Home Office

Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted July - December 2020 that require a retrospective assessment

Contents

1. Assessing metabolic effects of diabetes and metabolic disorders in the heart and other target organs

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

Heart, Metabolism, diabetes, magnetic resonance imaging, stem cells

Animal types	Life stages
Mice	adult, juvenile
Rats	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.

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 interplay between metabolic processes and tissue and organ function in health and disease, with a particular focus on how the development of diabetes affects liver and cardiac function. Damage to a tissue or organ can impair metabolic energy generation and vice versa. By understanding this interplay, we can develop therapeutic approaches to minimise or correct the damage caused by dysfunctional metabolic processes. As part of trying to understand these processes, we will develop new methods for non-invasively imaging them via MRI.

A retrospective assessment of these aims will be due by 03 March 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 it's aims and if not, why not?

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

Why is it important to undertake this work?

Metabolism underpins the function of all organs of the body and generates the energy needed for the heart and other organs to function. The heart is critical to maintaining the supply of blood and oxygen to the body, so that damage to the heart muscle, caused by a heart attack or an increased workload due to high blood pressure, reduces blood flow and energy generation in other organs. These effects are part of the reason why cardiovascular disease is responsible for 27% of all UK deaths. In the UK there are more than 100,000 hospital admissions each year due to heart attack resulting from a blocked artery.

This means that part of the heart muscle is not fed with the oxygen and nutrients that it needs, heart cells die and that part of the heart muscle is replaced by a scarred region that doesn't beat properly. The rest of the heart therefore has to work harder to compensate. Recent developments in clinical practice have meant that many more people now survive a heart attack, which is either treated surgically (via stenting to open the blocked artery) or with drugs, but in some cases the damage to the heart is so severe that the heart no longer functions well enough. Currently over 900,000 people in the UK are living with heart failure. By understanding what causes the heart muscle to die and how we can prevent or reduce the damage, we can increase survival and reduce the number of people for whom the damage is so great that the heart begins to fail. Additionally, sometimes patients who are admitted with a heart attack have received damage that is unlikely to recover if treated surgically, but are operated upon nevertheless. This exposes them to an unnecessary risk of life-changing or life-ending complications, such as stroke, ventricular fibrillation, or cardiac rupture, and it is often usually only realised in the surgical setting of the catheter laboratory that there is nothing that can be done. We are investigating the use of advanced MRI methods with injected compounds to determine whether or not the heart tissue at risk is still viable for surgery, or if it is dead and will not respond to surgical treatment. Also, by identifying exactly which areas of the heart require treatment, we are also investigating whether we can rescue the damaged region of the heart by treatment with stem cells, either injected directly into the heart or attached across the scar to form new viable tissue.

Damage to organs such as the liver and pancreas can alter the supply of sugar and fat in the blood and mean that the heart and other organs cannot generate sufficient energy to work efficiently. By far the most prevalent metabolic disease is diabetes, and its precursor known as metabolic syndrome. There are over 3.5 million people diagnosed with diabetes in the UK and this is estimated to rise to over 5 million by 2025. Worldwide it is estimated to affect 8.5% of the global population. The most common two forms of diabetes are type 1 and type 2 diabetes, of which 90% of people with diabetes have type 2 diabetes. Both type 1 and type 2 diabetes are associated with elevated levels of sugar in the blood and this, over time, leads to complications including vascular problems, high blood pressure and high cholesterol. These are major causes of coronary heart disease, which is recognized to be the cause of death for 80% of people with diabetes. Yet it remains difficult to exactly assess the degree of damage to the heart in diabetic patients, and to understand, non-invasively, what damage has occurred, or is at risk of occurring, or could be prevented in an individual patient. Metabolic syndrome and type 2 diabetes are associated with obesity, which is becoming increasingly prevalent, and which is also linked with sleep apnea, a

common breathing disorder that affects many people whilst they sleep. It is thought to affect 2-4% of the population and, in turn, has been shown to increase the risk and severity of type 2 diabetes independent of age and obesity. In diabetes, there is a mismatch between supply of fat and sugar to the heart and other organs, and the ability of those tissues to take up and use those fuels. This results in reduced tissue function and the build-up of unwanted and potentially toxic by-products. We are investigating why this happens and whether we can use drug molecules to restore a more normal metabolism, thereby preventing the development of further organ dysfunction.

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

Our research will provide valuable insights into the mechanisms behind damage caused by metabolic abnormalities. It will also develop new techniques for imaging this damage, these abnormalities, and their response to treatment. Our work will be published in peer-reviewed journals and presented at national and international conferences. New MR technology and techniques will be shared with other users in the field, in publications and at conferences and workshops, and translated to clinical MRI scanners in the local hospital.

The co-PIs have published over 200 papers which have been cited over 5000 times. One PI is part of the organising committee of our Metabolic Health network which promotes dissemination of research between clinical and pre-clinical departments. She is also an active member of Diabetes UK and is able to discuss her work with the clinical community and patient groups at the annual meeting of the society. Another PI is part of the BHF-funded Regenerative Medicine Network and her work is presented to the BHF community at their annual meetings. The third PI is an active member of the International Society for Magnetic Research in Medicine and his team regularly present at meetings of the society. All PIs are members of the British Society for Cardiovascular Research and encourage members of the group to present their results at the annual meetings of the Society. One PI works in the MR using at the local Hospital and so developments in MR imaging will be directly translated into the clinic, which we anticipate could be done within 12-18 months. Where we are exploring pharmacological therapies, we look first to see whether we can repurpose an existing drug to minimise adverse effects and so that potential therapeutic benefits can be rolled out more rapidly. For example, we are studying the effect on the diabetic heart of a drug which currently going through phase 3 clinical trials for anaemia and which could be directly repurposed for treating diabetic cardiomyopathy. Similarly, we have shown that daily treatment with a dietary supplement increased glucose oxidation in the diabetic rat heart. Where there are no existing drugs, we work in collaboration with colleagues in the Department of Chemistry, whose work focuses on the discovery of new drug targets and mechanisms, and the translation of these findings into new clinical candidates. We have clinical Fellows within our research team who undertake clinical studies linked to our preclinical work.

Our collaborator in the Department of Materials has an excellent track record in product commercialisation and has won awards for his innovative products. He is thus ideally placed to take forward successful tissue engineered scaffolds.

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

The immediate impact will be the sharing of new knowledge and techniques with the scientific research community. This will stem from presentation at conferences worldwide and the high impact publications that will come out during the project duration. Previously our research has been published in the national press, and future work will be promoted in

a similar way, so that the public will be aware of our work, and an increased understanding of the links between diet, diabetes and heart health may influence lifestyle choices. Research into the effects of particular diets, such as a high fat diet or a ketogenic diet, could result in changes to clinical advice within the lifetime of this licence.

