of acute pancreatitis, which in the long-term will reduce the number of animals required.

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

Cultured cells and acutely isolated mouse pancreatic acinar cells that represent “cellular models” for studying pancreatitis which in theory reduces the need for and are a convenient alternative to animal experiments.

Why were they not suitable?

1- Cultured cells—unfortunately their underlying biological function is just far too different and therefore unreliable.

2- Acutely isolated pancreatic acinar cells—Although these cells represent an excellent model for studying the mechanisms of cellular injury and insulin-mediated protection, these cell very rapidly die following isolation from their natural tissue environment.

3- Understanding severity of acute pancreatitis—A major problem with using “cellular models” of acute pancreatitis is understanding how cellular injury relates to whole organ pancreatic injury and in turn, how other organs respond to pancreatic injury to influence disease severity. Specifically, this project investigates how the pancreas and gut “communicate” with each other and how the injured pancreas might release toxic enzymes into the blood that lead to injury of other organs, such as the lungs. This could never be achieved using the “cellular model” of acute pancreatitis.

A retrospective assessment of replacement will be due by 02 February 2028

The PPL holder will be required to disclose:

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

Reduction

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

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

This was achieved by measuring blood amylase during intravenous caerulein infusion-induced pancreatitis combined with insulin infusion as the therapeutic intervention.

Amylase is a highly abundant pancreatic digestive enzyme secreted into the gut where it digests starch. However, it leaks out of the injured pancreas into the blood during pancreatitis and therefore is an accurate and easily quantifiable measure of disease severity. The magnitude of these responses and how they vary between animals can be used to estimate the minimum numbers of animals.

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

We will always aim to maximise the amount of data we get from each mouse by collecting as much tissue, blood and faeces as possible from each mouse. This guarantees that as many experimental measures of pancreatitis and gut function can be assessed as possible, rather than having to repeat experiments to capture additional responses. We may also employ national on-line design resources,
e.g. the Experimental Design Assistant (EDA) tool created by the NC3Rs, and consult with the NC3R team who provide periodic onsite help, to improve our approach.

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

Breeding mice—Breeding will be optimized by replacing breeders before their reproductive performance declines (15 months). We will also adopt staggered breeding cycles to ensure optimum production of litters at appropriate times when it’s logistically feasible to perform experiments.

Parallel in vitro experiments—The protective effects of “insulin-mimetics” will be tested on acutely isolated pancreatic acinar cells and any antibacterial substances will be tested on bacteria in a culture dish. Only those substances that show clear-cut therapeutic benefit will progress to pancreatitis experiments in mice. This will reduce negative responses and therefore avoid unnecessary suffering of animals.

A retrospective assessment of reduction will be due by 02 February 2028

The PPL holder will be required to disclose:

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

Refinement

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

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

Genetically altered mice— Diabetic mice will assess the effect of loss of insulin and high blood sugar on the severity of acute pancreatitis. PACIRKO mice will assess the effect of loss of insulin action of pancreatic acinar cells on the severity of acute pancreatitis. Using

diabetic mice for experiments at 6-9 weeks old minimises any adverse of diabetes and using PACIRKO mice within 5 days of drug-induced gene deletion also minimises harm.

Why caerulein-induced acute pancreatitis?—Caerulein-induced acute pancreatitis in mice has been extensively characterized, is easy to execute, reliably reproducible and the specific dose and number of injections can be adjusted to control disease severity. Unfortunately, this produces characteristic abdominal pain that is comparable to the human disease. However, the administration of adequate pain relief that does not interfere with the pancreatitis responses will minimise suffering and will reduce the number of animals required as results will be more reliable and reproducible.

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

The mouse is the lowest vertebrate animal that shares the most common biological and disease mechanisms to humans which can be genetically manipulated so that we can understand these mechanisms.

Animals cannot be terminally anaesthetized as this cannot be maintained over the prolonged periods required for the manifestation of pancreatic injury, systemic inflammation and distal organ injury required to assess pancreatitis (2-3 days).

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

Breeding and maintenance of genetically altered mice—Any adverse and serious clinical symptoms of diabetes, which normally occurs in older diabetic mice (12 weeks and older) are minimised by using mice for experiments at 6-9 weeks old. Similarly, any adverse effects of drug-induced gene deletion in PACIRKO mice are minimised by using the mice for experiments within a few days of inducing gene deletion.

Experimentally-induced acute pancreatitis—Although all experimental models of acute pancreatitis cause characteristic abdominal pain, we will attempt to reduce pain, suffering or lasting harm to the animals as much as possible by the use of appropriate pain management. Pilot studies will determine the most appropriate and effective pain relief, using well-established pain measurements (facial grimace score), without affecting pancreatitis responses.

Administration of substances—For repeated injection in the same mouse, injection sites within the lower abdomen will be rotated to avoid excessive injury at the injection site. Substances will be administered at doses known to be tolerated and only those that are effective in in vitro experiments will be used in animals.

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

We will be guided and directed by the most up to date documents on the welfare and use of animals in pancreatitis research. Specifically, a recent paper has identified a specific pain relief that is not only as effective as some of the more commonly used pain relief drugs, such as morphine, but also has no impact on the measurement of pancreatitis responses. Additionally, we will follow relevant ARRIVE guidelines to ensure that our studies are reported in enough detail to add to the knowledge base.

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

We will frequently liaise with the National Centre for the Replacement Refinement and Reduction of Animals in Research (NC3R) team who provide very helpful advice and consult their website (https://www.nc3rs.org.uk/resource-hubs) for updates on advances in 3Rs. We will also refer to peer- reviewed scientific papers, oral communications/posters at conferences and engage in regular discussions with scientific colleagues on the 3Rs specifically related to models of acute pancreatitis.

A retrospective assessment of refinement will be due by 02 February 2028

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. The Role of Complement (Innate Immunity) in Disease, With a Focus on the Kidney, Liver and Eye.

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

Complement, Immunity, Therapy, Cancer, Inflammation

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

Retrospective assessment

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

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Contains severe procedures

Objectives and benefits

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

What's the aim of this project?

The aim of this study is to advance our understanding of how complement (an evolutionary ancient part of our immune system) helps protect us from disease and helps our body’s normal processes to work on the one hand but on the other hand, can be hijacked or broken to such an extent that it leads to kidney diseases (C3 glomerulopathy (C3G) and atypical haemolytic uraemic syndrome (aHUS)) or to a common form of eye disease (age-related macular degeneration (AMD)) and a liver disease associated with poor diet and lack of exercise , (non-alcoholic steatohepatitis (NASH)) and even to liver cancer. Using our unique animal models, based on patient identified changes in the proteins of the

complement/immune system, we analyse their effects across the animals lifetime. We will also identify new drug targets and test new medicines for the treatment of these diseases.

A retrospective assessment of these aims will be due by 13 March 2028

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?

There are no cures or even effective treatments available for the dry form of AMD (the leading cause of blindness in the western world, affecting an estimated ~180 million people or roughly 1 in 10 of the population over their lifetime) or C3G (an ultra-rare kidney disease, affecting ~3 per million population), while aHUS, another rare kidney disease, is only partly understood (affecting ~3 per million population). It is worth noting that these rare kidney diseases have proven to be the gateway to bringing anti-complement drugs into the clinic and offer a mechanism to rapidly test and compare new anti-complement therapies. The complement system is the bed rock that our immune system is built on. It therefore influences all aspects of the immune system (and beyond) in ways we are only now beginning to understand. For instance, new data indicates important roles in ageing and cancer susceptibility. The cumulative incidence of hepatocellular cancer (HCC) is 2.6% in people with NASH- related liver injury. In the United Kingdom both the incidence and mortality rates of primary liver cancer, and HCC in particular, have risen dramatically. Liver cancer incidence increased from 4.4 per 100,000 in 1997 to 9.6 in 2017 (20.1 in Scottish men), while incidence of HCC has nearly trebled from 1.8 to 5.5 per 100,000 (15 in Scottish men). Any disease with inflammation - such as auto-immune diseases - rheumatoid arthritis and Systemic lupus erythematosus, elements of cancer as well as many kidney diseases are intrinsically linked to complement over activation (when all diseases are combined then a dysregulated complement system could be affecting a staggering 1 in 5 of us). However, complement is also critical to normal bodily functions in the brain, liver and lymphatic systems. Therefore, there is an urgent need to better understand how complement modulates health, how complement can be re- balanced or blocked in disease scenarios.

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

The complement system consists of more than 50 proteins, some activate to deal with threats (pathogens such as bacteria and viruses) while others defend the body from 'friendly fire'. Normally this is in balance but it is often skewed in disease to over activation of the complement system. There are now many drugs being tested that target the complement system and switch it off.

Our work will allow testing of multiple complement targeting 'drugs' or 'therapies', including gene therapies, in state of the art models. We will establish the parameters that will define

the best possible therapy for use in the rare kidney disease, aHUS, C3G and more widely into eye and liver diseases, as well as potentially cancer.

Work conducted under this project licence will generate data and new knowledge that will help advance our understanding of how over active complement changes molecules and cells to make diseases worse. Our work will help advance knowledge of multiple disease processes of the immune system and help to provide tools to further dissect these pathways.

Our work will shed light on why certain gene therapies result in unwanted immune responses

We aim to identify biomarkers of disease (e.g. proteins or DNA in the blood that are released from the damaged organs) or develop imaging tools to help assess is the immune system is over activated or provide information if a drug is working.

Our work will be presented at scientific meetings and published in research papers to share knowledge gained with the wider scientific community and help advance knowledge in the field, ultimately for patient benefit.

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

In the short-term, scientists in both academia and industry will benefit from the discoveries generated under this program of work. This could be due to the development of new research tools, experimental approaches, new animal models or identification of new pathways which when targeted yields therapeutic benefit.

Ultimately, the long-term aims are to benefit patients either through development of new diagnostics (biomarkers or imaging tools) or new treatment strategies, including gene therapy approaches.

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

By presenting our discoveries and national and international scientific meetings, publishing our research discoveries and through collaboration with academics or the pharmaceutical industry, we will be able to maximise the impact of knowledge gained under this program of work.

Wherever possible we collaborate with others to share tissue samples, lines or provide training in methods through collaborative research or participation in workshops.

Species and numbers of animals expected to be used

- Mice: 7600

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.

Diseases involving the immune and complement system can be acute and therefore may involve young animals or equally can develop over many weeks/years therefore adult/aged mice are used in those experiments (particularly relevant for eye disease and liver disease due to changed diet).

To study normal and disease biology and ask how a protein effects normal and disease processes we need to use genetically modified mice which do not express that protein or express a modified (or mutant) form of the protein that is found in patients, identified in genetic studies as increasing the risk to develop that disease or a more aggressive form of the disease. The breadth of genetically modified mice with altered complement genes in mice provide an unrivalled approach to study the disease as it impacts across the whole animal and across the lifetime of that animal. Additionally, as the mouse is widely used for this type of research, the research tools/reagents needed to investigate disease mechanisms and test therapies are readily available and allow our findings to be translated to higher mammals with greater certainty. With respect to the complement system human proteins can be substituted into the mouse allowing direct testing of drugs destined for man in the mouse. This is a huge advantage to other models (rats, rabbit, zebrafish etc). We are interested in study the role of complement proteins in modulating the road to liver injury and cancer, animals need to have been in the disease journey for a length of time for these changes to develop. We will use a range of genetically modified mice, which possess subtle changes in their immune system to understand the mechanism; each genetically modified mouse allows us to answer a very specific question. The genetically modified mice used will mostly be at a young age, typically 8-12 weeks but in some cases mice will be aged to much older (up to 24 months) to replicate the effects of old age in man.

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

Several models develop spontaneous kidney disease, this generally occurs within 3 months of birth, the disease is acute and animals will be treated with a therapy or placebo (either one off, multiple injections over many days or in their diet). In these cases, animals are generally genotyped and then tracked through weight and urine analysis until disease develops.

As an example - an animal will typically be bred using standard breeding practices and genotyped, usually once, using the least invasive method such as ear biopsy. They will then be subjected to a course of substance administration via the most appropriate route for the compound for a maximum of 21 days, at most twice weekly injections/gavage, with blood sampling occurring at most six times during the study, with the final experience being collection of available blood under terminal anaesthesia. Non-invasive monitoring may also be carried out to assess blood pressure or renal function in a small number of these study animals.

Chemical models of liver (including liver cancer) or kidney disease have been refined for many years and are very predictable models, therefore we know what the disease stage animals have reached at any given time point. We have lots of experience running drug studies in these models, therefore we know exactly when to give drugs and for how long to administer them. In the kidney and liver disease models mice may receive weekly or bi-weekly injections of the chemical into their abdomen. It is unavoidable to study liver disease without inducing some degree of lethargy. In certain models, we need to induce damage to the liver in a non-lethal way to study the disease process.

For other liver models, it takes many weeks to induce dietary disease and a sufficient disease stage needs to develop e.g. establishment of cancer and to test the ability of anti-inflammatory therapies to reverse this. As cancer formation is a moderate/progressive symptom, we need to run the model to a point where a natural cancer occurs and advanced enough to observe changes in the level of or number of events due to a particular genetic background or a particular anti-complement therapy (likely a gene therapy).

To better understand the mechanisms controlling liver cancer as well as test novel therapeutics which modulate these biological processes animals will be aged after chemical treatments to provoke cancer development. In all these models, we endeavour to use the earliest scientific endpoint possible to avoid unnecessary suffering.

For models that change the animals genetic profile (conditional knockout of genes), either hormone is given daily for 4 -5 days (or as part of their diet for up to 6 weeks) and disease develops over the following 14 days or one off injections delivery modified viruses to reduce or overexpress proteins.

Surgery: Pain relief is always given as needed. To mimic transplant of a kidney, blood supply is stopped from entering the kidney or to induce kidney disease the ureter (a tube connecting the kidney to the bladder) is surgically tied to prevent urine flowing from the kidney to bladder, which causes kidney injury and fibrosis.

To model diet induced liver disease or eye damage, we will feed mice a modified diet (e.g. high fat) either with or without sugar water (comparable to coke). These animals gain weight and develop features of fat induced liver disease/or oxidative stress in the eye.

In many of these models we may wish to perform non-invasive imaging whilst the animals are asleep. We are likely to take blood samples to assess disease stage or drug metabolism. In the liver disease models we might perform glucose tolerance tests.

Some mice in the licence will be aged and will suffer the effects of normal aging, these can result in cancer or inflammatory disease, with or without additional modification. These will be carefully monitored to ensure animals suffer the least possible harms.

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

Typically, any mouse in an experiment will be monitored by weighing and collection of urine, during routine handling – for basic analysis of kidney and liver function. Any animal showing significant distress due to detected illness will be rapidly put on a treatment or culled. Animals on treatment are normally monitored twice daily and with the help of a clinical monitoring sheet, a decision to increase monitoring or terminate the experiment is quickly reached to ensure animals experience the minimal adverse effects.

General maintenance of mice

In our mouse models, mice may show signs of sickness e.g. hunched posture, diarrhoea, ruffled fur, look pale or feel cold. Supportive care and pain relief will be given and if the clinical condition does not improve within 24h mice will be humanely killed. Animals suffering acute kidney failure (urine dipstick measure) or showing recognisable and significant pain/distress will be humanely killed.

In the dietary models most mice will gain weight, but not to a point where mobility is impaired, these are key for liver and eye disease models – which are often chronic. Eye disease models in this licence are sub clinical (subtle) but the act of aging mice can lead to natural harms.

Some mice may be housed singly in cages with special sand to collect urine to track disease for no more than a few hours and no more than 6 times overall, this may cause mild distress which may exacerbate disease. Animals will be closely monitored by non-invasive tests, i.e. weighting and urine dipstick tests.

Kidney injury as a result of cancer drugs; mice will develop kidney disease over a period of up 18 days and can lose up to 10% of their starting weight during the first week of the models but regain weight after this time.

Induced kidney or liver disease will be in the form of a mimic of a common infection or the infection itself, (so called ‘trigger’ events or ‘triggers’ in the case of aHUS), where possible, practical (i.e. in some cases symptoms would be analogous to the flu and/or Covid-19) or agents that mimic mild infection or caused by insufficiency of immune proteins or through chemicals designed to alter kidney or liver function or that mimic the conditions associated with organ transplant. The use of influenza and particularly SARS viral strains can be highly stressful and fatal to the mice if left untreated. The large majority of animals in our studies will only experience minor effects of disease before intervention is applied. Animals will be carefully monitored throughout. In most cases agents are given for a limited number of times (often once) and animals are in experiments for a short period of time, less than two weeks. Monitoring of the animals during the induction phase is tailored to the experiment, based on our current experience, generally including routine 2x daily checks (weight and urine analysis as noted above) or live video monitoring for interventions where the outcome is not predictable (for instance: in the case of new models of in-born errors of immunity).

Liver Injury leading to inflammation and Cancers. Complete Liver failure and death in these models is very unusual and mice tolerate liver damage as it has a remarkable ability to regenerate. Some animals will receive chemicals dissolved in olive oil, these mice will undergo liver injury followed by a natural wound healing response, any pain would be transient. In the chronic model, mice will need to receive repeated injections of olive oil or the chemical dissolved in olive oil. The animals are expected to develop liver failure and to show poor condition, altered behaviour and lose weight as a result of the liver injury over a 6 to 8 week period. In other experiments, mice will be treated with inducers of liver damage (which creates transient inflammation and pain). Some mice may be given a second dose of damaging agents after the initial administration to further induce damage, which will again lead to transient pain, mice recover within a period of 24-48hrs. In some cases mice will be fed a modified diet for up to 60 weeks (including supplementation of drinking water, e.g. sugars to equivalent to full sugar coke) and they may develop many small tumours (around 60% of animals), mice generally tolerate these small tumours and remain bright and active. Ageing mice can be used for both analysis of liver and eye phenotypes associated with a broken immune system (complement). In some cases, animals on particular diets may be maintained for up to 24 months of age to more closely replicate the human experience. It is possible that some of our genetically modified mice will display a premature ageing phenotype and so mice will be carefully monitored for the signs of aging guided by a clinical monitoring sheet.

Surgery; mimicking post kidney transplant or chronic disease/fibrotic injury. Less than 1% of mice may experience complications after surgery and/or use of drug delivery devices which may induce distress. These can be managed effectively through use of pain relief and antibiotics. However, if infections or complications persist animals will be humanely killed.

Imaging: mice may experience a small weight loss post imaging but this should recover within a few days.

Therapy: we do not anticipate any therapies (gene therapy via AAV, protein based therapy etc) will cause significant adverse effects. Equally, we do not anticipate any significant harm or lasting effects of multiple blood collections or IV drug dosing protocols.

In all models pain relief is given when needed.

The large majority of the animals at the end of experiments will have their blood withdrawn under anaesthesia followed by humane killing.

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)?

Only mice will be used in this program of work. We estimate that ~65% animals used in procedures will be mild including breeding mice to maintain a colony, for experiments and to take tissue or perform milder models of disease such as ageing or dietary models. Approximately 15% of animals will be of moderate severity, whilst up to 20% may fall into the severe category.

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

- Killed

A retrospective assessment of these predicted harms will be due by 13 March 2028

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 the immune system in both natural processes and complex diseases over many years. Wherever possible, we will use human tissue/cells or cell culture systems to replace our animal models. The group have accumulated archival tissue banks of frozen and formalin fixed tissues from our previous models and human

normal and diseased tissues. These samples are used in multiple on-going projects to minimise the number of animal disease models used.
However, human tissue (including whole kidney) or cells can be used to answer questions/ and provide some outcomes. We use these where possible. However, inflammation and immune responses are complex events and processes. They involve multiple cell types and pathways (blood based pathways in intricate cascading systems with 100's of proteins involved) and often modulated by environmental inputs - including diet and time (age); therefore, it cannot be modelled in the laboratory in any realistic manner and animal studies are necessary to fully dissect the mechanisms of natural processes and particularly, chronic inflammatory diseases, such as age-related macular degeneration which take a lifetime to develop.

This research will identify proteins and pathways which either cause or limit inflammatory disease (and/or cancer). To prove that particular proteins modulate disease, models may be performed in mice which are "genetically engineered" and lack or overexpress the protein of interest. Alternatively, we may wish to test drugs which we believe will limit inflammation or blood clots. Genetically engineered mice allow us to find out if changes in normal protein expression in the body will have a major effect on organ function or disease progression. This is particularly important in deciphering the impact of in- born errors of the immune system.

Cancer, is also a complex disease, which involves many different cell types but is more likely to develop in an organ that is hyper-inflamed and diseased e.g. liver/kidney disease.

For these reasons we need to perform some of our research and drug testing in animals.

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

We routinely use cells in culture (including cell lines) to understand and model the biological processes involved in the immune system or to perform drug testing.

We are also involved in kidney transplant studies and we do use whole human kidney (which clinicians have declined to use for transplant) on a special circuit that can keep them alive for up to week, although most studies are completed over 6-10hrs due to continual staffing required to maintain organs on the circuit. We are using these to test some of the therapies we have developed, and these studies are invaluable.

Why were they not suitable?

Whilst these are useful tools, there are limitations of cell culture or whole organ systems, these include;

1. Cells grown in petri-dishes sit on plastic, which is much stiffer than where they reside in the body. The increased stiffness can change their behaviour and they become "super activated" or fail to do the job they would in the body. These abnormal behaviours could lead to the identification of non-relevant pathways or fail to predict drugs which are likely to be ineffective in the disease.

2. Organ disease development and resolution is regulated by many different types of cells communicating with each other within the damaged organ as well as through communication with white blood cells and receiving signals from other organs (delivered

by circulating blood). Recreating all of these internal and external organ damage signals is extremely difficult to model in culture.

3. Analysis of systems (inborn errors of the immune system) - immune, clotting and cell activation systems involve 100's or even thousands of proteins which cannot always be provided in cell cultures.

4. Maintaining cultures or organs alive and sterile for weeks, months or years is very challenging and will not mimic all the natural events cells or organs deal with and so will lack context that is very important in most diseases or normal processes.

5. Using genetic engineered mice will help advance our understanding of both natural processes and those in of disease to help identify new targets for drug discovery.

A retrospective assessment of replacement will be due by 13 March 2028

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 knowledge from previous studies to mathematically calculate the minimum number of animals we need to breed (around 7600, 95% of which will be used in some form of experimentation, including provision of blood products) in order to generate the data which allows us to answer our scientific questions. By doing this we can minimise use but be confident that the differences in a scientific measurement between two groups is meaningful and has not been obtained by chance.

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

We have taken multiple approaches to ensure that we use the minimum number of animals for research purposes. These include strict adherence to our S.O.P. for searches to be carried out prior to conducting in vivo research. The use of sample power calculations and as per our recent paper Kamala et al, 2021 Frontiers in Immunology– experiments were we successfully used only two controls but linked back to previous studies with 8 animals – showing appropriate spread, mean and standard deviation of our 2 controls.

We have also performed audits of our previous research studies (principally through home office returns and for recent publications) and assessed research plans of current projects to predict use under 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?

Statistical analysis is performed to determine the minimum numbers of animals need to generate biologically meaningful data. If this is not possible then pilot studies are performed to reduce numbers and inform future studies going forward.

We employ efficient breeding strategies and where possible we use both male and female mice to minimise numbers of animals used.

Tissue is regularly shared between projects to maximise outputs from animal procedures and minimise numbers of animals used.

Wherever possible, we will use human tissue/cells or cell culture systems to replace our animal models, as noted above.

A retrospective assessment of reduction will be due by 13 March 2028

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 of the disease models chosen are the lowest severity model that can be used to answer our research questions. Accumulation of inflammatory damage in response to immune activation can affect any organ in the body and is caused by many different types of injury. Inflamed organs are also at a higher risk of developing blood clots and cancer. Therefore to understand how this disease develops, determine if this process is common to all organs and test new therapies, we need to perform different models of tissue injury each is chosen and carefully managed in a way that causes the least amount of lasting harm.

Our main model is based on spontaneous disease as a result of a small change in a key protein in the blood. It is a direct copy of the human disease. It therefore provides the most realistic system to test drugs and interventions that would protect patients, there is no other way to replicate this therefore it is the only model available, and we have developed excellent standard approaches to ensure animals suffer only transiently before treatment or being culled.

Chemically induced models of liver and kidney damage have been refined for many years and are very predictable, therefore we can predict the disease stage at any given time

point. We now have lots of experience running drug studies in these models, therefore we know exactly when to give drugs and for how long. In such a way, we can ensure the negative effects on animal welfare are minimised while experimental results maintained.

Gene therapy is an evolving science and we will also look at immune response to the agents and how that results in unwanted responses. In order to model this, we may need to create some disease.
Again, this is essential to understand the processes that occur in these circumstances. It will be limited and where possible pain relief will be given while animals are diseased.

Surgical models will be used to study kidney disease. Pain relief is always given as needed. To either mimic transplant scenarios or induce kidney disease - the main artery into the kidney (closed for up to an hour, usually 35mins) or the ureter (closed for 3 to 10 days, usually 5 days) is surgically tied, which causes kidney injury. These methods will be used sparingly and only if absolutely necessary to prove very specific questions around drug utility.

To model diet induced liver and eye disease we will feed mice a modified diet (e.g. high fat) either with or without sugar water (comparable to coke). These animals gain weight and develop features of fat induced liver and eye disease.

The most refined model is always used.

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

The mouse is a well-established model for experimental kidney (liver and eye) disease, and there is a large body of published data in this species and because of the complex nature of the disease process it is not possible to recreate this using cells in culture dishes, therefore we need to study disease progression and test medicines in the whole animal.

In respect to the immune system, it is well characterized in the mouse and is highly similar to humans, particularly the blood based immune system. Although there are differences between human and rodent immune responses, these have only minor effects when studying inflammatory disease models and rodent models provide important insights into the causes of these diseases and also the normal processes that immune proteins alter. Mice are the species with the lowest capacity to experience pain or distress that are likely to produce satisfactory results. For instance, fish and insects (e.g. flies) lack the same broad range of immune cells found in humans, which means that there are differences between fish and mammals that could affect the disease biology. Some genes are not conserved between these species and mammals therefore some of the disease mechanisms may not be the same. Therefore, drugs that target those cells or disease pathways may not work in these systems due to fundamental differences in the biology of the species compared to humans.

Genetically engineered mice for which a specific gene is either deleted or reintroduced are very useful tools for the study of gene function. Use of genetically engineered mice with specific genetic alterations believed to affect disease development, particularly in immune and clotting pathways, will allow us to identify and determine the influences of specific genes (and by consequence specific molecules) in many diseases. Indeed, use of gene conversions may allow us to move from models with uncertain outcomes to reliable models that are less likely to generate significant harm yet still provide models to test drugs destined to treat man.

Some experiments will monitor the animal across its life, the mouse life span is a good compromise and allows many environmental influences of the immune system and inflammation to be modelled in a highly realistic manner. Age is an important modifier and there is no culture system that can account for the myriad of complex interactions that occur over a life time.

That said, we continually refine our experiments and plans to include animals in an immature life stage and we will continue to collect blood products from terminally anaesthetised animals to carry out experiments in culture - these include mixtures of immune cells in the petri dish. As noted above, these are limited to simple interactions.

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

All animals, regardless of disease model are checked regularly and supportive care is readily provided to minimise distress or suffering and improve animal welfare. By working with the vet team, NACWO and published works from animal behaviour/welfare scientists we have developed a clinical scoring system to help assess the animal's wellbeing and level of disease that is applicable in the majority of our models.

For all surgical models, we would consult with the vet team/NACWO/NTCO to establish surgical/pain relief refinements and ensure training and assessment of that training is as needed. We use good surgical techniques and operating theatres/equipment to minimise the risk of infection. The kidney transplant model is a surgical model of post-transplant injury. In this model mice receive pain relief and a high level of post-operative care including soaked diet, a warm environment and fluids as required to minimize stress and suffering.

All protocols allow for appropriate supportive care in consultation with experienced technical and veterinary staff. Induction of disease will be carefully planned/staged to ensure the lowest level of useful effect of infectious or chemical agents is applied. Development of 'trigger' specific models will allow us to progress from using models with unpredictable outcomes and increase the usefulness of the studies as a whole.

Where possible we will also follow the following strategies:

Cryopreserving animals where appropriate e.g. earliest opportunity after import of new strains or after backcrossing to ensure strains are preserved with a minimal amount of genetic drift and therefore preserving their integrity.