The design of new MR imaging techniques and hardware will be able to be put into clinical use immediately in the MR research unit at the local hospital. Whereas MR imaging is used routinely in the clinic, MR spectroscopy is still largely a research tool. Research into the effects of re-purposing drugs for improving cardiac function in diabetic patients or following a heart attack would be able to go directly to phase 2 clinical trial, since any adverse effects of these drugs would already be known. Nevertheless, it would take around five years for a drug to be validated through phase 2 and 3 clinical trials. For novel drug compounds, testing in animal models, determining appropriate dosage levels and safety testing in a phase 1 clinical trial would add a further five years to the process. Work in the BHF-funded regenerative medicine network includes feasibility studies in large animal models, so that advances in stem cell therapy could be tested by collaborators within the network. Unlike novel drugs, stem cells do not need to be tested for toxicity and so advances in stem cell therapy could advance to phase 2 clinical trials in the same way as repurposed drugs.

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

We work in close collaboration with other research groups in the field and share our knowledge through Diabetes UK, the British Heart Foundation Regenerative Medicine Network, the International Society of Magnetic Research in Medicine and the British Society for Cardiovascular Research. It is difficult to publish unsuccessful approaches but new data archives such as Data Brief are making this more feasible.

We have collaborations with companies which provide hardware for imaging scanners and with the diabetes research company Novo Nordisk which funds research and Fellowships. From time to time we have students from the Doctoral Training Centres which have links to the pharmaceutical industry.

Species and numbers of animals expected to be used

- Mice: 1700
- 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.

As metabolism and energy generation are linked to the function of the organs of the body, we need to study these effects in a living animal. In order to study how damage to the heart can change the way the blood and oxygen are delivered to the rest of the body, or how metabolic diseases such as diabetes can stop the heart working efficiently, we need to use rodent models which have a four chambered heart and respond to changes in metabolism in a similar way to that in humans. The bulk of our work is done in young adult animals. However, for development of deteriorating organ function and/or metabolism, animals may need to be kept for sufficient time to allow the changes to develop. In some

cases we may study juvenile animals, for example to investigate the effects of the onset of diabetes in young adults. We will use genetically altered animals which have a mutation which causes a change to the way the body metabolises fats and sugars. For example, we use animals with a mutation in the leptin receptor so that they continue to eat even when they don't need to and become obese and diabetic. We also use an animal which is used to model muscular dystrophy, as we have found these animals don't respond to insulin as they should.

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

A wide range of experiments are described in this project. The majority of experiments will be performed in wild-type rodents although we may use genetically altered animals where we want to investigate the role of one particular gene or pathway in the way the body handles fat and carbohydrates or in the development of diabetes or of heart failure.

Protocol 1: In most cases, animals will undergo terminal anaesthesia for removal of the heart or other organs while the heart is still beating. This will ensure that organ function, metabolic proteins and circulating metabolites are representative of the levels *in vivo* as many of these deteriorate very rapidly when the heart stops beating. Animals may be fasted overnight, prior to anaesthesia, as this results in more consistent data since there is no risk of one animal having eaten more recently than other. In some cases, before it is killed, the animal will be given an injection of a substance such as insulin which will cause a change in the way the body handles fats and carbohydrates. Tissue from these animals will be used for *ex vivo* experiments such as a) whole organ perfusion to measure the rate of uptake and metabolism of fats and carbohydrates, b) measurement of oxygen consumption by isolated cells or c) molecular biology to measure levels of genes and proteins.

Protocol 2. A lot of our *in vivo* work involves imaging or spectroscopy to measure function and metabolism of the heart and liver. The early stages of the process development are done using ‘phantoms’ designed to mimic live tissue. However, we need to image live animals where we are measuring heart function so the final stages of method development will use animals. These may be imaged on several occasions but generally no more than once or twice per week and no more than 10 times in total. These animals may have an injection under anaesthesia to deliver contrast agents or drugs to increase the heart rate temporarily. Under this protocol we may image genetically altered animals which are predicted to have problems with heart or liver function as a result of, or resulting in, impaired metabolism.

Protocols 3 and 6. As diabetes is a metabolic disease, we are particularly interested in the changes induced by obesity and diabetes at various stages of the disease. Diabetes can be induced in animals using a compound called streptozotocin (STZ) which kills some or all of the insulin-producing cells in the pancreas. To induce type 2 diabetes, animals are fed a high fat diet for 3 weeks and then have a low dose of STZ. This model mimics the early stages of the disease as these animals become obese and show increased levels of glucose and insulin in the blood. To induce type 1 diabetes we give a higher dose of STZ which kills the majority of insulin-producing cells so these animals have very high levels of glucose and low plasma insulin. We may also use genetically altered animals which become diabetic where these give a more appropriate phenotype for the question we are asking. The animals will have blood samples taken at intervals to determine development of the disease and may be imaged using techniques developed in protocol 2. Some animals will be given a drug in their food or drink or, if necessary, by injection to establish

whether this changes the disease progression. At the end of the experiment we will take tissue for *ex vivo* experiments as in protocol 1.

We also use animals with diabetes on protocol 4 as we know that diabetic patients are at greater risk of heart failure after a heart attack and we are trying to establish why this happens and how to prevent it. In these animals, we will ensure that the diabetes is established and stable before proceeding to further experiments.

Protocols 4 is a surgical protocol designed to mimic the impaired heart function resulting from a heart attack caused by a blockage of the coronary artery. The animals may be kept for several weeks to allow the changes to the heart to develop. They will probably be imaged to measure the changes to heart function and may be given a modified diet or drug treatment to determine whether this improves organ function and/or substrate metabolism. This treatment could include injection of stem cells into the heart muscle or attachment of a tissue-engineered scaffold across the damaged area of the heart to study the effect of adding new, healthy cells or tissue on the function and metabolism of the heart. Again, at the end of the experiment we will take tissue for *ex vivo* experiments as in protocol 1.

Protocol 5 is used to test which tissue-engineered scaffolds survive best after being implanted in the body, but without the complex surgery needed to access the heart. These animals will have scaffolds implanted under the skin in areas of the back where the scaffold does not affect movement. The animals may be imaged to measure the development of blood flow into the scaffold.

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

Animals will be used for method development on our imaging protocol. These animals will by anaesthatised several times, although normally this will be no more than once a week. However, the animals may become averse to the anesthesia over time.

Animals which undergo surgery to induce a heart attack may die during or after surgery. Once they have recovered, they rarely show signs of pain or abnormal behaviour or weight loss. We may keep these animals for up to six months as the heart gradually remodels after the surgery and we are interested to see whether therapies such as treatment with stem cells will mean that the heart function does not deteriorate so much over time.

We use animal models of diabetes which will have increased thirst and urination. The type 2 animals will become obese whereas the type 1 diabetic animals may show some weight loss. For most projects, the animals will be diabetic for up to a month, but if we are looking at a potential therapy that takes longer to take effect we may keep the animals for longer. We use diabetic animals on our surgical protocol to induce a heart attack as we are investigating why diabetic patients recover less well. These animals may not recover from the surgery as well as wild-type animals and will be monitored particularly carefully.

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 do a lot of experiments using the *ex vivo* perfused heart or isolated mitochondria. These animals will be on protocol 1 and will generally only undergo terminal anaesthesia.

Some will have an overnight fast so that levels of metabolic substrates are not affected by a recent meal. Some animals will be used for method development on our MRI protocol and undergo general anaesthesia with administration of substances but no other interventions. These animals will predominantly have a mild experience although some on the imaging protocol may become averse to the anaesthesia and have a moderate experience.