Backcrossing of mouse strains at appropriate points such as every 5-10 generations, dependent on colony size and backcrossing of both sexes to ensure good refresh of both sex chromosomes. This will preserve genetic health and the increased reproducibility by limiting genetic drift that occurs naturally across generations.

Utilising Single Nucleotide Polymorphism (SNP) analysis to ensure correct back crossing to preserve genetic health. Analysis will occur either when animals are frozen and/or before backcrossing occurs to establish the correct choice for background. In exceptional circumstance backcrossing will not be appropriate and working with the colony management team we will find alternative approaches to support preservation of genetic integrity.

Utilising the breeding system that is most suited to the efficient production of that strain of mouse, this will be based on data or information available in mouse passports e.g. pairs/trios.

Active monitoring of defects in breeding colonies will occur with support from the colony management team and technicians. This will identify early reoccurring health defects that may indicate genetic drift. Likewise, we will remove non-standard animals from breeding programs.

We will adhere to the local AWERB standards: Rodent 12 Month Breeding Age Limit and Rodent Breeding Defect Management.

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

Alongside the guidelines listed below, I will also adhere to local AWERB standards for research animals, and where appropriate, support the development of new local standards for refinements discovered during the project licence.

Code of Practice for Housing and Care of Animals Bred, Supplied or used for scientific purposes

LASA Guidelines

RSPCA Animals in Science guidelines UFAW Guidelines and Publications NC3R's and Procedures with Care
I will consult with the Colony Manager to review genetic health, breeding practices and overall colony health and management at regular intervals.

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

There are many sources in which provide information regarding 3Rs advances, these include; the NC3Rs website, NC3Rs seminars/events and emails, scientific publications and published guidelines as well as continued professional development e.g. local seminars, regular communication with the NACWO and veterinary team and academic collaboration with the welfare group.

The local AWERB, NIO, NACWO, NTCO and Veterinary team regularly inform, and disseminate improvements and recent studies involving reduction, replacement and refinement

Alongside external resources including (but not limited to); collaborators, peers, conferences and lab animal and animal welfare bodies.

During the 1, 3 and 5 year review of the project licence I will update on implementation or consideration of the 3Rs that has occurred during the previous period, alongside a review of the linked training plan, score sheets etc. in collaboration with the NACWO, NTCO and Veterinary team with a particular focus on refinements

As information on welfare or technical improvements, alternative less severe models or new non- animal model systems becomes available an appropriate strategy within the

research group and veterinary teams will be implemented to ensure that animal use and suffering is minimised. This will include testing new models (animal or non-animal) and modifying procedures.

A retrospective assessment of refinement will be due by 13 March 2028
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. Genetic Identification of the Cellular Interactome of Blood Stem Cells and Leukaemic Stem Cells.

Project duration

5 years 0 months

Project purpose

(a) Basic research

Key words

Stem cells, Leukaemia, Bone marrow transplantation, Bone marrow niche

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

Retrospective assessment

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

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Contains severe procedures

Objectives and benefits

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

What's the aim of this project?

We aim to identify molecular and cellular regulators of normal blood functions and provide mechanisms through which stem cells undergo leukaemic transformation and become leukaemic stem cells (LSCs). This research will allow us to define new means to expand in vitro normal healthy blood stem cells and therapeutically target LSCs/cancer stem cells.

A retrospective assessment of these aims will be due by 09 March 2028

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?

Blood stem cells (BSCs) produce all red and white blood cells that the body needs to survive and fight infection. BSCs sit in specialized pockets, so-called “niches” of cells in the bone marrow (BM). These niches are critical to regulate BSC health, and therefore help to generate all blood cells. The precise identity of the cells that form these niches is unknown.

As we age, the environment that surrounds BSCs changes, and this can lead to health issues including anaemia or a weaker immune system. Moreover, if BSCs are damaged they can transform into leukaemic stem cells (LSCs). LSCs are able to further modify BM niches for their own benefit to promote cancer progression. Thus, it is very important to know exactly how the BM environment changes with age or disease so we can prevent and treat associated conditions, such as leukaemia.

BSCs are used in BM transplantation to treat different blood diseases including leukaemia, anaemia, or sickle cell disease. Patients also need them to recover from anti-cancer treatments such as chemotherapy. Every year worldwide, thousands of people require a BM transplant. For the transplant to be successful, the donor has to be compatible with the recipient. Due to donor shortages, this can leave some patients without the transplant they need. To eliminate the continuous need of BM donors, we aim to expand BSCs in the laboratory, as this will lead to never-ending supply of suitable donor cells. Future banking of expanded BSCs from different groups of donors (defined by histocompatibility genes) will allow appropriate donor BSCs to be readily available for transplantation and to minimize graft rejection. Unfortunately, this is currently not possible as BSCs cannot be efficiently expanded in the laboratory.

Additionally, the precise composition of the niches that support leukaemic stem cells is also unknown. LSCs produce large numbers of immature cells, known as blasts, which rapidly divide, and interfere normal blood cell function. Chemotherapy is able to kill these blasts, but sometimes does not eradicate all the LSCs. Following therapy, the remaining LSCs are thought to fuel disease relapses. Since LSCs rely on their niches which nurture them, we aim to unveil alternative therapeutic approach cutting off the lifeline of LSCs by targeting their niche cells.

In sum, this research is of critical scientific and clinical importance and will result in: 1) eradication of LSCs, avoiding leukaemia relapses, 2) treating the effects of ageing on blood, 3) the expansion of BSCs in laboratory culture dishes for transplantation, which will decrease the need for bone marrow donors and will allow for the investigation of other blood diseases.

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

This project aims to investigate the cellular and molecular interactomes that support normal and leukaemic stem cells and to determine the role of genes of interest in leukaemogenesis. Blood stem cells (BSCs) produce all red and white blood cells that the body needs to survive and fight infection. BSCs sit in specialized pockets, so-called “niches” of cells in the bone marrow (BM). These niches are critical to regulate BSC health, and therefore help to generate all blood cells. The precise identity of the cells that

form these niches is unknown. If BSCs are damaged they can transform into leukaemic stem cells (LSCs). LSCs are able to further modify BM niches for their own benefit to promote cancer progression. Thus, it is very important to know exactly how the BM environment changes with disease so we can prevent and treat associated conditions, such as leukaemia.

Moreover, BSCs are used in BM transplantation to treat different blood diseases including leukaemia, anaemia, or sickle cell disease. Patients also need them to recover from anti-cancer treatments such as chemotherapy. Every year worldwide, thousands of people require a BM transplant. For the transplant to be successful, the donor has to be compatible with the recipient. Due to donor shortages, this leaves some patients without the transplant they need. Unfortunately, BSCs cannot be efficiently expanded in the laboratory. We aim to learn how BSCs divide naturally within the body, to imitate this in the laboratory to get continuous supplies for BM transplants. Particularly this project aims to understand how the BM niche supports BSC division in vivo with the aim to mimic this in vitro.

At the end of this project we expect to publish multiple high-impact papers in the stem cell, cancer and leukaemia biology fields.

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

Understanding the molecular and cellular processes governing normal and malignant blood stem cell biology is incredibly important. It has essential implications in hematology and oncology and on how to treat leukaemia and other blood conditions. This research project is of critical scientific and clinical importance and will provide multiple novel means to 1) expand HSC in vitro to improve bone marrow transplantation by decreasing the need for bone marrow donors and to model blood diseases ex vivo;

and 2) to generate novel therapeutic strategies to treat leukaemias by producing niche-based anti- leukaemia therapies.

Our research is therefore of an immense strategic importance and addresses key areas of unmet clinical needs.

The key beneficiaries are:

Patients requiring stem cell transplantation: the expansion of BSCs in laboratory culture dishes for transplantation will be a breakthrough for patients who require a stem cell transplantation. This includes patients with many disorders including severe autoimmunity disorders, immunodeficiencies, bone marrow failure syndromes and to recover from anti-cancer treatments such as chemotherapy employed to target multiple cancer types.

Leukaemia patients: We aim to achieve efficient eradication of leukaemic stem cells (LSC) by informing novel niche-based anti-leukaemia treatments through the identification of niche components which specifically support chemotherapy-resistant LSCs. Patient benefit will depend on the efficient translation of our work to the clinic. We have strong links with clinical haematologists in the UK and the US and will work closely with them to achieve this as rapidly as possible.

Patients with other blood malignancies: Once we provide a proof of concept in acute myeloid leukaemia (AML) treatments, we will test whether similar therapies can be applied to other blood malignancies (e.g. chronic myeloid leukaemia, myeloma, lymphomas).

Cancer patients: In the longer term, we will collaborate with our colleagues focusing on different cancers to test whether our niche-treatment strategies can be employed in other cancers. To tackle this, we will work to adapt our technologies to the investigation of the niches that support other cancer stem cell types.

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

We will strictly adhere and support our funders' policy for research data sharing and management. Our data will be presented during multiple conferences/workshops and published in open-access high quality journals. After publication, data will be deposited in an open access digital repository. Published materials/reagents/mice will be available upon request to allow for collaboration and dissemination of new knowledge. Together, our data will be safely stored to ensure their longevity and that they can easily be shared, uploaded or reanalysed by everyone.

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.

Many dish-based or computational-based experiments are unsuitable to study human blood stem cells or cancer stem cells in leukaemia. As such, we need to employ mice to recapitulate these complex biological processes. Our lab uses mice for research because mice and humans share the same genes of interest as well as the cellular processes involved in stem cell biology and leukaemia. Mus musculus is the most widely used system to study haematopoiesis, because of the similarities among mouse and human haematopoietic cells, allowing for translation to the human system.

In general, we will use 2 types of mice.

Firstly, in the majority of our experiments we will study genetically altered mice - mice lacking or expressing genes of interest or mutated genes specifically within the blood system. We have recently identified genes with a role in blood stem cell biology. To further explore these results and identify cellular and molecular components in the bone marrow niche, and prepare our data for publication and translation into clinical trials, we plan to examine the impact of removing or expressing these genes in mice. Typically, 8-12 week-old mice will be humanely killed to obtain haematopoietic organs, which will be used for analyses, dish-based tissue culture or transplantation experiments. In some cases, we will age these mice for 60 weeks to study the role of genes of interest in the ageing of the blood system.

Secondly, we will use non genetically altered mice as recipients of bone marrow or stem cell transplantation. Transplantation of blood cells into recipient mice is the gold standard way to analyse the activity of normal stem cells or leukaemic/cancer stem cells. These experiments allow us to study how normal stem cells regenerate blood or how leukaemic/cancer stem cells generate leukaemia.

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

Most of our mice will be used for breeding to generate cells, tissues and organs lacking or expressing genes and mutated genes of interest. The mice will be humanely killed to obtain this research material, typically at the age of 8-12 weeks. We will also breed our recipient mice which will undergo bone marrow or stem cell transplantation. In this procedure, recipient mice undergo a controlled irradiation procedure to remove their bone marrow cells, and are then injected with new stem cells or bone marrow cells. These recipient mice will be kept for 16 weeks after transplantation. In some occasions, we will humanely kill these mice, collect the bone marrow and re-transplant the cells into other recipient mice, which will be also kept for 16 weeks. This experiment, known as a secondary transplant, is required to test the self-renewal capacity of blood stem cells, i.e. their ability to continually generate all types of blood cells. Some of our transplanted and non-transplanted mice may develop blood cancers. All mice will be very carefully monitored and appropriate humane endpoints will be applied to avoid further suffering of the animals. However, due to the nature of the leukemic disease, a very small percentage (<5%) of sudden acute deaths may happen (despite an intensive program of care and welfare, including regular daily /twice daily monitoring). All mice will be humanely killed at the end of each experiment.

In some cases, we will need to withdraw small quantities of blood from both non-transplanted and transplanted mice by inserting a fine needle into a vein. This causes short-lasting discomfort, similarly to patients who give a blood samples for testing.

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

The expected impacts and adverse effects for animals in this project vary depending on their use. In the majority of cases, breeding involves no adverse effects, pain or suffering. In some rare cases we may have to breed healthy mice that are susceptible to leukaemia. These mice will be carefully monitored on a daily basis, so their suffering will be minimised.

In transplantation experiments, after irradiation, animals may experience weakness, modest weight loss and some abnormal behaviour, such as withdrawal from the group. Animals may also experience some pain or discomfort and may be given pain killers. These adverse effects, caused by the controlled irradiation itself, normally last for several days and then animals are expected to fully recover. During this period (5-14 days after irradiation), mice will be assessed every day according to the scoring system we have developed and refined. This includes assessing body weight, body condition, animal posture like hunching, piloerection, facial expression of discomfort and/or reduce activity/social interactions. This system gives us an objective way of assessing animal health and allows us to identify and apply a humane killing point at the earliest opportunity, both to achieve our scientific goals and to minimise suffering.

Some of our mice may develop blood cancers. Mice will be very carefully monitored and evaluated, and any animals showing the earliest clinical side effects will be humanely killed. However, due to the nature of the disease, a very small percentage of sudden deaths may happen. All mice will be humanely killed at the end of each experiment.

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

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

A large number of our mice will be used for breeding, where we expect to see no adverse effects. When breeding mice susceptible to leukaemia (which covers approximately 4,000 of our mice for the period of 5 years), approximately 10% of mice will experience adverse effects such as weakness, modest weight loss and some abnormal behaviour, such as withdrawal from the group. About 15% of the mice harbouring Dnmt3a-fl-R878H mutation, which confers leukaemia susceptibility, may develop ulcerative dermatitis (UD). This will be monitored closely using a specifically designed monitoring system for these UD. Based on the degree of the UD lesions, animals will be treated following veterinary advice, and closely monitored to assess the progression and successful healing of these lesions. If lesions reach a % of skin surface and/or show tissue penetration, suppuration and/or blood discharged- animals will be immediately killed.

We plan to use approximately 5000 mice to investigate the functions of normal and leukaemic stem cells following gene deletion. Some mice in these experiments will develop leukaemia, but due to close monitoring, the vast majority of mice will be humanely killed before they develop significant adverse effects. We estimate that 10-25% of mice may experience moderate adverse effects as a result of leukaemic disease.

Approximately 10,000 mice will be used for transplantation assays to study functions of normal and leukaemic stem cells. Of these experiments, approximately 10% will be injected with leukaemic cells, and we expect 20-80% of those to suffer moderate and progressive adverse effects. Despite an intense welfare monitoring screening, acute sudden unexpected death related to leukaemia may be observed in these leukaemic cell transplantation studies –such acute mortality is expected to be <5% in these experiments.

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

- Killed

A retrospective assessment of these predicted harms will be due by 09 March 2028

The PPL holder will be required to disclose:

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

Replacement

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

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

In some instances, the use of animals is currently unavoidable as many facets of stem cell and leukaemia biology can only be studied in animals, such as mice, where these cells and diseases naturally occur. For example, current knowledge does not allow us to properly culture blood stem cells in a dish – once plated into a dish they die and lose their potential. Current culture conditions also do not allow us to test many key properties of leukaemic cells, including their ability to cause leukaemic disease, critical interactions with bone marrow cellular and molecular components that affect the behaviour of leukaemic cells, how leukaemic cells modify the niche to thrive at the expense of normal haematopoiesis or how a fraction of leukaemic stem cells avoid chemotherapy by remaining quiescent. These in vivo models allow to functionally test the role and effect of mutations found in leukaemia patients and provide critical information that can be brought from the bench to the bedside.

Moreover, this project aims to identify bona fide cellular components of the niches that support blood stem cells (BSCs) and leukaemia stem cells (LSCs). To preserve the full plethora of unknown cell types that form these niche cells in the bone marrow (BM) it is critical to perform these experiments in vivo.
Unless an animal model is employed, it is currently not possible to maintain the integrity of native cell- to-cell-interactions. This would require controlling for any unknown critical extracellular matrix components or O2 tension, both of which could critically affect the location preference of BSCs or LSCs. Importantly, the use of mouse models maximizes the scientific relevance of our studies: 1) due to the availability of critical genetic murine tools (e.g. numerous Cre recombinases…) allowing inducible gene expression and 2) because it provides a mammalian system harbouring critical cellular components in the bone marrow niche as shown by their ability to allow the engraftment and function of transplanted human BSCs and LSCs, something that it is not possible in other less sentient species. Therefore, as such it is impossible to perform these experiments without the use of murine models.

Therefore, at the moment, many important experiments need to be conducted in mice. Notably, my research will contribute to the development of in vitro model systems to study BSC and LSC niche cell interactions, which may reduce the use of mice.

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

Where possible, we strive to use non-animal alternatives in our research. Transplantations of blood stem cells or leukaemic stem cells into recipient mice are essential experiments for the study of stem cell and leukaemia biology. However, these experiments require large numbers of mice, both mouse donors, from which blood cells are derived, and mouse recipients, into which blood cells are injected. Whenever possible, we replace mouse transplantation with dish-based tissue culture experiments, so called long-term culture-initiating cell, colony-forming cell assays and long-term of hematopoietic progenitor cells which require very few animals. We apply the same type of cell cultures to study genetic pathways in stem cells, whenever possible.

Notably, we are active in developing techniques that would improve our ability to study aspects of blood stem cell and leukaemia in a dish. Particularly, this project aims to identify bona fide cellular components of the HSC-niches and LSC-niches. Thus, we expect our project to contribute to the development of in vitro model systems that should allow to faithfully study blood stem cell and leukaemia stem cell biology by reproducing the cellular and molecular components that support HSC and LSC in a plate.

Why were they not suitable?

Blood stem cells and leukaemia stem cells reside in bone marrow niches, very specialised pockets within the bone marrow which provide stem cells with a specific microenvironment composed of multiple biological factors that support their functions. Culture conditions in the dish fail to reproduce this complex microenvironment. Indeed, when exposed to culture, stem cells lose their activity and adopt characteristics of non-stem cell types, making them unusable for research. As such, non-animal alternatives are sometimes unsuitable and we must use animals to accurately study stem cell and leukaemia biology in a whole animal context.

A retrospective assessment of replacement will be due by 09 March 2028

The PPL holder will be required to disclose:

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

Reduction

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

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

The numbers of mice are carefully planned based on our long-term experience and consultations with our expert collaborators. Based on this, our group performed careful estimations to ensure that the number of animals used in our experiments is the minimum number required to generate statistically significant results (i.e. statistically convincing results). Designing animal experiments to produce statistically significant results means that we can generate more powerful data. This data can then be translated into clinical applications, such as curative novel anti-leukaemia therapies, as quickly as possible.

We plan to breed and generate 20,000 mice for this project for the period of 5 years. 5,000 of these mice will be used for cell-based tissue culture assays. 5,000 will be used to study stem cells functions, and 10,000 will be employed to examine stem cell functions upon transplantation. In all experiments we will use mice of both sexes to avoid having to cull a surplus of mice of a particular sex.

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

As key means of achieving reduction we have and will continue to carefully design our experiments and mindfully estimate the number of animals required in order for the results of our experiments to be statistically convincing. To minimise the number of animals used in each experiment, we will use power calculations, calculations that allow us to gauge how statistically convincing a given result is.

Experiments will be carefully planned to maximise the information obtained per animal and thus limit the subsequent use of additional animals. Experiments requiring cells from animals will be carefully optimised in order to minimise the number of animal cells required.

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

An important means of achieving reduction is to apply the most efficient breeding strategies. My group has extensive expertise with mouse colony management (i.e. monitoring large cohorts of mice) and we will strive to employ the best breeding schemes. We will replace breeders before their reproductive performance declines, and non-productive breeders will also be replaced.

My group is fully aware of the ARRIVE 2.0 and PREPARE guidelines and experimental design tools provided from NC3Rs. As such, we employ the optimal experimental designs (EDA from NC3Rs) to implement the 3Rs and optimise the number of mice for each experiment.

Importantly, experiments will be carefully planned to maximise the information obtained per animal and thus limit the subsequent use of additional animals. For example, haematopoietic organs, i.e. organs involved in the blood system, including bone marrow, lymph node and spleen cells will be stored and used for multiple experimental purposes. All experiments requiring cells from animals will be carefully optimised in order to minimise the number of animal cells required.

A retrospective assessment of reduction will be due by 09 March 2028

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 majority of our experiments, we will study mice lacking or expressing genes of interest or mutated genes specifically within the blood system. These 8-12-week old mice will be analysed to study the haematopoietic organs (bone marrow, spleens, thymi and lymph nodes), which can also be used for tissue culture or transplantation experiments. In some cases, we will age these mice for 60 weeks to study the role of genes of interest in the ageing of the blood system. These experiments do not involve any invasive procedures and therefore any pain, suffering or distress will be minimal.

As this project aims to study the leukaemia stem cell niche and leukaemogenesis, in some cases we will need to inject leukaemic cells into recipient mice or induce leukaemia in mice carrying specific mutations. Following this, these mice will develop leukaemia. In 50% of cases they will be analysed before they develop clinical symptoms of leukaemia. In another 50% of cases, when the progression of leukaemia is of scientific interest, mice will be monitored and humanely killed at a point such that our scientific research is achieved, but pain, suffering and distress is minimised. When performing xenotransplantation of human blood stem cells, we will employ immunocompromised mice as recipient, which do not need to be irradiated to enhance human haematopoietic chimerism in peripheral blood, bone marrow, and provide a level of chimerism similar to that seen in irradiated NSG mice, allowing us to avoid irradiation and additional suffering of recipient mice.
All these models are the current state-of-the-art and the gold standard in cancer and stem cell research. If/when other improved models become available, we will immediately implement them in our research. We are very active in trying to refine our procedures. For instance, we employ bone marrow imaging techniques to better monitor leukaemia, maximise the information obtained and minimise suffering.

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

Given that our aim is to target human cancer stem cells in leukaemia or expand adult stem cells, we will need to employ mice for these purposes. Mice and humans share the same genes of interest and the cellular processes involved in stem cell biology and leukaemia are very similar. As such, mice are ideally suited for our work as they allow us to gain insight into human stem cells and leukaemia biology without having to work on human patients. Furthermore, all the reagents necessary for our research are mostly developed for the mouse and human systems. Other less sentient animal models (e.g. flies, fish or worms) are excellent model systems to study some conserved biochemical pathways, but do not replicate complex human or mouse blood stem cell and leukaemia biology. However, we do collaborate with several experts around the world to obtain maximum relevant information from less sentient animals (e.g. zebrafish, via our collaborators).

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

To ensure technical competence, all staff will be directly supervised by the project license holder. To minimise infections of mice with impaired immune systems, the animals will be housed in barrier caging under sterile conditions and handled in a sterile environment. Whenever appropriate, in order to prevent pain, pain killers will be given as directed by veterinary staff. Genetically modified animals exhibiting any unexpected pain or suffering will be humanely killed. For protocols involving bone marrow transplantation and leukaemia experiments, we will employ a stringent scoring system that we have optimised with our collaborators, which allows immediate identification of mice displaying adverse effects. Furthermore, we will closely monitor the emergence of ulcerative dermatitis in some of our mice via the use of an optimised scoring system. These systems give us an objective way of assessing animal health and allow us to clearly identify and apply a humane killing point at the earliest opportunity, both to achieve our scientific goals and to minimise suffering.

Over the years we have optimised and refined many procedures. For instance, as mentioned above, we have collaborated to generate a clinical scoring sheet allowing us to

rapidly identify any animals which are likely to develop post-irradiation sickness and clinical symptoms of leukaemia and kill them humanely before they start suffering.

Notably, our current transplantation experimental protocol requires that the recipient animals undergo an irradiation procedure. Without irradiation, the immune system of the recipient mice would reject all donor cells, rendering the experiment ineffective. Irradiation procedures allow us to preform successful transplantation experiments, generating important data. However, irradiation can have unwanted health effects on animals, which is why we are seeking to replace irradiated recipients with immunodeficient mice in our transplantation experiments. Due to their impaired immune system, immunodeficient mice will already not reject donor cells, and so do not require irradiation. Optimising transplantation experiments using immunodeficient mice will reduce pain and suffering, and so will be a major refinement to our procedures. Moreover, whenever possible we are avoiding transplantation by employing genetic mouse models that allow the expression of the mutated gene of interest inducing a particular type of leukaemia without the need of irradiating these mice and transplanting leukaemia cells. Additionally, we will employ immunocompromised mutant mice as recipient mice when performing xenotransplantation of human blood stem cells (BSCs), which allows to avoid irradiation and additional suffering of recipient mice while we still obtain the best engraftment from transplanted human BSCs and the quality of our research. These immunocompromised mice will be provided with special husbandry and care in isolators.

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

Our experimental designs are based on our long-term experience in this field (we have already refined many protocols and experimental designs) and peer-reviewed high-quality literature and grant applications. We always seek to apply the most refined methods which are published in the field. We also base our knowledge on ample literature disseminated by NC3R and interactions with many expert colleagues and collaborators. We will always continue to refine our protocols based on the literature and knowledge exchanged with skilled collaborators and we are very proactive in this area.

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

Many advances in cancer research, including advances in mouse cancer models, are disseminated during scientific conferences and seminars, which we frequently attend. Further, we continue to update our knowledge through the literature NC3R disseminates. We also have an extensive network of local and international collaborators who use similar state-of-the-art models, and by frequent exchange of information we will always stay informed about the best advances in the field. Given that our group is extremely committed to the implementation of the 3Rs, we will utilise any useful knowledge learned during these events and the literature to improve mouse procedures, apply petri dish-based models where possible, improve statistical methods, and minimise pain and suffering of our experimental mice.

A retrospective assessment of refinement will be due by 09 March 2028

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. Breeding and Therapy of the De50-Md Dog Model of Duchenne Muscular Dystrophy

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

Duchenne Muscular Dystrophy, Therapy, Animal model, Dog, Genetic disease

Animal types	Life stages
DE50-MD Beagle cross	juvenile, neonate, adult, embryo, pregnant, aged

Retrospective assessment

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

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- 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?

This project aims to maintain a colony of Beagle cross dogs (DE50-MD) with naturally-occurring muscular dystrophy that will be used to test promising therapies prior to clinical trials in human patients with Duchenne muscular dystrophy (DMD).

A retrospective assessment of these aims will be due by 28 March 2028

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?

DMD is a disease first recognised in young boys caused by mutations in an X-linked gene that is critical for muscle function but that also causes behavioural and cognitive problems. Sufferers are confined to a wheelchair by the age of 12, are effectively paralysed by their 20s and all die in their late 20s or early 30s due to progressive wasting of all muscles including the heart and in particular, respiratory muscle failure. The disease is the most common genetic disorder diagnosed in childhood and has a worldwide prevalence of 1 in 3600-5000 male births. Optimal medical management has improved quality of life and increased lifespan from an original age of death at 16, but can do little to prevent the relentless muscle wasting. Pet dogs also are affected with this condition, so our work will also benefit pet dogs and their owners in the future.

The development of treatments for this condition mostly relies on cells in culture and the mdx mouse model of the disease. The mdx mouse is a good biochemical model but does not show the clinical signs typical of the disease in boys and the immune system in mice differs to that of humans such that responses to (for example) gene therapy viral vectors can be very different between these species.
Consequently, there is doubt about the ability to directly translate results in the mouse into a human clinical trial. In contrast, dystrophic dogs show similar clinical and pathological progression to humans and have similar immune responses, so can serve as a final test to enable rational decisions about which treatments are most likely to be successful in humans.

In this programme, we will test treatments (such as gene therapies) planned for boys with DMD and we will also be examining the optimal way to deliver these treatments, comparing, for example, whether a sustained administration over several hours is better than a single short dose of a treatment.

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

This work is to be conducted with a primary aim of finding and optimising effective treatments or a cure for Duchenne Muscular Dystrophy. We will investigate novel treatments (such as gene therapies) or ancillary treatments, publish the results and promote the licensing of drugs that are to be taken into human clinical trials.

A secondary aim of this work is to provide much needed information (via publication) regarding the underlying disease mechanisms at play in Duchenne Muscular Dystrophy. A species comparison approach (for example between mice, dogs and humans) with the same disease is a powerful method by which to examine conserved disease mechanisms, which themselves can then be a target for future therapeutic investigations.

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

The work will be of primary benefit to human patients with Duchenne Muscular Dystrophy and their families and carers. Furthermore, the work will have a secondary benefit to society because the emotional and financial costs for dealing with this currently fatal disorder are very substantial.