Our type 2 diabetic model does not routinely result in more than increased weight gain and increased thirst and urination. We ensure that these animals have sufficient water and are on absorbent bedding and so many of these animals will have a mild experience. In some cases, higher levels of blood glucose will mean that the animals have a moderate experience. The GAA models of type 2 diabetes may develop increased levels of blood glucose and have a moderate experience.

The type 1 diabetic model will mean that animals develop high glucose and low insulin and these animals, either STZ-induced or GAAs, will have a moderate experience.

Animals which undergo surgery for myocardial infarction will have a moderate or severe experience, depending on how quickly they recover from the surgery and whether they progress to heart failure. Mice recover less well than rats and are more prone to developing life-limiting heart failure. Diabetic animals on this protocol are more likely to develop severe adverse effects.

Based on this previous experience, and the number of animals on each of our protocols, overall we expect around 50% of animals to have a mild experience; around 40% of animals to have a moderate experience and 10% of animals to have a severe experience.

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

- Killed

A retrospective assessment of these predicted harms will be due by 03 March 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?

We study the interplay between organ function and substrate metabolism, with a particular emphasis on the heart. To obtain relevant measurements of heart function it is necessary to use an animal model with a four chambered heart like the human heart. As metabolism and contraction of the heart are inextricably linked, it is essential to study metabolism in the intact beating heart. Impaired delivery of metabolic fuels, due to decreased heart function or an imbalance such as is seen with diabetes, will mean that the organs of the body cannot function properly. This may, in turn, cause a further change in the metabolic

balance of the body which could feed back to damage heart or liver function. As a result, these effects need to be studied in animals over time.

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

We use mouse HL1 cells, a cell line that behaves like mouse heart cells, to determine the mechanisms behind effects observed in our *in vivo* studies. We also use this cell line to explore new theories before taking experiments into animals. We have developed a protocol to make the cells insulin-resistant and have found that these provide a good *in vitro* model of the changes we see in the diabetic rat heart. For example, we used these cells to investigate molecular changes occurring in diabetic animals after a heart attack, which lead to one of our recent publications. As it is not easy to get human heart cells, we use human stem cell-derived cardiomyocytes to help us determine whether effects seen in rats or mice are likely to also occur in humans.

In order to minimise the number of rodents used for developing cardiac imaging sequences at an early stage of the process, we are using an "eMouse", developed by collaborators, that generates a 'heart beat' and signals to mimic the movement due to breathing. We can mimic biological tissues, by adding chemicals to the gel, which has enabled the creation of "blocks" of tissue with much closer MR properties to certain tissue types of interest. This improves our ability to robustly test hardware and software prior to using it in animals.

Why were they not suitable?

The insulin-resistant HL1 cells provide a useful model but they only enable us to understand what is happening in heart cells in a monolayer, isolated from other cell types and from the mechanical stresses encountered by cells in the heart. We can generate engineered heart tissue, which is a 3D construct where the cells contract against flexible posts, and this is a better model of the environment cells encounter in vivo. However the cells retain a very immature phenotype and do not behave like a true adult heart cell. We are working to mature the cells and to adapt the insulin resistant model we use in the HL1 cells. Nevertheless, these cell systems can only ever provide a part of the picture when one is studying the complex metabolic interplay between different organs in the body. In addition, the heart contains many other cell types in addition to the beating cardiomyocytes. For example, after a heart attack blood flow to part of the heart muscle is cut off so that the cells do not get enough oxygen or nutrients to continue beating efficiently. We can mimic this in our cell models. However the dying cells send out signals to the body's immune system and this attracts immune cells to the damaged region. Some of these immune cells are beneficial and others are not, and it is much harder to mimic the effects of this immune reaction in a dish. Non-beating cells in the heart, called fibroblasts, start to change and reinforce the dying muscle, forming a stiff scar and changing the workload on the rest of the heart which again it is hard to reproduce in a model.

Similarly, although the "eMouse" is extremely valuable for testing new hardware and software for MR imaging, it cannot provide the imaging problems associated with blood flow and movement due to breathing and does not provide any cellular or functional information.

A retrospective assessment of replacement will be due by 03 March 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?

We have been working with rodent models of disease for many years and use the information we have from previous studies to determine the number of animals we think will be needed for particular studies. Based on our current and planned funding, we know which types of experiments are proposed for the next few years.

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

We take statistical advice from experienced researchers in the field, combined with our own previous experience of working for over 15 years in this area. We use statistical power calculations to estimate the number of animals we need in each experiment to detect metabolic and/or functional changes. We have developed new statistical methods that are unique to MR experiments that others in the field now use, which increase statistical power. Where we are testing a new drug compound, after appropriate *ab initio* calculations, we will use a small number of animals to determine the dose of drug to give before undertaking the experiments with sufficient animals to determine whether the drug has the effect we predict. In all cases we aim to pick the animal model which provides consistent results in our experiments, to reduce the number of animals we need.

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

We use MRI to measure cardiac function and metabolism on the same animal over time as the disease progresses. The MRI does not affect the animals adversely and allows us to reduce the number of animals required to follow the disease progression. We can measure substrate metabolism *in vivo* in the heart and the liver in the same animal, further reducing the number of animals needed; and we have refined our *ex vivo* heart perfusion measurements so that we can measure metabolism of both glucose and fat in one heart where previously we would have needed to use of two animals.

Where possible we use cryoinjury in the rat to provide a damaged region of heart tissue. The previous ischaemia/reperfusion protocol meant the animal was kept under anaesthesia about 90 minutes and also resulted in a large variation in tissue damage. The new protocol comprises placing a liquid nitrogen-cooled probe on the heart tissue for 10-15 seconds and means that the animal is under anaesthesia for about 30 minutes. This protocol produces a more reproducible infarct size and therefore results in fewer animals being used. It is also a refinement because the animals are under anaesthesia for about 30 minutes rather than 90 minutes.

However, the type of damage does differ from that resulting from a blocked artery and so in some cases we have to use the previous protocol where we block the blood flow through the artery, either permanently or for just under an hour.

We have been using omics-based technology to investigate the metabolic changes occurring in diabetes or after chemotherapy. We have discovered that the high rate of reproducibility and low error measurements from these mass spectrometry-based techniques have allowed us to decrease our numbers needed to find the information we need.

Where possible, we use tissue or organs from control animals not required for the designated study, or animals that do not fit the criteria for the designated study, for method development on other studies, such as imaging protocols or *ex vivo* mitochondrial analysis.

A retrospective assessment of reduction will be due by 03 March 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 use rats and mice because the hearts and other organs are structurally and metabolically close to the human heart. Although we have to generate disease models for our experiments, we use non- invasive techniques wherever possible, for example we feed high fat diet to elevate plasma free fatty acids and give therapeutic compounds in drinking water if applicable. We use a model of cryoinjury to induce damage to the rat heart but maintaining an open artery, as is seen clinically, because this generates more reproducible results, thereby reducing the number of animals needed, and exposing animals to a shorter period of anaesthesia than required for ischaemia/reperfusion. For experiments to design cell scaffolds for heart repair, we first test whether these are tolerated by the body, by implanting them in a pocket under the skin, before we progress to the more invasive surgery to attach scaffolds to the heart.