The work has secondary benefits in other diseases because many of the approaches we plan to take can be applied in other disorders.

An additional longer term benefit might be in treatment of the same condition in pet dogs in the future, thereby benefiting the animal and the owner.

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

In all our work to date we have promoted our findings (via the BBC, national press, in patient led meetings). We will discuss our work at domestic and international research meetings and publish our work in high impact, peer reviewed research journals under an open access policy so that it is available to everyone.

We recognise that it is important to publish negative findings in order to prevent other researchers repeating our work. We will do this as well.

Species and numbers of animals expected to be used

- Other dogs: No answer provided

Predicted harms

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

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

There are various animal models of Duchenne Muscular Dystrophy that have advantages and disadvantages for research. Mouse models in particular are useful for initial screening of drugs, but they generally do not display clinical signs of disease so they are not suitable for testing treatments designed to promote functional improvements. Pig models of DMD (kept in other countries) have a very severe phenotype and most die or are euthanased within the first few weeks of life. They are therefore unsuitable for testing treatments in longer duration trials which are important for prolonged efficacy and safety evaluation.

We will use dogs with a naturally-occurring form of Duchenne Muscular Dystrophy caused by a mutation which is in the identical gene that is mutated in humans with the same disease and in a region of the gene that is most often affected in humans. We will only breed animals that are required for maintaining the colony, studying the disease and for the therapeutic trials. We will study animals throughout their lives - up to approximately 1-2 years of age. The colony will be maintained for the duration of the programme. Additional healthy animals that are bred that are not required for the trials will be rehomed whenever possible, usually after weaning as is done for pet dogs. We have placed over 120 dogs in this way in our prior work since 2016.

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

Generally the procedures conducted on dogs within this project are no different to those that might be conducted on pet dogs during investigation of disease by qualified veterinary surgeons. Animals will undergo non- or minimally-invasive procedures such as blood sampling, ultrasound and clinical examinations. Some dogs will undergo procedures (such as muscle biopsy and MRI) under general anaesthesia, but we will follow the same procedures that are conducted routinely by vets in pet dogs. Some dogs will undergo functional assessments to measure their muscle strength by stimulating muscle contraction whilst under anaesthesia and by examining activity and walking, by videoing and through the non-invasive use of activity monitors on the dogs' collars.

All dogs have access to grassy paddocks and are kept in groups (except when whelping) to enhance their welfare. They have daily human interaction and are assessed daily for their welfare. They are fed the same as pet dogs.

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

We do not expect any adverse effects of the therapies or research that we are conducting. All therapies will have first been tested in cell culture and/or in rodent or other animal models. Of the procedures that we are performing, muscle biopsy is associated with mild discomfort in people that can readily be controlled with pain relief. We will routinely use pain relief in our dogs whenever needed or other medication as recommended by vets. Other procedures are not expected to be associated with any discomfort. Some procedures (for example MRI imaging) are conducted under general anaesthesia. Muscular Dystrophy in humans is not associated with pain. As in humans, affected dogs become weaker as the disease progresses and sometimes the dogs can have problems with swallowing, so we carefully monitor these aspects in particular and have defined humane endpoints so that these problems do not compromise the welfare of the animal.

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

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

The majority of animals that will be covered by this licence will be categorised as 'subthreshold' or 'mild' as these are breeder animals and unaffected littermates which are then rehomed. Breeding of affected dogs with Duchenne Muscular Dystrophy is categorised as 'moderate' due to their genetic disease and to the minor surgical procedures that they undergo. Up to 90 animals will be in this category.

We maintain typically no more than 12 carrier female dogs of breeding age and up to 3 normal male adult stud dogs. Of the puppies that are born, 75% of animals are either normal or carrier animals. 25% of puppies are affected male puppies.

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 28 March 2028

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 studies are required because of the complex disease processes that occur in humans with this disease that cannot recapitulated fully in cell culture. Because mice do not display clinical signs of Duchenne Muscular Dystrophy, we cannot assess response to treatment in mice, and instead need to study a model that displays the weakness and muscle problems that are seen in humans.

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

Therapies might have first been tested in cell culture before animal studies or might have been tested by other groups using organoid type preparations.

Why were they not suitable?

Our work covers the final investigations that are required before moving into therapeutic trials in humans. Cell culture studies are often used in early investigations but they are not suitable for assessing final therapy development as it is currently not possible to generate mature muscle and other relevant tissues in a cell culture system because cultured muscle cells fail to differentiate to become mature muscle fibres that are found in live mammals. Cultured cells for example might lack the relevant receptors necessary for drug entry into a muscle fibre. Even organoid-type preparations do not have a mature blood or nerve supply or the immune system and local tissue fibrosis that has to be taken into account when doing our work.

A retrospective assessment of replacement will be due by 28 March 2028

The PPL holder will be required to disclose:

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

Reduction

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

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

For each study we will use the minimum number of animals that are required to prove or disprove the efficacy of a possible treatment. We have already performed very extensive testing that allows us to know with high confidence the minimum number of animals that are required to produce a robust result. For example, we might be interested in determining whether a specific treatment can improve an affected animal between 25% to 50% towards normal for a specific issue (such as muscle strength). We know from our prior work, the number of animals that would be required to demonstrate this difference (if a drug is effective) with a high likelihood of success. As such, the animal numbers proposed here ensure that their ethical use is maximised because we will use sufficient numbers of animals to ensure success of our experiments, but avoid use of more animals than are needed.

The majority of the healthy animals generated in this project are rehomed as pets and a minority are used for ongoing research.

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

We use the results of previous work, and where possible, stored tissues from previous studies, further to reduce the number of animals required. Experiments are typically conducted according to ARRIVE (2.0) guidelines and design is examined and scrutinised closely by external or professional statisticians and a separate Scientific and Ethical Advisory Board who are independent from the researchers to ensure that the proposed work is valid and ethical. This is an additional level of scientific and ethical scrutiny that occurs beyond normal regulation. The NC3Rs Experimental Design Assistant will be used for algorithm-generated feedback on adjustments that could be made (such as identifying potential sources of bias/nuisance variables) and, where appropriate in representing experimental design visually for group or external discussion. We utilise other online resources (such as GLIMMPSE) to ensure that numbers of animals used will maximise the chance of a positive outcome, when using repeated measurements, to increase the power of our statistical comparisons.

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 share results of animal use between studies where possible and in the course of this work will continue to generate a database of results that can be used as historical information to enable us to compare different treatments. Our goal is to generate a complete online dataset that can be openly accessed for historical natural history data from this colony so it becomes a resource for other researchers.

A retrospective assessment of reduction will be due by 28 March 2028

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.

Whilst other dog models of Duchenne Muscular Dystrophy are used in research, the model we use (unlike others) has a naturally occurring mutation that is in the region of the dystrophin gene that is most commonly mutated in humans. This means that this model is more applicable to several of the most promising treatments currently being evaluated. In addition, the dog breed we use weighs less than other dog models, and as a result, they are less affected by the muscle weakness that develops as they get older.

Generally the methods we use are similar or identical to those used by veterinary surgeons when investigating disease in pet dogs. Like them, we use pain relief medications for procedures that might cause discomfort.

We take welfare aspects of this project especially seriously. All dogs are socialised and have access to outside runs and paddocks to play with other animals and humans. We monitor the dogs very closely for signs of progression of their disease and make decisions to end the studies before the dogs reach the end stages of disease that occur in humans with this same disorder.

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

Mouse and fish models of Duchenne Muscular Dystrophy are used by other researchers to evaluate Duchenne Muscular Dystrophy and they are key components in the drug evaluation pathways. These animals however do not sufficiently reproduce the clinical disease features (muscle weakness) that occurs in this disease and they have immune systems that behave differently to those of humans. As such, these animals are often not suitable for functional efficacy testing of therapeutics or for studies for which the subject's immune system is relevant (such as gene therapies).

For our work, we are often interested in long term functional efficacy and safety of therapies, such that terminally anaesthetised animals are not suitable for the vast majority of procedures.

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

All animals used in our work are socialised through human interaction and we spend a lot of time training them (as with pet dogs) so they are familiar and accustomed with the procedures they undergo. Animals are maintained in groups and have access to outside runs and grassy paddocks and toys to run and play.

Any procedure that might be associated with discomfort is performed under local or general anaesthesia and we always provide pain relief drugs of the type used in pet dogs and people.

When we can, we minimise use of blood collection needles through use of a preplaced intravenous catheter, of the type that is used in pet dogs.

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

We will follow guidance generated by the NC3Rs in terms of husbandry: https://www.nc3rs.org.uk/3rs- resources/housing-and-husbandry-dog

All our work will be done in accordance with the ARRIVE 2.0 principles, and we always provide an ARRIVE statement in our publications confirming that this was done. We follow LASA guidelines whenever appropriate for administration routes and blood sampling protocols.

In addition, work conducted within our facility, in conjunction with BSU staff, is performed with attention to the Culture of Care, promoted by the PREPARE guidelines to which we espouse in ongoing work and in particular, when planning and preparing future projects.

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

We will conduct regular 3Rs assessments for our work and utilise online tools and information and advice from the NC3Rs including their Resource Library and Resource topics. We will communicate with our NC3RS liaison officer and institute changes whenever we can to improve animal welfare, reduce numbers of animals required and refine the methods.

A retrospective assessment of refinement will be due by 28 March 2028

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. Effects of Human Gene Mutations and Follicular T Cell Products on Immune Responses and Disease

Project duration

5 years 0 months

Project purpose

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

Key words

Autoimmunity, Autoantibodies, Follicular T cells, B cells, Tumours

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

Retrospective assessment

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

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Contains severe procedures

Objectives and benefits

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

What's the aim of this project?

The aim of this project is to understand how a healthy immune system is normally regulated to avoid reacting against innocuous environmental antigens (to prevent allergies) or self-antigens (to prevent autoimmunity) while still being reactive to self-tumour antigens, microbes, and infected cells.An important parallel aim is to trial new therapeutic agents emanating from our work to prevent and/or treat autoimmune disease (like lupus, pemphigus, multiple sclerosis, autoimmune arthritis and colitis) and allergies.
A retrospective assessment of these aims will be due by 04 May 2028

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?

There are over 100 different human autoimmune diseases and the incidence is increasing at rates of 3- 9% per year. Together, these autoimmune diseases affect ~ 5% of the population. These diseases like lupus, pemphigus, rheumatoid arthritis, inflammatory bowel disease (colitis) and multiple sclerosis, can cause severe symptoms that can be debilitating and sometimes life-threatening. To date there are no cures for any of these diseases. They are generally treated with high dose steroids and treatments that tend to dampen the entire immune system and can lead to multiple side-effects and organ damage over time. Furthermore, sometimes, some of the most serious disease manifestations including kidney damage and leukopenias (e.g. thrombocytopenia) do not respond to these treatments. Although new treatments are being developed, it is impossible to know which patient will respond to which therapy, so a lot of time and money is wasted prescribing these treatments one after another until one works.
For treatments to be more effective, it is very important to improve our understanding of the root causes of each autoimmune disease in each patient or group of patients.

Allergies are also on the rise and can cause lethal anaphylactic shock in susceptible individuals. The limited treatment options are not effective in a large proportion of cases.

Cancer is a leading cause of death worldwide; the prognosis and life expectancy of many cancers is improving thanks to developments in immunotherapy, where boosting our own immune systems provides the ability to better fight tumours.

At the core of all these pathologies is the establishment of immune cell tolerance processes that in healthy individuals prevent reactions against self or innocuous environmental antigens, while allowing immune cells to fight tumours. How these processes work is incompletely understood.

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

New knowledge and information on the causes of autoimmune disease and allergies, or on how to better fight cancers, that will peer reviewed and published in respected international publications. The new insights will also be communicated at national and international conferences. Some of the knowledge may be translated into new treatments. We will also be testing novel therapeutics we are developing, mostly based around a small soluble protein shown by our laboratory to be effective at suppressing autoimmune B cells and IgE production (allergies associated antibodies) in mice. We will test the therapeutic potential of neuritin and neuritin derivatives in several autoantibody mediated disease in mice (e.g. collagen-induced arthritis, K/BxN arthritis, pemphigus, lupus, inflammatory bowel disease and a multiple sclerosis models). We currently hold a 'method of use' patent and are aiming to obtain a 'composition of matter' patent and develop the product for commercial application. This will be done with the help of a translation team, venture capitalists and

pharmaceutical partners. Other treatments will also aim to silence TLR7 signaling in mice with lupus-like disease. We are interacting with pharma companies that have developed specific RNA-based TLR7-blocking compounds, which we will be trialling alongside other commercially-available inhibitors.

Our work investigating the effects of follicular regulatory T cells and their products on B cells and antibodies in cancer may reveal novel immunotherapy targets.

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

This knowledge will allow clinicians to refine the diagnosis of patients and will provide information as to which groups of patients will respond better to which treatments. This may be undertaken in different ways. For example, we have determined that TLR7 overactivity drives the expansion of a rare B cell subset known as DN2. A simple flow cytometric assay enumerating DN2 B cells in patients with autoimmunity may therefore identify those that will more greatly benefit from TLR7 inhibition. Whilst the few individual patients whose gene variants have been introduced into mouse models may benefit from refined diagnosis and/or more targeted treatment within the life of this project license, larger groups of patients will see the benefit after we have completed the project. Some of our findings (i.e. inhibition of autoantibody-producing plasma cells by Tfr products) may be applicable to a large number of B cell- driven diseases (e.g. pemphigus, rheumatoid arthritis, lupus, thyroiditis, anti-phospholipid Sd, Sjögren's Sd and vasculitis). Other findings, such as the therapeutic value of TLR7 inhibition, may be selectively beneficial for patients with systemic autoimmunity to nucleic acids (i.e. lupus). While the latter may be translatable to the clinic within the time-frame of this PPL (there are already biologicals in pre-clinical trials inhibiting TLR7), taking our novel compounds to the clinic may take longer.

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

We will maximise the outputs of this work through already established collaborations with clinicians and scientists in the UK, US, Australia, China and several European countries. We will also collaborate with pharmaceutical and commercial companies. The principal investigator has done extensive outreach work to patient groups or societies in her previous position, and will aim to continue to do this in the UK. The knowledge will be disseminated in publications and conferences.