We use genetically altered animals (GAAs) when we are investigating the role of a specific gene in a disease pathway or to mimic a particular aspect of a disease. In general, mutations in metabolic pathways may cause the animal to gain or lose weight but do not cause significant pain or suffering. If the disease phenotype may deteriorate with age we will use the animals before they reach that point.

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

In order to examine changes to metabolism and organ function in disease, we need to use a model that matches the human physiology, rather than a non-mammalian system. For some of our experiments we take a wild type animal and terminally anaesthetise it before imposing metabolic changes on an isolated organ. For these experiments, we need to perfuse the heart in a system which mimics the delivery of oxygen and substrates and for this we need a heart from an animal the size of a mouse or rat. Similarly for our MR experiments we need enough tissue to get a measurable signal from the heart. These experiments would not be possible in a species that is the size of a fish.

For most of our *in vivo* experiments, we look at how a disease such as diabetes or a heart attack changes the function of the heart over a period of weeks and we cannot keep an animal terminally anaesthetised for that length of time.

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

We have purchased a Mouse Monitor from Indus Instruments that includes a heating pad and records ECG, body temperature and pulse oxygenation from both rats and mice. We use this to monitor animals during surgery and it has been very useful whilst establishing the cryoinjury model, as we can detect changes on the ECG which can indicate whether that the damage to the heart is too severe for the animal to be safely recovered.

We've introduced double-HEPA filtered air handling systems as much as possible into our workflows, both MRI and otherwise. As well as protecting operators, these will have positive beneficial effects on rodents undergoing procedures: as well as increasing sterility, there is a lower likelihood of airborne scents being communicated between animals.

A dedicated rodent echocardiography machine has been purchased for cardiovascular research, which is more efficient for scanning animals than human systems previously used, and therefore can achieve better results with less time under anaesthesia for each animal.

This will be particularly useful for screening animals after myocardial infarction or aortic banding where the surgery can give variable results. Where the surgery is unsuccessful or the effect is too severe, the animal can be removed from the study at an early time point. Similarly, we monitor blood glucose levels regularly, using a pinprick sample of blood and a glucose monitor. Again, where an animal is not sufficiently diabetic or blood glucose levels are too high, the animal can be removed from the study at an early time point.

In order to observe breathing motion of the animals in the MRI scanner, we have previously used an induction loop across the chest. This loop is effective, but the signal it provides can be obscured briefly during each MR pulse. We have recently installed a small warm plastic balloon, to be placed underneath the animal, that enables more robust monitoring of breathing, thereby increasing scanning efficiency and decreasing the time the animal is under anaesthesia.

We have close links with the cardiac groups at the Wellcome and other imaging groups in the University and discuss techniques and share hardware and software advances between the groups. We liaise with the veterinary staff and keep up-to-date on LASA guidance to minimise harms.

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

We will use the NC3Rs experimental design assistant when planning our experiments and will follow the LASA guidelines on aseptic surgery. We will keep up-to-date on advances publicised in the NC3R newsletter which provides information on the most refined techniques, such as new guidelines on non- aversive methods of picking up animals, single-use of needles and blood sampling. We will adhere to updated ARRIVE guidelines on reporting work with animals as now required by many journals.

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

The PPL holder or a designated representative attends the departmental 'gold standard meeting' each term at which the 3Rs are discussed. Members of the team attend workshops and courses run by the NC3Rs and all staff receive the NC3Rs newsletter from the Home Office Liaison team. Relevant advances from these outlets and from reading the literature are discussed at the weekly lab meeting.

A retrospective assessment of refinement will be due by 03 March 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?

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

Key words

gene therapy, neurodegeneration, genetic brain disorders, molecular therapy, genetic rodent models

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

Retrospective assessment

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

Objectives and benefits

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

What's the aim of this project?

To develop and test new genetic therapies for currently untreatable brain disorders.

A retrospective assessment of these aims will be due by 26 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?

Faulty genes are a significant cause of severe brain disease, especially those affecting children. We are currently undergoing a revolution in how we understand and envisage treating highly debilitating genetic disorders of the brain. The focus of this work is on childhood onset neurological disease for which there are no current effective treatments. Genetic brain diseases are often lifelong, affect a considerable number of individuals globally and place a huge socioeconomic burden on families, carers and health services. Childhood onset brain disorders are especially costly with regards to treatment, lost earnings, care and support for children and adults. The revolution in genetic medicine now means that we are diagnosing these diseases and their underlying genetic causes very effectively. However, this is not matched by treatment options and it remains the case that there is no effective therapy for the majority of severe brain disorders. That said, advances in gene therapy (replacing or fixing a faulty gene) offers the option of developing genuinely transformative treatment options. The reason that genetic approaches to therapy has such potential is that they offer to fix the root cause of the disease rather than just some of the symptoms. The goal of this programme of research is to develop and establish the effectiveness and safety of gene therapy across a number of significant brain disorders. For this we use rodents in which the equivalent gene that causes brain disease in humans had been disrupted or inactivated. Assessing the impact of gene therapy drugs in such animals allows us to predict the extent to which the gene therapy is likely to be safe and effective in people with severe brain disorders.

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

This project will provide vital new data on the potential utility of gene therapy in pediatric onset neurological indications. Based on our work to date, it can be expected that outputs include publications, public dissemination, patent applications and lead molecules for preclinical and clinical development.

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

There is currently intense research in the genetic therapy space and any early publications on innovative therapeutic approaches or tractability of disease phenotypes will be of significant impact in the short to medium terms (1-3 years). Data generated by this project will allow us to refine our approaches to hone in on the most likely effective interventions in childhood onset severe brain disorders. The longer terms benefit of these outputs, especially patents and therapeutic molecules will be new treatment options in hitherto untreatable pediatric disorders. We have a track record in this area with genetic therapies we have developed being destined for first in human gene therapy trials.

Beneficiaries include patients of childhood genetic brain diseases and well as scientific and medical researchers in this fast moving field.

It is expected that the innovative genetic therapies proposed will facilitate the rapid advances being in this sphere of translational research and will have direct potential for very rapid (in drug development terms) therapeutic impact. In addition, the parasite therapy is a highly innovative therapeutic approach which is much earlier in terms of translational

development but has the potential to deliver therapeutic cargoes that are difficult to deliver by established methods. It thus has the potential to treat patients with mutations in very large or complex genes or even multiple genes.

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

It is vital to the ethos of the laboratory that we work in collaboration with other laboratories both within and without the research institution. We have established links with laboratories across the UK and internationally with whom we share new knowledge, techniques and ideas. This informal 'peer review' process enables us to interrogate paradigm and experimental designs, enabling us to target those experiments we feel will generate the most useful and transferable.

Species and numbers of animals expected to be used

- Mice: 10000
- Rats: 1800

Predicted harms

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

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

This project will require the use of animal models that exhibit the clinical features of patients with neurological disorders under investigation. As our focus is on assessing potential treatments for pediatric and juvenile-onset disorders, we require the use of rodents at neonates, juvenile and adult stages of development. Rodents used in this work are genetically altered to model genetic brain disorders, with rats and mice being well suited for assessing cognitive (learning and memory) and functional outcomes.

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

Experimental animals will be enrolled in therapeutic studies. Animals will be assigned to a treatment group and novel genetic therapies administered, typically via a single injection. Animals will then be monitored for clinical signs of neurological disease to assess whether the therapeutic intervention impact aspects of the disease. Tests may include assessment of activity, walking, learning and memory, anxiety or functions such as breathing control.

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

The therapeutic agents being tested are known as gene therapy and target the root-cause of the diseases under investigation. As such, there is an expectation that the therapy may be highly impactful in ameliorating the clinical features of the disease. We expect that the therapy may result in correcting highly debilitating features of neurological disease including motor function (control of muscles), breathing control, seizures as well as higher level brain functions such as cognition. It is possible that we will observe unexpected adverse effects due to the novel therapeutic agents. Where these occur, experiments will be stopped immediately.

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 the majority of the work the severity will be in the mild to moderate range, especially in neurological disease models where the main clinical feature is intellectual disability. In some instances, the rodents will model very profound neurological disease and in order to assess the impact of gene therapy in these instances, the procedure will be considered severe.

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

- Killed

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

The neurodegenerative diseases under investigation in this project are complex conditions exhibiting a range of physical, neurological and learning impairments. We aim to test the impact of gene therapy on these complex aspects of neurological disease. As such it is not possible to model these conditions in isolated systems such as single cells grown in a dish. Therefore, the use of animal models is unavoidable when investigating these disease types.

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

We have made significant efforts to replace experimentation on live animals to investigate complex neurological disorders. This includes the use of neuronal and other stable cells lines for the testing of genetic therapies at a cellular and molecular level. We also study gene structure and sequences using human post-mortem tissues. We collaborate with groups working on human-derived cell lines from patients with intellectual disability disorders.

Why were they not suitable?

None of these alternative approaches enable the assessment of gene therapy on key neurological features that characterize childhood-onset neurological disorders such as intellectual disability or motor control. Instead, the therapeutic potential of agents in neurodevelopmental and childhood-onset disorders require the study of an intact nervous system as they involve combined problems in brain circuitry, connectivity, neurochemistry

and brain cell physiology which develop over time. It is only by creating accurate genetic models of these disease which closely mimic the major features of the human condition, that one can study how these processes interact. Moreover, whilst cellular level assays are used to test whether pharmacological and genetic interventions can rectify disease at the single cell level, it is only by testing these at the whole brain/animal level that one can truly assess whether these translate to improvements in behaviour, learning or corrected brain development.

A retrospective assessment of replacement will be due by 26 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 principles of our experiments are twofold. One is to characterise the development of the disease in the nervous that underlies Rett syndrome and related disorders so that we can identify avenues of potential therapy. This mainly includes the use of genetically altered animals and wild-type controls to provide tissues necessary for investigation outside the animal, such as neurochemical, molecular, electrophysiological and anatomical profiling. The second is to utilise this knowledge to develop and test the efficacy and safety of therapeutic interventions. Whilst as much of this as possible is done at the cellular level, for instance testing genetic therapies in cultured cells, Rett syndrome is fundamentally a nervous system disorder and for this we need to test putative therapies in the whole animal.

Our research conducted using animal tissues (electrophysiological, biochemical and morphological assays and genetic and pharmacological interventions) is based on the minimum number of animals required to produce sufficient biological replicates for robust statistical comparisons. Our use of cultured cell systems enables the preparation of multiple cell-based assay systems from a smaller number of animals.

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

We have developed cellular assays to characterise the properties of our molecular therapies prior to any animal work. This acts as a triage even prior to any pilot rodent study. Where organismal level work is required, we will use the minimal numbers to achieve robust scientific outcomes. All experiments are conducted according to 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?

For experiments on living animals, our previous experience has shown that 15 animals in each control and experimental group will provide a 90% chance of detecting a significant change in respiratory, motor and other behavioural phenotypes. We employ the services of a professional biostatistician to advise on all areas of our gene therapy work.

Pilot experiments on a small sample of animals (maximum n=5) will be conducted to select appropriate drug doses and for viral studies, to assess effectiveness of transgene expression.
A retrospective assessment of reduction will be due by 26 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.

The majority of experimental procedures will be performed on mouse models of RTT. In additions, mouse models of CDKL5, Syngap, Pten deficiency, Angleman syndrome and other NDD models will be used during this project. We intend to develop gene therapy treatments for RTT which will inform our approach in developing gene therapies for other conditions. Whilst RTT-like phenotype is severe in rodents, our experienced and knowledgeable scientists will closely monitor the animals and will take the following steps to ensure that pain, suffering and distress are kept to a minimum.

We have adopted a revised phenotype scoring system which we use at least weekly to assess, in a semi-quantitative and objective fashion, the onset and development of RTT-like symptoms in mice and rats. We have refined the scoring system so that there are now two separate instruments - while sharing most of their criteria, one is deployed to assess the phenotype of the mice for use in analysis, and the other is deployed to assess whether welfare-related actions are required, including closer monitoring, a vet-supported husbandry package, and clearly-defined humane endpoints.

Male Mecp2 mutant mice have an early-onset phenotype that becomes progressively more severe. They are therefore not kept alive long enough to become severely symptomatic unless required for a specific experiment. Treated mutant mice used to provide tissue for cell culture and other ex vivo experiments are euthanised while still presymptomatic.

Neonatal dosing is used to assess early genetic interventions in NDDs. Our breeding strategies generate mixed litters of GA and non-GA animals. We have therefore adopted a rapid genotyping protocol to identify mice with the appropriate genotype and avoiding

unnecessary injection of the wildtype mice. For this approach it is necessary to conduct tail biopsies.

Gene therapy is generally considered an irreversible treatment in that, once delivered, the treatment cannot be withdrawn. Part of our programme of works is to develop safety features whereby a pharmacological agent can be used to turn down/shut-off the transgene expression at the earliest sign of any adverse effects. This is a safety feature for translational purposes but is also a refinement during preclinical testing of gene therapy cassettes to mitigate against potential adverse effects in mice.

Use of rat models: In addition to mouse models, rat models of CDKL5 deficiency, SynGAP deficiency and Fragile X syndrome will be assessed. Models of these disorders show less overt outward phenotypes (motor, autonomic and peripheral phenotypes) and are therefore more dependent on detecting more subtle behavioural phenotypes that are more clearly expressed in rat models. In terms of refinement, this enables the finer grain testing of therapeutic interventions in domains (e.g. cognitive) that are most pertinent to the disorders in patient. It is a key scientific objective to assess whether cognitive features are reversed by genetic therapies targeting Rett syndrome and for this reason we wish to extend experiments into rat models which enable the assessment of more robust behavioural outcomes. For SynGAP knockout rodents, the phenotype is severe in hemizygous male animals. In limited proof-of-concept experiments, we wish to establish whether early genetic intervention can ameliorate the onset of these overt phenotypes in male animals. As a refinement, we would aim however to progress the study to heterozygous females as quickly as possible. All rat studies will be in collaboration with groups within the centre that have existing extensive expertise in dosing, maintaining and phenotyping rat lines.