Species and numbers of animals expected to be used

- Mice: 31500

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 because their immune system is 99% identical to that of humans, and we have been able to recapitulate some human diseases in mice, by introducing the genetic mutations found in the human patients. As a result, the models are valid for the study of the immune pathways that are faulty because of the mutations.

For most studies we will use young and adult mice, since the diseases we are interested in develop in late childhood, adolescence and adulthood.

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

For this project, we have developed new mouse models of human disease by introducing the genetic mutations found in the patients. We first need to validate these models and see if they develop the clinical manifestations of autoimmune disease found in humans (for example the swollen joints and tiredness that accompany several forms of autoimmunity). We will take blood samples and cells from the mice to understand the abnormalities that are linked with disease development. Development of disease manifestations can take from weeks of life to 6 months of age, depending on the symptoms: for example, in lupus mouse models, autoantibodies usually appear in the first 2-3 months, whereas kidney disease usually takes 6 months to develop.

The first few experiments with each new line will validate the model by allowing the mice to develop end-organ damage, and once the model is validated, further experiments will not go as far and we will use the earlier manifestations as readout of disease.

Some mouse lines do not or may not develop disease overtly. For those lines, we will inject them with substances that mimic environmental or other known disease triggers (like viral infections). In some

case we will expose mice to a transient increase in temperature that might change the presentation of self-proteins and make them immunogenic and accelerate the onset of autoimmune disease.

A particular strain of mouse will develop tumours spontaneously and these mice will be used to investigate how B cells contribute to the control of the tumour and how T cell-derived factors may or may not limit B cell-dependent tumour control.

In order to understand whether the immune system is generally overactive or there are selective defects, we will immunise the mice with proteins or other agents that are typically used to induce antibody responses, allergies, and/or inflammation, and investigate whether such responses are protective or cause unwanted reactions. These experiments typically last several weeks.

In order to understand which cells are responsible for causing disease, we will irradiate mice to remove their immune cells, and then inject them with a mixture of immune cells that have or do not have the disease-causing mutation, with a marker that can tell apart their origin. This will help us pinpoint very precisely the cells that trigger disease, so they can be targeted for treatment. Normally, we need to wait at least 8 weeks after the mice have been irradiated and injected with the new immune cells, for the cells to reach stable numbers and the mice to be analysed.

In some cases, we will need to remove components of the immune system, to check whether they are necessary for disease development. This will be achieved by injecting antibodies that neutralise such components, or by genetic manipulation of the mice. In some cases, the mice will need to be treated with a substance like a hormone or an inactivated toxin, in order to activate the genetic deletion in a particular cell type. From the moment the substances are injected, the experiments typically last a few weeks.

Finally, some mouse models of autoimmune disease will be treated with therapies based on natural products of our immune system that we have published, for which we understand at least in part the mechanism of action, aimed at improving the condition. These treatments are expected to last for a few weeks to a couple of months.

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

Immunization experiments are expected to cause no or minimal side effects.

Injection of antibodies or substances to delete immune components will also cause minimal adverse effects in mice bred under SPF conditions, although elimination of immune subsets like T cells may make mice more susceptible to infections by commensal organisms and may need antibiotic treatment.

Irradiation and reconstitution of mice is expected to cause mild transient adverse effects and rarely death due to poor immune reconstitution. Mice poorly reconstituted or undergoing an alloreaction will show signs of illness that will prompt increased monitoring and if needed, humane killing.

Exposing mice, especially female to a transient increase in body temperature should not cause any harm except for mild transient discomfort.

In systemic autoimmune disease models exemplified by lupus, mice will develop manifestations of autoimmune disease that typically include tiredness, general malaise, abdominal discomfort and decreased survival. These manifestations tend to be chronic and will prompt closer monitoring and humane killing when needed. More specific ones will depend on the autoimmune disease:

In mouse models of arthritis, the majority if not all mice will develop swollen and painful joints, which may affect mobility and food intake (causing weight loss). In the spontaneous model of autoimmune arthtitis (K/BxN) these manifestations tend to be chronic and will prompt closer monitoring and humane killing at the indicated stage. In the induced model of autoimmune arthritis (collagen-induced arthritis), joint inflammation lasts for 10 days to 3 weeks after the second injection, after which mice typically recover. Mice will typically be culled as soon as they reach the peak. Only when the effectiveness of treatments or removing immune system cells or components is being investigated will mice with swollen and painful joints be kept alive (being closely monitored), but mice will be treated with regular analgesics.

In mouse carrying mutations or receiving treatments like DSS that cause inflammatory bowel disease (IBD), a proportion of mice may develop diarrhoea causing weight loss. DSS-induced colitis peaks in severity at day 6 and mice gradually recover. In most of our experiments mice will be taken down as soon as they peak or even before then.

In a mouse model of pemphigus, the majority of mice develop irritation in the mouth and skin from 1-2 weeks after cell transfer. Minor mucosal/skin lesions can be detected by 4 weeks and disease peaks at 6 weeks post transfer. The skin and mucosal lesions may be irritating and painful, and therefore need to treated regularly with analgesics, and given soft food. Mice will be killed humanely as soon as skin erosions (i.e. ulcers) are found.

In mouse models of multiple sclerosis (experimental allergic encephalomyelitis (EAE)), mice will develop progressive paralysis starting from the tip of the tail from 8-10 days after immunization, with the course of disease rarely exceeding 14 days. Most mice will be humanely culled as soon as they reach the severity limit.

In mouse models of allergies, mice could develop anaphylaxis within 10 minutes from the second allergen challenge that will prompt humane killing.

Mice developing mammary tumours may experience mild discomfort at the site of the tumour and compromised overall well-being.

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)?

Immunisation experiments: MILD

Injection of substances that deplete components of the immune system or induce genetic deletions: MILD

Sublethal irradiation and immune reconstitution experiments: MODERATE in up to 5-10% in which immune reconstitution fails or there may be some unwanted alloreactivity. Otherwise, this is a mild procedure. Very rarely, and only in a particular type of experiment in which the mutant donor cells

trigger autoimmune disease, the proportion of mice developing moderate disease may increase to 50%.

Heat exposure: MILD

Mouse models of autoimmune disease:

a) Lupus: most genetic mutations will induce MILD (80%) to MODERATE (20%) disease, with exceptions like TLR7 gain-of-function mice (kika/ramos) in which disease may be SEVERE (20%) in mice over 12 weeks of age.

b) Autoimmune arthritis models: Collagen-induced arthritis: Expected severity = MILD (70%); Highest severity that can be experienced = MODERATE (30%). In K/BxN mice, most mice develop arthritis spontaneously and may peak within 2-3 weeks after weaning, which means that nearly 100% will reach MODERATE disease severity. These mice will be monitored very closely and culled humanely to avoid severe disease.

c) Colitis models: MILD (50%); highest severity that can be experienced = MODERATE (50%)

d) Pemphigus vulgaris model: MODERATE in the majority of the mice due to the development skin and mucosal lesions that can eventually ulcerate, at which point mice will be immediately culled.

e) Experimental autoimmune encephalitis: SEVERE in the majority of the mice (80-100%). Even though mice will be culled immediately if they develop total hind limb paralysis, mice may experience reduced mobility, eventually developing partial hind limb paralysis.

f) Allergies: SEVERE in less than 10% in WT and up to 100% in GA animals because anaphylactic shock develops from 5-10 minutes from the second injection of allergen, and even though each individual mouse is closely monitored during those 10 minutes and culled as soon as the first symptoms are observed, the very rapid fatal progression makes it very difficult to assess the degree of suffering.

g) Tumour models: Expected severity = MILD (50%); Highest severity that can be experienced = MODERATE (50%)

h) Infection models: Expected severity = MILD (20%); MODERATE (70%); Highest severity that can be experienced = SEVERE (10%),

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

- Killed

A retrospective assessment of these predicted harms will be due by 04 May 2028

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 focus of the experiments in this application is the discovery of genetic and cellular mechanisms that regulate antibody production relevant to vaccination and immune diseases including autoimmunity and allergies. Over the last few years, we have managed to undertake close to 50% of all our experimental work using human primary cells, or cell lines. There are however a few areas of our work that unfortunately, to date, still require experiments using mouse models:

1. Obtain definitive proof that a particular genetic mutation found in human patients causes disease, particularly in the context of rare diseases in which there are few patients in the world that carry mutations in a given gene. Before generating the mouse model, extensive functional screens are performed to obtain biochemical and molecular evidence that a gene mutation alters protein function. Only when this evidence is compelling, we need to prove that alteration is the cause of the disease. It is then necessary to isolate that mutation from the other hundreds of rare gene variants found in each individual and introduce that single mutation in mice in which their genetic make-up is otherwise identical and are also exposed to the same and highly controlled environment. Pathogenesis of immunological disease is generally very similar in mice and humans, and the function of

individual genes and mechanisms can be resolved in the uniform genetic and environmental background of laboratory mice.

2. Establish the mechanism by which a gene mutation causes disease or breaches immune "tolerance checkpoints". These checkpoints can act during B cell development in the bone marrow, or during their selection and transition through a number of maturation stages as lymphocytes recirculate through different organs, or during interactions with specialised T cells at defined niches in secondary lymphoid organs (i.e. "germinal centres") that spontaneously occur during infection or vaccination. None of these developmental stages nor germinal centres have yet been recapitulated in vitro and therefore require animal models.

3. To elucidate in which cell a particular mutated gene is acting to cause disease. Mixed bone marrow chimeras (ie reconstituting the immune system of sub-lethally irradiated mice by injection of a mixture of labelled mutant and wild-type stem cells) allow us to determine in which cells the mutation is acting to cause disease.

4. To identify which diseases can be treated with our newly discovered regulatory proteins produced by follicular T cells.

Our program is generating the most accurate models of disease to date, because mice are generated to harbour the mutations found to cause disease in patients. In turn, these more animal models will be more relevant and therefore immediately translatable to human disease and therefore accelerate the path to discovery of more effective treatments.

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

We have considered non-animal alternatives, some of which we will still use.

1. Before undertaking any research in mice to understand mechanisms of action of the human mutations, we try and use a broad range of in vitro reporter assays to try to narrow down the pathway. We transfect a spectrum of human B, T, myeloid and embryonic kidney cell lines with plasmids containing the human gene variants, to inform as to which may be the affected pathway, and whether a particular mutation is pathogenic, gain-of-function or loss-of-function.

2. We use short term cultures of fresh human tonsil cells in which most cells including germinal centre B cells can survive for some amount of time.

3. We routinely model the effects of mutations on protein structure and function using a number of bioinformatic tools. These insights help focus the experimental work and formulate more specific hypotheses.

4. We routinely investigate the consequences of genetic mutations on protein production, stability, localization and cell migration using in vitro assays. For example, we test whether a plasmid carrying a human mutation within the coding sequence can make the normal amount of RNA and protein, or whether the mutation results in nonsense-mediated decay, less protein expression, or the function of the protein in well-validated assays.

These efforts have proven very successful at reducing the use of mice. Indeed, my laboratory was 100% mouse-focused 5 years ago, and today, mouse work represents less

than 50% of our research activity, as we have gradually designed better assays that can be performed in-vitro using human peripheral blood mononuclear cells (PBMCs) from both patients and healthy controls, as well as tonsils extracted during routine pediatric tonsillectomies. We are also trialling thick-tonsil section based organoid cultures, although survival of B cell subsets of interest is still severely compromised.

Why were they not suitable?

Further reduction using some of the alternatives described above is impossible at the moment, given that protective immune responses in the context of infection or immunisation involve interactions between T and B cells, innate cells and stromal cells in various organs both as the cells mature and as they encounter microbes, commensal bacteria, or physiological stressors. Such interactions and effective activation to mount a productive long-lived memory antibody response to date have not been successfully recapitulated in vitro.

In a similar line, despite ongoing efforts by many groups around the world including ours, negative selection of self-reactive B cells during their different developmental stages cannot be induced in cell cultures. These cellular processes and the tissue in which they physiologically occur, are inaccessible in live humans and, with rare exceptions, the complexity of human genetic and environmental variation makes it impossible to tease out the critical genes and pathways without experimental validation in laboratory mice.

We continuously read and screen the literature, conference presentations and abstracts, and wherever possible develop tissue culture assays to investigate genes and mechanisms for vaccine development and autoimmune disease prevention and treatment without the use of animals once these mechanisms have been established by in vivo experiments.

A retrospective assessment of replacement will be due by 04 May 2028

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?

Estimating the numbers required for breeding and propagating gene variant and transgenic strains while generating sufficient mice for experiments: We estimate 40 non-genetically-altered mice will be needed as breeding partners per year per strain. Our breeding strategies are often complex and entail generating a large number of mice per year. We often need to intercross strains carrying several alleles of interest that: a) reduces the frequency of mice of interest per litter and b) often results in an inability to use

mice until the 2nd or 3rd generation. We also regularly back-cross our strains to avoid genetic drift (these mice are of no use experimentally but are necessary to ensure animal welfare in adoptive transfer and bone marrow chimera experiments). Therefore, we anticipate we will need 3 breeder pairs per strain. Breeder mice produce on average a litter of 6 mice every 5 weeks. That means 10 litters x 6 mice/litter x 3 breeders, per year and per strain = 180, plus 6 breeders = 186/strain. For 30 strains = 5580/year.

Typically, each experiment will include 5-10 mouse per genotype, depending on how large the expected change is between experimental and control groups in order to detect statistically-significant differences. Most experiments will require 3 different genotypes (i.e wild type mice, mice with one copy of the mutated gene and mice with two copies of the mutated gene). Thus, for protocols that do not require intervention (e.g. injection), we will typically use 15-30 mice. By contrast, protocols that require challenges and analysis of tissues/organs before and after challenge, will require additional groups of treated mice (the total number per experiment will depend on the number of time-points that need to be evaluated by tissue dissection).

To ensure reproducibility, we will repeat experiments at least 2 times. For experiments where we expect, based on previous experience or pilot projects, a small effect size or larger variability between animals we will increase the experimental groups to achieve a similar statistical power.

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 the numbers used we will:

Have a very clear idea of the question we want to answer and the required readout.

Plan carefully every experiment, to include every necessary control in all experiments, as well as include randomization and blinding at all required steps.

Perform the required small pilot projects to estimate the size of effect we are expecting and narrow the number of doses to be used etc.