We aim to also test an unconventional approach to deliver therapeutic protein to the brain by delivering engineering attenuated strains of Toxoplasma that can secrete the MeCP2 protein into neurons. Having shown that this approach is effective in delivering MeCP2 to neurons in vitro, we reply on experiments in mice to show that the approach has therapeutic application in vivo in terms of establishing whether this delivery approach can impacton RTT-like phenotypes.

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

The use of genetically-modified mice is frequently the only viable strategy for studying neurodevelopmental genetic disorders. Indeed, they are especially well suited for studying single-gene disorders of brain in which accurate genetic mouse models recapitulate many of the cardinal features that characterise the disorder in humans. Intellectual disability is characterised by impaired cognition and at present this can only be tested in the live animal. However, we have developed sensitive assays for changes in motor function that can detect nervous system abnormality at a very early stage (at 4-8 weeks). This enables us to follow the trajectory of the disorder phenotype from an early stage and assess whether therapeutic interventions affect the onset and development of disease-like characteristics rather than simply testing for the reversal of established and more severe symptoms.

RTT is a severe neurological disorder and is diagnosed once patients develop overt symptoms. The aim of the research is to develop effective therapies for RTT. As such, animals are therefore required to develop overt RTT-like signs in order to test the impact of putative therapies. Protocols 5, 8 and 8 are categorised as severe for this reason.

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

As detailed in the project plan, we have implemented a very rigorous monitoring system for our Mecp2 rodent lines. This include regular detailed monitoring of welfare and an escalation of monitoring frequency and enhanced husbandry (bedding, soft food etc) as the phenotypes progress as well as
clearly defined humane endpoints. Any of studies involving surgical or invasive intervention will adopt appropriate pain management and post-operative care. For mouse lines that have a propensity for benign tumours, only young mice will be maintained unless on a dosing procedure. In these cases, mice will be monitored frequently and with clear humane endpoints upon tumour discovery.

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

Guidance on the Operations of ASPA - https://www.nc3rs.org.uk/

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

Our technical staff are members of professional bodies (Institute of Animal Technology, Royal Society of Biology) and take part in continuing professional development to ensure their knowledge is as current as possible. Regular updates from bodies such as the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) and Understanding Animal Research (UAR) mean new developments and best practices can be evaluated and implemented where they might improve animal welfare without inhibiting the integrity of the research. Regular communication with our veterinary staff will ensure techniques are reviewed and refined where necessary and appropriate.

A retrospective assessment of refinement will be due by 26 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?

3. Autoimmune and inflammatory 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

Key words

Autoimmune, Inflammation, Arthritis, Inflammatory bowel diseases, 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.

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 identify and investigate novel molecular targets and mechanisms and develop new medicines for the treatment of autoimmune and inflammatory diseases.

A retrospective assessment of these aims will be due by 08 January 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 it's aims and if not, why not?

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

Why is it important to undertake this work?

Despite the progress in the discovery and development of medicines, there remains a significant unmet need in the treatment of autoimmune and inflammatory diseases. Such diseases contribute to more than 100 serious chronic illnesses involving almost every organ system in the human body, affecting an estimated 7-9% of the world population, and this incidence has been rising by 3-9% over the last few decades. Women account for 80% of those individuals with autoimmune diseases (Invernizzi et al., 2009). For example, women account for 80–95% of patients with primary Sjögren's syndrome, systemic lupus erythematosus (SLE), and about 60% of arthritis patients are women (Beeson, 1994).

Autoimmune diseases present a group of common and highly disabling long-lasting conditions with similar features making diagnosis and treatment extremely difficult, which are among the world's leading causes of death. Autoimmune and inflammatory diseases are frequently associated with additional serious healthcare issues, including severe pain, fatigue, depression, anxiety, chronic morbidity presenting a considerable burden on healthcare systems. In addition, a patient with an autoimmune disease such as rheumatoid arthritis or IBD, is significantly more likely to develop others, leading to more complex healthcare needs and lives that are considerably more challenging.

Autoimmune and inflammatory diseases such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), inflammatory bowel disease (IBD), Sjögren's syndrome (SS) and psoriasis are all characterised by a breakdown in function of the immune system and/or activation of pathological mechanisms that remain poorly understood. These diseases vary greatly in the tissues and/or organs they affect and how they are presented, with some being limited to a particular tissue and others affecting the entire body. Through the assessment of information obtained from individuals living with autoimmune and inflammatory diseases, identification of the biological mechanisms and specific genes involved can be identified as novel approaches for treating such diseases. The use of animal studies is fundamental for the validation of these mechanisms and genetic targets to support the research and development of potential new medicines.

In addition, the production of autoantibodies in autoimmune diseases has a profound involvement in the development of pain associated with such diseases. Cellular responses involved during acute inflammation leading to activation of the immune system, trigger mechanisms involved in pain signalling. However, recent studies suggest that the pain associated with autoimmune diseases such as RA develops in the absence of inflammation and is primarilly driven theough the production of autoantibodies (Wilderbrand et al., 2016). Although there is a large variety of autoimmune disorders with different symptomologies, pain appears to be a common factor in most of these conditions. The pain associated with autoimmune diseases is considered as one of the leading causes of comorbidities such as depression, resulting in a significant impact on quality of life and disease burden.

Increased understanding of the immune system and the mechanisms involved in the development and maintenance of autoimmune and inflammatory diseases has resulted in the important discovery of many effective treatments that manage the symptoms, but have little or no effect on the underlying causes of the disease. In addition, with undesirable adverse effects and a high proportion of non- responders associated with current treatments, there still remains a high unmet need to develop new medicines that are effective against multiple autoimmune and inflammatory diseases, offering a significant improvement over currently available treatments.

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

This licence supports a wide range of methods to enable the identification and evaluation of inflammatory and immunological systems involved in autoimmune diseases, to enable the investigation and development of potential new medicines to act against those diseases. The information generated from this work will enable scientific programmes to increase understanding of how mechanisms could drive development and maintenance of disease or why a condition may not heal or resolve. Studies targeting the identified and validated biological mechanisms will enable the effectiveness of therapeutic agents to be tested and developed as new medicines. This will provide information on common mechanisms that underlie different systems and disease indications (to enable subsequent expansion of research into a wider range of disease indications when mechanisms in common exist). It is also anticipated that results generated from these studies will be shared through scientific meetings and literature to further increase scientific knowledge and understanding in this field.

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

The information generated from this work will contribute to the development of new medicines to address the unmet medical need and improve the lives of people suffering with autoimmune and inflammatory disease.

Early research and discovery efforts will continue with the identification and validation of new disease related targets, which will form the basis of continued discovery and development of novel and effective therapies to benefit patients and increase knowledge available to the scientific community. Patient benefit will occur over the longer term due to the long timescales involved to develop new medicines. The wider scientific community will benefit from published work presented at scientific meetings and in scientific journals to further increase knowledge and understanding in the field.