Take advantage of a number of online tools including the NC3Rs Experimental Design assistant.

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 to test the doses of the products we are going to inject as well as to estimate the effect sizes mentioned above.

We plan to collaborate with other groups interested in exploring similar pathways so we distribute the work and do not duplicate efforts
We will maximise the use of genetically altered animals so we take all the required readouts from the smallest number of animals (serial serum samples, lymphoid organs for flow cytometric analysis and preparation of cell suspensions for in vitro cultures, tissues at necropsy for histological examination)

For mouse models in which there are few mice of the desired homozygous genotype born (i.e. Prkcd-/- mice), we will generate bone marrow chimeric mice from a single mutant donor, to save having to breed large numbers of mice to obtain the required genotypes.

A retrospective assessment of reduction will be due by 04 May 2028

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.

There are two types of models that we use depending on the project:

1. In order to understand the genes and pathways that cause systemic autoimmunity (diseases like lupus), we are introducing in mice the same gene variants that we find in humans. This ensures that the models of disease are the most relevant to human disease. In these models, mice develop lupus- like disease and other related systemic autoimmune conditions that closely recapitulate the human condition. When possible – i.e. for studies of cell types and general mechanisms, we will prioritise the use of our newly generated lupus models that cause less pain and distress (i.e. Tlr7 gain-of-function). Those that cause more severe manifestations (such as for example Trex1-deficiency) will only be used when we need to understand the differences between pathways and find the key biomarkers that can identify each pathway.

2. In order to test the efficacy of our recently discovered therapies in autoimmune and allergic diseases (i.e. autoimmune arthritis, inflammatory bowel disease, multiple sclerosis) we need to use models of disease that are antibody-driven, and in which production of antibodies to a specific antigen can be monitored.

Given the mechanism of action of neuritin (preventing the generation of autoantibodies), we have no choice but to use the only validated models of arthritis and pemphigus, that have been proven to be antibody-dependent in serum-transfer assays. These are the K/BxN model of arthritis driven by GPI autoantibodies, and the model of pemphigus induced upon transfer of desmoglein3-deficient cells into C57BL/6 mice, driven by DSG3 autoantibodies. Experimental models of allergies such as OVA or peanut-induced allergies are typically mediated by antigen specific anti-IgE antibodies, that can be monitored .

For experiments where we want to understand other roles of Tfr cells, we will choose collagen-induced arthritis as the best accepted and mildest model of arthritis.

In the case of multiple sclerosis, mouse models are somewhat limited: some do not fully recapitulate the human relapsing remitting disease, and the ones that do, are not B cell dependent. Thus we are constrained to use the only mouse model that is B cell-dependent so we can test our B cell-modulating therapies. This is the modified EAE model selected in our protocol.

Monitoring the development, cell of origin and kinetics/persistence of these antibodies in the presence or absence of neuritin-based or similar treatments will be crucial readouts, besides monitoring the development of clinical signs and symptoms.

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

Development of autoantibodies requires a mature immune system, and autoimmunity is not observed during immature life stages or in species that are less sentient. The pathogenesis of these diseases is studied over time and cannot be studies at a single time point.

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

We will pay a lot of attention to define very carefully the question we want to answer so that we can identify and put in place the earliest possible end points. For example, in order to find out if our treatment with neuritin is effective at preventing pemphigus disease, valuable readouts can be measured before mice develop the full clinical manifestations (i.e. appearance of autoantibodies), therefore preventing the suffering associated with these condition. So whenever possible, early readouts will be prioritized and experiments terminated before mice become unwell.

We also plan to undertake very close monitoring of the animals. We have developed a separate clinical scoring sheet for each model of autoimmunity so that we can very quickly and continuously assess the more likely sources of pain and distress in each model so as to manage them efficiently.

We will also have in our protocols very clear pain management plans to prevent suffering. While the arthritis and pemphigus autoimmune mouse models have been widely used in the literature without the use of analgesia, we are aware that they can involve significant discomfort or pain to the animals. We are planning a trial on the K/BxN arthritis and pemphigus mouse models to test if the use of Buprenorphine has any significant effect on the production of total and antigen-specific antibodies, inflammatory cytokines, and clinical scores.

In the case of mice developing tumours, besides mice being very closely monitored, the tumours themselves will be examined regularly and experiments will be terminated if tumours ulcerate, metastasise or reach a certain diameter or growth.

Environmental enrichment will be provided, guided by the most up-to-date practices in our facility. These will include more and softer bedding/nesting material for mice with arthritis and sore skin due to pemphigus, food on the floor for animals with arthritis and EAE, and mashed food for animals with pemphigus.

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

We will follow the local guidelines, as well as those from NC3Rs (https://nc3rs.org.uk/) with particular attention to the themes of Analgesia" and "Humane end-points"; ARRIVE (https://www.nc3rs.org.uk/arrive-guidelines) and PREPARE guidelines.

Important guidance for pain management will be sought from the online book: Recognition and Alleviation of Pain in Laboratory Animals (National Research Council (US) Committee on Recognition and Alleviation of Pain in Laboratory Animals; Washington (DC): National Academies Press (US); 2009.

We will also follow best practice and refinement as published in the following papers: For administration of substances:
D. B. Morton , M. Jennings , A. Buckwell. Refining procedures for the administration of substances. Laboratory Animal Limited (LAL) 2001.
https://doi.org/10.1258/0023677011911345

For arthritis research:

Hawkins P, Armstrong R, Boden T, et al. Applying refinement to the use of mice and rats in rheumatoid arthritis research. Inflammopharmacology. 2015;23(4):131-150. doi:10.1007/s10787-015-0241-4

For EAE research:

Wolfensohn S, Hawkins P, Lilley E, Anthony D, Chambers C, Lane S, Lawton M, Voipio HM, Woodhall
G. Reducing suffering in experimental autoimmune encephalomyelitis (EAE). J Pharmacol Toxicol Methods. 2013 May-Jun;67(3):169-76. doi: 10.1016/j.vascn.2013.01.009. Epub 2013 Jan 26. PMID: 23357188.

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

I will seek to read the communications and advice produced by our NC3R liaison officer on advances in the 3Rs. I will continuously read the literature to find progresses that may enable substituting some of our models for better ones and further reducing animal work. My team will continue to optimise the use of organoids using human tonsil to understand immune cell interactions so that we reduce the need to investigate these in vivo.

A retrospective assessment of refinement will be due by 04 May 2028

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. Studies in the Later Stages of Prion Disease in Mice

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

Prions, Therapeutics, Drugs, Creutzfeldt-Jakob Disease, Transgenic animals

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 purpose of this project is to determine the efficacy of therapeutics in the treatment of prion disease when administered once neurological symptoms are manifest.

A retrospective assessment of these aims will be due by 21 June 2028

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?

We are interested in studying the efficacy of therapeutic drugs in established neurological disease in mice at a stage which corresponds to the clinical stage at which human patients are diagnosed.

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

This work aims to facilitate assessment of drugs that affect the clinical phase of prion disease once neurological signs are established (the stage at which most patients present to clinicians). At the end of the project, we hope to see significant mitigation of severe prion disease symptoms in response to the various therapeutic compounds we will be administering. Our findings from this project will be shared with the scientific community through presentations at conferences and publications so that our results will inform the work of others in the field and those working in other neurodegenerative diseases.

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

Potential benefits can be extended survival time, less severe disease or improved motor functions such as grip strength etc. The results will provide the basis for the design of future clinical trials.

Benefits will be both short and long term. In the short term, this work enables feasibility testing of therapeutic strategies in late stage prion disease, and informs on whether late stage prion disease (or related neurodegenerative diseases with underlying prion mechanisms) can be mitigated or reversed. Also, through the development of a severity scale it will provide a higher resolution readout of the progression of prion disease for future studies.

In the long term, this work will be directly informative for patient clinical trials.

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

The research of the Unit is multidisciplinary and strategically inter-connected, thus maximising a range of skills and expertise to ensure that the most important questions in prion biology are being tackled from many different angles. In addition to the internal close working relationships, we also work with several external collaborators on specific aspects of our research programmes. The Unit provides an internationally recognised centre of expertise in post-graduate training, prion biosecurity and specialist technical and advisory expertise to academic centres, industry, WHO and Government at a national, EU and international level. Any new knowledge acquired through the conduct of this project will facilitate the dissemination of knowledge and experience nationally and more widely.

Species and numbers of animals expected to be used

Mice: 300

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 mice will be used as their genetics, physiology and neuroanatomy are very well-characterised and their breeding time and lifespan allow experiments to be performed on manageable and affordable timescales. Their similarity to humans in terms of genetics and neuroanatomy, and their genetic tractability has led to the generation of many successful models of neurodegenerative disease and the identification of potential therapeutic avenues. Prion-infected mice faithfully recapitulate the clinical and pathological features of human prion disease. For these reasons, the mouse is our model of choice for efficient translation of our findings to humans.

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

This project on prion diseases has 1 Protocol. Some animals will be inoculated with prions under general anaesthesia, followed by administration of therapeutic agents through various routes of administration as stated in the Protocol Steps. Other animals will be used as control and will be inoculated with normal brain homogenate or vehicle only under general anaesthesia or left uninoculated. Animals inoculated with infectious material are expected to develop neurological disease after variable clinically silent incubation periods followed by rapid deterioration. Animals will be inspected regularly for onset of clinical signs, and daily thereafter. Any positive effects of the administered therapeutic agents in mitigating disease will also be observed.

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

- Animals undergoing surgical procedures carried out aseptically under general anaesthesia may rarely develop post-operative complications. Such animals will be killed unless, in the opinion of the NVS, such complications can be remedied promptly and successfully using no more than minor interventions. In the case of wound dehiscence, uninfected wounds may be re-closed on one occasion within 48 hours of the initial surgery.

- Animals may respond adversely to some of the administered therapeutic agents perhaps as a result of direct intolerance or by immunological hyper-responsiveness. All animals will be carefully observed to ensure continued good health following treatment with substances. Animals will be killed by a Schedule 1 method or perfused culled under terminal anaesthetic if there are any severe adverse reactions. Should the animals not experience severe disease, they will continue to the experimental end point when they will be killed by a Schedule 1 method or perfused culled under terminal anaesthetic.

- Animals inoculated with prions are expected to develop clinical prion disease after a clinically silent incubation phase, which typically manifests as a degenerative neurological condition. Animals diagnosed with severe signs of prion disease will be immediately killed by a Schedule 1 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)?

About 75% of animals used in this protocol will experience the Severe threshold.

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

- Killed

A retrospective assessment of these predicted harms will be due by 21 June 2028

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 animals to study how candidate therapeutic agents may mitigate late stage prion disease in order to mirror the stage at which Creutzfeldt-Jakob Disease (CJD) is diagnosed in patients. Clinical disease cannot be studied in cell culture. Key prion disease parameters such as clinical duration and features, behavioural changes, brain damage and the spread of the abnormal protein within the body leading to fatal brain damage, can only be studied in an animal. For this PPL, we need to study the effects of therapeutics in mice that are already showing signs of prion disease, with treatment continuing to cull point at diagnosis of severe signs of prion disease (see the Scrapie Diagnostic Criteria, under general humane endpoints), that is beyond the point where mice are currently being culled on welfare grounds.

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

Non-animal alternatives are currently not available for this project.

Why were they not suitable?

Not applicable

A retrospective assessment of replacement will be due by 21 June 2028

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

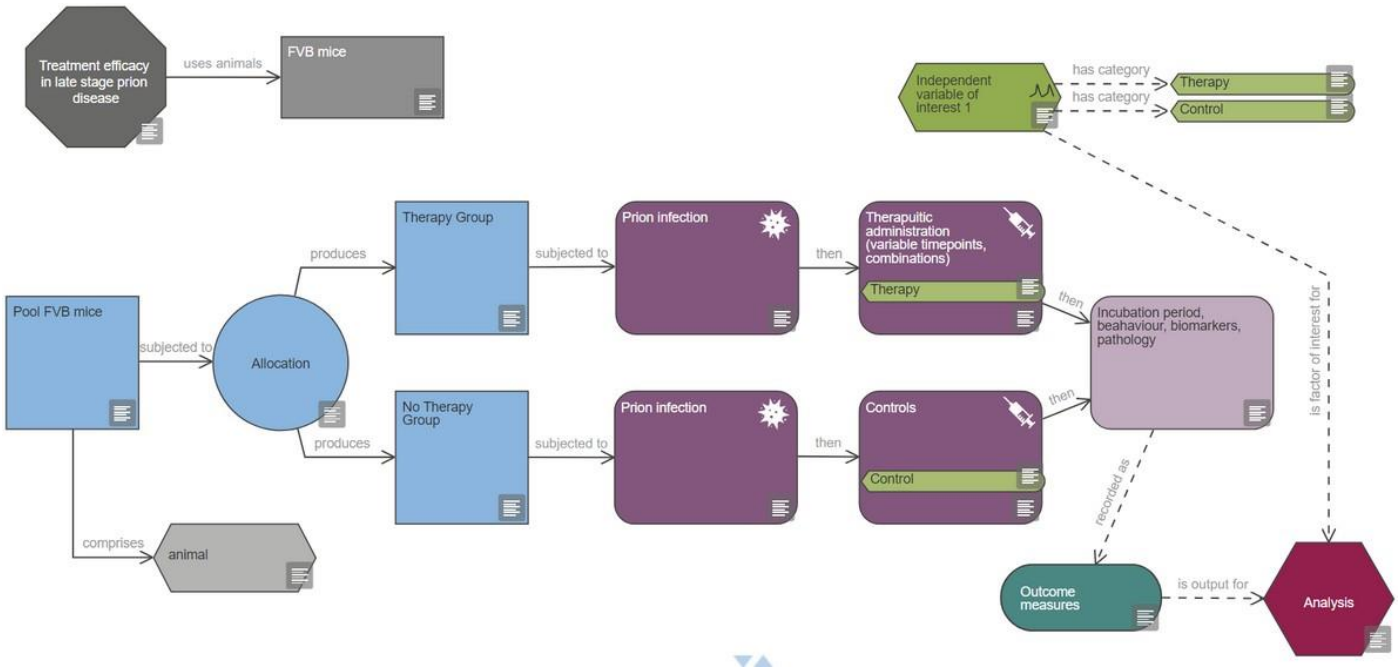
Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may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

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

For these studies we will use a combination of well-characterised mouse strains and prion isolates where the incubation periods and disease phenotype would have been previously determined. As well as giving us reference points against which success of the administered candidate therapeutic agents can be measured, the optimum number of mice would also have been determined from previous studies.

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

The NC3Rs' Experimental Design Assistant (EDA) will be used in the design phase of individual experiments where short incubation periods are expected. The graphical output of an experiment designed using EDA is shown below. The full EDA report is too long to present here, and will be made available if requested.



For prion transmissions that involve longer incubation periods or when multiple analysis of similar tissue, but with different processing is required group sizes of 15-20 mice will be used. This allows for sufficient surviving animals at the end of the experiment to provide adequate tissue for biochemical, cellular analysis and immunohistochemical analyses.

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 ensure that animals are bred to requirements, and that all animals left on the shelf have a justifiable reason for being kept. Only a limited number of animals will be used in this project.

A retrospective assessment of reduction will be due by 21 June 2028

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 as their genetics, physiology and neuroanatomy are very well characterised and their breeding time and lifespan allow experiments to be performed on manageable and affordable timescales. Their similarity to humans in terms of genetics and neuroanatomy, and their genetic tractability has led to the generation of many successful models of neurodegenerative disease and the identification of potential therapeutic avenues. For these reasons, the mouse is our model of choice for efficient translation of our findings to humans.

The large majority of testing of potential therapeutics in prion-infected mice has involved their administration to mice before the onset of symptoms to determine if onset can be delayed, with the mice being culled following the onset of defined neurological symptoms. However, to determine the ability of treatments to slow disease progression in humans who are already showing symptoms of prion disease it is necessary to test therapeutics in mice at a comparable point in the disease course.

Animals will be observed at least 2 times daily and will be culled as soon as they reach the severe end point. Every effort will be made to avoid animals being found dead, as in addition to reducing animal suffering, this will also ensure that we obtain brain samples suitable for histological analysis.

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

We need to observe late stage clinical disease that is reminiscent of patients who come to the clinic with disease symptoms. Cardinal signs of prion disease cannot be recapitulated in less sentient models such as flies. It is often necessary to study the specific effect of the PRNP codon 129 polymorphism which is a powerful modifier of human prion disease, and such studies require the use of genetically modified mice with specific PRNP genotypes.

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

By using anaesthesia and pain killers as necessary, and through careful monitoring, animal suffering will be reduced to an absolute minimum. We will also minimise harm by implementing high standards of care for each animal, by ensuring that the maximum severity for this protocol is adhered to, and by using well-defined humane end-points.

Detailed behavioural and motor tests will be performed to more accurately assess disease progression to determine relevant symptomatic timepoints for therapeutic administration, and to develop a severity scale of disease (as exists in clinical practice) which will be used to improve the definition of both moderate and severe disease stages, and inform on humane endpoint refinement.

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

We will continue to follow the NC3Rs' ARRIVE Guidelines 2.0, and LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery (https://www.ubs.admin.cam.ac.uk/files/lasa_aseptic_surg.pdf) as best practice to ensure that our experiments are conducted in the most refined way.

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

In keeping with the Unit's commitment to the 3Rs promotion, a 3Rs Champion has been appointed to lead our efforts to comply with all aspects of the 3Rs. The 3Rs are a regular feature of the meetings of the AWERB and local Animal Research Scientific Committee (ARSC) which both monitor and ensure that animals are bred to requirements, and that all animals have a justifiable reason for being kept. The ARSC meets once a month and the 3Rs Champion, who keeps an eye on information coming out of the NC3Rs and other relevant sources, has the opportunity to report on 3Rs advances that can be implemented in our practices during the duration of the project. Unit-wide presentations relevant to 3Rs are incorporated into our seminar programme.

A retrospective assessment of refinement will be due by 21 June 2028

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. The Safety Evaluation of Pharmaceuticals in Dogs and Pigs

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

Non-clinical, Safety, Dog, Pig, Toxicology

Animal types	Life stages
Beagles	juvenile, adult, embryo, neonate, pregnant, aged
Minipigs	juvenile, adult, embryo, neonate, pregnant, aged
Pigs	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.

- 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 project aims to test pharmaceuticals (for human and veterinary use) and Medical devices to determine the scientific and/or regulatory endpoints in non-rodent (dog, pig or minipig) toxicity, pharmacokinetics and metabolism for submission to regulatory authorities, to satisfy governmental regulatory requirements, for safety assessment purposes or for substance candidate selection.

These studies are run to satisfy the requirements of UK/EU (and sometimes international regulatory authorities) who are independent of governments) which require the testing of pharmaceuticals in a non-rodent species. Study designs are based on OECD, ISO and ICH guidelines for Pharmaceutical and medical device testing.

A retrospective assessment of these aims will be due by 21 January 2028

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?

New medicines have the potential to be of 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 pharmaceuticals we test in this programme will be designed to be better than existing treatments, possibly with fewer or less severe side effects.

At present there are no alternatives that don’t use animals that are scientifically, ethically or legally acceptable as replacements for systemic toxicity or safety assessment. Most new medicines are tested in rodents (mainly rats and mice) before being tested in a second, non-rodent species like the pig, minipig or dog.

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 or animal patients by generating high quality data that is acceptable to regulatory authorities and enables internal decision making within our client's organisations. 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 or animals are exposed by the use of new drugs. Supporting studies, including preliminary studies and candidate selection, will enable appropriate dose selection and appropriately focussed observations and investigations in the definitive regulatory studies.

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 and animal 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 or animals. These drugs that will be tested are for a variety of 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 or animals, to make sure any starting dose in a clinical trial is safe for the patients taking it.

Our customers 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 customers 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 candidate selection or to support drugs progressing to clinical trials). Where appropriate, we collaborate with our customers to share data we have produced in the form of scientific publications that are in the public domain.

We are able to advise our customers on which studies are required in their development programme and on suitable study designs, based on our experience and on knowledge gained from previous post- registration feedback from customers and/or regulators, leading to focussed and effective studies.

It is difficult to predict how the benefits of any work done on this project will be seen in the future due to confidentiality issues. However this work will contribute to the safety of pharmaceuticals that can be administered to humans and animals, (either by informing on safety and allowing to progress to clinical trials, or preventing pharmaceuticals reaching the market due to safety issues), which in itself reduces the overall number of animals used (by preventing further testing).

Species and numbers of animals expected to be used

- Pigs: 450
- Minipigs: 4000
- Beagles: 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.
The dog, pig or minipig are used in these studies, as they are well characterised species with a lot of background scientific data available over many years. They also satisfy the requirements of global regulatory authorities for safety evaluation in non-rodent species, which is required by law prior to testing in humans (or in some cases prior to conducting veterinary clinical trials).

As stated earlier, most pharmaceuticals are tested in a rodent species prior to testing in a non-rodent species, as covered by this project.

It is a legal requirement in the UK that dogs (or cats or equidae) may only be used in a programme of work involving regulated procedures when the objectives of the work cannot be achieved by using another species. In this project, the dog will only be used when use of the pig or minipig would not achieve the aims of the experiment, or satisfy regulatory authorities. All requests for studies using dogs are assessed by means of an internal review process; the review panel, including scientists, project licence holders and responsible persons under ASPA, consider the information presented to reach a consensus decision, and will only approve the use of dogs where there is robust justification that the study could not be successfully performed using pigs or minipigs instead.

Most studies would be carried out in adult animals; juveniles would only be used for specific studies for pharmaceuticals targeted at juveniles. Similarly, studies in pregnant/breeding animals will only be conducted where there is a need to assess safety of test items to which reproductively active humans or animals may be exposed.

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

Animals are dosed by the intended/likely route of human or animal exposure (for example oral administration, injection, infusion or inhalation), and observed regularly to monitor appearance, behaviour and clinical health.

Some animals may undergo a surgical procedure under general anaesthesia, eg placement of a deep vein catheter for intravenous infusion, or implantation of a monitoring device or minipump. Investigative procedures carried out in these studies are similar to diagnostic procedures that might be used medically to monitor progress of a human patient and include, for example, collection of blood and urine samples for laboratory investigations, or ECG monitoring to assess heart rate/function, or examination of the eyes using an instrument similar to those used by opticians. 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.

If we need to take a urine sample for analysis, we would put an animal into a special collection cage which is smaller than their normal cage. The animal can still move around.

Other more unusual tests might include assessment of retinal function, assessment of neural function, taking small samples of tissue under general anaesthesia, collection/examination of body fluids such as tear fluid or semen, collection under general anaesthesia and examination of lung washings or spinal fluid, body temperature by rectal thermometer. A minimal degree of restraint or confinement may be required for some

procedures. Where appropriate, positive reinforcement training (using treat rewards) is used to encourage co-operation in (and minimise any stress of) handling/procedures. Some animals may be used on procedure on more than one occasion (re-use); such re-use is limited and strict criteria are applied, eg veterinary examination indicates that it is appropriate to do so. Some animals (dogs only) may be re-homed via the establishment's rehoming scheme if it is in their best interests, but most animals are humanely killed at the end of the study to allow detailed examination of the organs.

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 degree of pain or discomfort an animal feels is similar to what a patient would feel having an injection done by a doctor

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.

Dosing with drugs may cause adverse effects in some studies. Experience from the last licence shows that the majority (~90%) of animals display only mild severity with the remaining 10% displaying moderate severity. Lethality and/or severe effects are not expected to occur, in any of the protocols in this licence.

We 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 consult vets and other senior animal care staff for advice and guidance in its care.

Most animals are expected to experience no, or only mild, adverse effects during the course of the study such as slight weight loss. A small percentage of animals may show more significant adverse effects, such as more marked weight loss, reduced activity, vomiting or tremors. No animals would be expected to die or to suffer prolonged adverse effects as a result of the procedures, and where necessary early humane end-points are applied, under veterinary guidance as necessary, to prevent this; such end-points might include interventions to discontinue dosing, or to provide supportive treatments, or if necessary to humanely kill the animal.

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 experienced mild severity, the rest were classified as moderate the moderate severities in the last project would have been due to treatment-related signs of moderate severity (mostly in prelims) or because a surgical procedure e.g. cannulation was involved. 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. However, a distribution between 'mild' and 'moderate' severities similar to those in the last project are anticipated.

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
- Kept alive
- Rehomed
- Used in other projects

A retrospective assessment of these predicted harms will be due by 21 January 2028

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 in animals is a mandatory legal and regulatory requirement and provides information on risks to people and animals 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.

We maintain a constant awareness of regulatory guidance and ensure that where non-animal methods exist which fulfil the regulatory requirement, they are used in preference to animal studies.

The regulatory requirements are mainly for UK/EU regulators, but occasionally other regulators in other countries like the US for example. If the requirements for these non-UK/EU tests are over and above the requirements for a UK/EU regulators, or the test required is more severe, then we consult the Home Office to ask for prospective authority to run such tests.

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 in vitro 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 in vitro tests that brings all these complex events together, as in the whole (animal or human) organism.

That is why we need to test 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 are subsequently used in humans.

A retrospective assessment of replacement will be due by 21 January 2028

The PPL holder will be required to disclose:

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- 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.

The numbers of animals used in each study are in some cases specified in the regulatory guidelines; where not specified, numbers are based on established minimum regulatory expectation, or on scientific estimates of the minimum numbers required to meet study objectives.

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 to get as many outputs as we can from a single animal where possible, without adversely affecting its welfare. So if we need to take several different samples, for example, we will often do that in the same animal, rather than using 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 preliminary studies are important as they give us confidence that the doses we are using are correct prior to testing them in bigger groups of animals as required by global regulators.

A retrospective assessment of reduction will be due by 21 January 2028

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 dogs, pigs and minipigs We only use dogs when pigs or minipigs are unsuitable for scientific reasons.

The models we use are the least invasive procedures, for the least amount of time necessary to get the information we need. They are carried out using standard and recognised techniques by fully trained staff. We also have veterinary clinicians on hand for advice and on the occasions we have to anaesthetise the animals and for general advice on animal welfare.

If we have to repeatedly inject animals or withdraw blood using a needle and syringe, we would choose different sites to do this where possible to minimise local adverse effects.Where appropriate we place temporary cannulas in blood vessels to reduce the number of needle punctures necessary. If we can take blood samples when an animal is deeply unconscious then we do so. .

For all surgical procedures pain relief will always be provided. Surgical procedures will be carried out aseptically and to at least the Home Office minimum standards for aseptic

surgery, and in accordance with the principles set out in the LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery (2017) (LASA is the Laboratory Animal Science Association). Any animals that undergo surgery will get the same standard of care as a patient who needed surgery in hospital.

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

There is a scientific and regulatory requirement for safety/toxicity data in non-rodent species such as dogs or pigs to supplement rodent data and enable a complete risk assessment. We use pigs in preference to dogs wherever possible; (a legal requirement in the UK), and dogs are only used where necessary to achieve the study objectives, ie when the pig is unsuitable (for example due to species- specific differences from humans, confounding pharmacology or toxicological responses, or practical limitations due to anatomy or physiology).

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 animals are recovering from surgery, we give them extra heat and monitor them closely until they are fully recovered and showing normal behaviour. We then check them at least twice daily before they go on study.

During dosing and restraint, animals are constantly and closely watched for signs of distress. If equipment is used to enable us to achieve the scientific aims of the study (e.g. confinement in a metabolism cage for urine collection), then we would habituate animals to this equipment prior to dosing. Most animals habituate well to this equipment, but if they don't (rare) we remove them from the study

If we have to repeatedly inject animals or withdraw blood using a needle and syringe, we would choose different sites to do this where possible to minimise local adverse effects.Where appropriate we place temporary cannulas in blood vessels to reduce the number of needle punctures necessary. If we can take blood samples when an animal is deeply unconscious then we do so. All personnel performing these procedures are trained to a high standard to minimise adverse effects.

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 any adverse events and provide supportive care and treatment as appropriate.

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, and calming measures such as stroking/gentle talking are used to help animals have a better experience of restraint. We have dedicated

working groups on animal welfare for each species with a permanent brief to identify potential measures to improve animal welfare, and to trial such measures and make recommendations for adoption.

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

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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 Animal Technology, and by attending appropriate training courses and conferences, or getting feedback from such events.

A retrospective assessment of refinement will be due by 21 January 2028

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?