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

It is expected that the information generated from the work carried out under this licence will be included in scientific publications as part of the process of investigating and validating new disease mechanisms and targets, and developing new treatments for disease. Where work is considered to be 'pre-competitive' (e.g. method development or model validation work), whereby it does not contain information that is subject to intellectual property constraints, it will also be considered for publication. In addition, the company also supports the view that publication of unsuccessful approaches ('negative data') is a valuable scientific output from properly conducted research, and this type of data would not be excluded from a publication strategy.

Information generated using this licence is maintained in a long-term, secure company database, that is continually available to other internal company researchers. Therefore, data will be recoverable in the future, even after likely project and personnel changes, and the information will be a valuable future resource to reduce the need to repeat and re-establish expertise in a field of research.

Species and numbers of animals expected to be used

- Mice: 13,500
- Rats: 2350

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 the aim of this project to identify and develop new medicines to treat diseases affecting the immune system. Prior to performing in vivo experimental models under this licence, in silico and in vitro human and animal models are regulary employed to study biological responses and mechanisms related to the human disease. In vitro biological cell based assays are reproducible, reliable, robust and biologically relevant enabling the screening of many compounds to identify those with the desired biological effect for progressing in vivo. Currently, animal models cannot yet be replaced in all pre-clinical research as they are required to provide basic information relating to the function of specific mechanisms and targets, and for assessing the activity of potential new treatments in complex, integrated systems only found in living animals. For most of the proposed work an established immune system is required that is able to respond to a challenge and/or develop disease symptoms, and is suitable to investigate the roles of genes of interest that may be involved in human disease. For rodents, this would typically require animals of at least 8 weeks of age. Animals may be genetically altered resulting in developing diseases naturally (spontaneous), and/or can be treated (induced) to develop the required response and/or relevant disease symptoms which are similar to those observed in humans. Animal models are essential for providing such conditions, as they present with many similarities regarding the features and characteristics of the human disease they intend to reproduce, therefore there is a high level of translation between species. The recognised similarities validates rodents as highly relevant and extremely valuable model systems for the work proposed in this licence. The animal models used for this work have been developed to be the least severe, and are well characterised and validated to investigate the role and function of mechanisms and pathways involved in immunological and inflammatory changes. The processes involved in disease states are extremely complex, thus new treatments need to be tested in a whole living animal to ensure effects produced on immune responses are accurately predicted. Within this project licence, it is proposed to use adult rats and mice with mature immune systems to test the effectiveness of new treatments.

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

An animal used under this project licence will typically experience the following.

Protocol 1 (Tissue supply)- Animals will not undergo any disease induction, and will be used to supply tissues and/or fluids to support work performed in culture (e.g. ex vivo). Under this protocol, animals will be humanely killed to allow blood and/or tissues to be collected. As part of this protocol, animals may receive either a single or repeat course of drug treatment prior to collection of samples. Drug levels and/or markers of treatment effects in the blood may also be measured. At the end of the study, animals will be humanely killed, and further blood and/or tissues may be collected for further analysis.

Protocol 2 (Mechanistic challenges) - Animals will be injected (typically on a single occasion) with an inflammatory agent (e.g. LPS) to produce an inflammatory/immune response that could lead to moderate suffering and last up to 7 days. An animal could receive either a single or repeat course of drug treatment throughout this duration. The effect of any given drug treatment on the inflammatory/immune response produced will be assessed by measuring drug levels and/or markers of treatment effects (i.e. cytokines) in

the blood. Animals will be weighed and general health assessed daily throughout the study duration. At the end of the study, animals will be humanely killed, and further blood and/or tissues may be collected for further analysis.

Protocol 3 (Immune responses) - Animals will be injected (typically on a single occasion) with an antigen (e.g. KLH) to produce a primary immunisation response. A second injection (typically on a single occasion) may be provided to produce a secondary immunisation response (e.g. DTH) that could lead to moderate suffering and typically last up to 14 days. Animals could receive either a single or repeat course of drug treatment during this duration. The effect of any given drug treatment on the inflammatory/immune response produced will be assessed by measuring drug levels and/or markers of treatment effects (e.g. antibodies) in the blood, measurement of tissue responses (i.e. ear swelling), and/or via imaging capabilities (e.g. MRI). Animals will be weighed and general health assessed daily throughout the study duration. At the end of the study, animals will be humanely killed, and further blood and/or tissues may be collected for further analysis.

Protocol 4 (Mouse arthritis) - Animals will receive a single injection of an initial sensitising substance (e.g. collagen/antibody cocktail) followed by a further single injection (boost) of a secondary inflammatory agent (e.g. LPS) up to 1 week later to induce arthritis. Animals will start to show signs of joint swelling in all paws within 2-3 days following the second injection which could last up to 3 weeks which could lead to moderate suffering. Animals will be weighed and general health assessed daily throughout the study duration. Animals are likely to receive either a single or repeat course of treatment during this duration. The effect of any given treatment on the arthritis produced will be assessed by measuring drug levels and/or markers of treatment effects (e.g. antibodies) in the blood, physical observations (i.e. paw swelling) and/or via imaging capabilities (e.g. MRI). At the end of the study, animals will be humanely killed, and further blood and/or tissues may be collected for further analysis.

Protocol 5 (Rat arthritis) - Animals will receive an initial single injection of type II bovine collagen usually combined with an adjuvant (e.g. IFA) followed by a further single injection (boost) 7 days later of type II collagen combined with an inflammatory agent (e.g. LPS) to induce arthritis. Animals will start to show signs of joint swelling in the hind paws within 5-7 days following the second injection which continues until day 21 post initial collagen injection that could lead to severe suffering. Animals will be weighed and general health assessed daily throughout the study duration. Animals are likely to receive either a single or repeat course of drug treatment administered during this duration. The effect of any given drug treatment on the arthritis produced will be assessed by measuring drug levels and/or markers of treatment effects (e.g. antibodies) in the blood, physical observations (i.e. clinical scores, paw swelling) and/or via imaging capabilities (e.g. bio-luminescence). At the end of the study, animals will be humanely killed, and further blood and/or tissues may be collected for further analysis.

Protocol 6 (Mouse colitis) - Animals will receive either a single injection of T cells (adoptive transfer model), or antibody (e.g. aCD40), or will be administered with a chemical substance (e.g. DSS) in the drinking water, to induce colitis. Animals will typically start to show symptoms of colitis (e.g. body weight loss) 2-3 weeks following T cell transfer or 2-4 days following either antibody or chemical administration. Animals will be monitored daily and general health assessed. Animals will be weighed every other day until disease symptoms are observed, then every day thereafter. Animals could receive either a single or repeat course of drug treatment during this duration. The effect of any given drug treatment on the colitis produced will be assessed by measuring drug levels and/or markers of treatment effects (e.g. proteins) in the blood and/or faeces, or physical observations (i.e.

endoscopy). At the end of the study, animals will be humanely killed, and further blood and/or tissues may be collected for further analysis.

Protocol 7 (Mouse Lupus) - Animals that are genetically altered (e.g. mouse strains NZB/W F1 and MRL/lpr)) will either spontaneously develop lupus symptoms, or animals will have a single injection of a disease accelerator (e.g. pristane) or an immune trigger such as an adeno-virus (e.g. Ad-IFN) to initiate lupus development. Genetically susceptible mice and those challenged with an accelerator will typically start to show lupus symptoms (e.g. protein in the urine, enlarged lymph nodes, enlarged spleen, arthritis) from 2-6 months of age, while those challenged with adeno-virus will show symptoms from 6-12 weeks of age. Animals will be weighed and general health assessed daily throughout the study. Animals could receive either a single or repeat course of drug treatment during this duration. The effect of any given drug treatment on the lupus produced will be assessed by measuring drug levels and/or markers of the treatments effects (e.g. auto-antibodies) in the blood, and/or via imaging capabilities (e.g. MRI). At the end of the study, animals will be humanely killed, and further blood and/or tissues may be collected for furtheranalysis.

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

Animals exposed to an inflammatory/immune challenge agent (protocols 2 and 3) are likely to experience short lasting local or systemic inflammatory and/or immune responses, although certain models may be associated with weight loss (e.g. 5 - 10% of highest achieved body weight) lasting for a few hours or several days after. Additionally, adverse effects such as hunched posture, piloerection, subdued responsiveness, ocular-nasal discharge and diarrhoea may be observed, although these usually subside and animals recover within a few hours/days depending on the challenge agent and model used.

Animals that develop arthritis (protocols 4 and 5) may display signs of weight loss (e.g. 5 - 15% of highest achieved body weight), hind limb swelling and/or loss of weight bearing, reduced mobility, scabbing at the injection site, and may experience some degree of pain during the development (e.g. 5- 7 days following boost) and maintenance (e.g. up to 21 days from initial collagen injection) of arthritis symptoms.

Animals that develop colitis (Protocol 6) may display signs of weight loss (e.g. 5 - 20% of highest achieved body weight), develop diarrhoea, hunched posture, blood in faeces (e.g. DSS model), swelling and reddening of skin, especially around the eyes, and may experience some degree of pain

Animals that develop Lupus (protocol 7) may display signs of weight loss (e.g. 5 - 10% of highest achieved body weight), develop protein in the urine, enlarged spleen, enlarged lymph nodes, skin rash, arthritis, and may experience some degree of pain.

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

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

Experience during work under previous licences indicates that at least 25% of animals are expected to show no more than mild effects following treatment. Up to 60% of animals will likely, experience a cumulative moderate severity, or at least a period of moderate severity

at some point during experiments. Up to 15% of animals will likely, experience a cumulative severe severity (e.g. rat CIA model).

Protocol 1 is non-recovery (100% of animals), Protocols 2-4 will be moderate (40% of animals), Protocol 5 will be severe (up to 40% of animals may enter severe category, but all attempts will be made to work within the moderate severity), protocol 6 is moderate (40% of animals) and protocol 7 is moderate (up to 100% of animals have the potential to enter moderate severity).

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

- Killed

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

The aim of this programme of work is to identify and develop new treatments for extremely complex human conditions that affect many organs and/or systems in the body that have many different causes (e.g. hormonal, genetic, cellular). While it is possible to reproduce some aspects of these systems and study certain aspects of these functions in isolation (e.g. in vitro), the complex processes and interactions that work in different ways and at different times, either alone or together, cannot be completely and accurately replicated outside of the whole animal. The immune system is a very complex, highly coordinated network of specialised organs and cells that protect the body from potential harmful substances (e.g. bacteria, virus, infection), which can respond quickly and efficiently (i.e. immune response) to eliminate such harms. These responses involve several cell types (e.g. white blood cells) and signalling proteins (e.g. cytokines), and consist of a sequence of events that are common across mammalian species, and hence results from experiments carried out in rodents can be compared and translated to humans. Responses are coordinated to identify potential disease causing organisms (e.g. a virus or other pathogen) and/or damaged tissue, and act to alert and recruit other cells and signalling molecules and/or activate processes that can lead to structural changes in tissues and organs. An inefficient or malfunctioning immune system can result in the body mistakenly begins attacking its own healthy cells, tissues and organs leading to autoimmune diseases that may be restricted to certain organs (e.g. Lupus) or involve a particular tissue in different places (e.g. rheumatoid arthritis).

Additionally, investigations into disease states that are the result of altered or mis-functioning immune mechanisms, or investigations into highly organised body systems

require the use of mature functioning tissue(s) that cannot currently be fully replicated, grown or kept alive outside animals (ex vivo).

Rheumatoid arthritis (RA) is an example of an autoimmune disease primarily affecting joints of the hands and feet resulting in inflammation, pain and reduced function. The underlying cause of the disease is unclear, however due to the nature of the immune response and the structure of the joints/tissues involved, it remains too complex to replicate the cellular responses, tissue damage, symptoms and course of the disease in a plastic tube. Whilst a wide range of information from isolated cell systems is generated as part of the initial investigatory process to further increase our understanding of the basic mechanisms and how potential treatments target or affect many functions, understanding the integrated response in a whole animal with a physiology that is common with humans is vital to direct potential medicine progression to human clinical trials.

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

Various in silico and in vitro assays are used to predict and/or investigate whether a novel therapy can directly effect isolated cellular processes involved in immune diseases. For example, cells and/or tissues may be isolated from naïve, diseased or challenged animals for use in biological assays. Cell populations may be isolated from tissues and cultured as cell suspensions, or tissue pieces may be cultured in plastic tubes as explants or organoids. Models using types of isolated human and animal cells have been developed and widely published in the scientific literature, and are being increasingly used as part of the development of new medicines. Currently, the vast majority of these models use flat cultures of cells growing on plastic that have a limited level of complexity, and are only capable of addressing specific questions. The continued development of organoids (e.g. intestinal epithelial system) and three-dimensional (3D) modelling systems (e.g. gut-on-a-chip) have enabled some of the challenges of investigating the cellular interactions to be addressed, however, the full level of complexity required to replicate the human disease situation are yet to be achieved.

Why were they not suitable?

Despite much progress over the last few years, the three dimensional (3D) systems such as gut-on-a- chip and joint-on-a-chip are limited in their availability and they do not model the complex processes of the immune system. Immune responses involve the integration of genetic, molecular and cellular factors, across multiple systems. Additionally, systems using cells are not yet adequately able to model potential drug absorption, distribution, metabolism and elimination (ADME) properties throughout the body resulting from the administration of a therapeutic agent passing through organs such as gut and liver to enable delivery to the target organ/tissue. Thus, these non-animal systems are not yet fully characterised and/or validated to provide confidence in the clinical relevance of data generated from using them. While these models provide great value in predicting the potential effects of a novel therapy, Given this level of complexity, there are currently no suitable alternatives to assess the efficacy of a novel therapy on the ability to produce a desired or intended result, without the biological changes seen in the animal models.

A retrospective assessment of replacement will be due by 08 January 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 estimated number of animal that are likely to be used over a five year period are based on the experience of related work and projects previously supported under licence authority within the company. There is an anticipated steady rate of projects likely to require information that cannot be gained from non-animal alternatives. Also considered in these estimates is the likelihood of changes to research priorities as a result of continuing scientific advancements in many areas of science. Because
we are familiar with the types of models that are likely to be used and know the resources that we have available we can estimate the number and type of studies that will likely be required over the life cycle of a licence.

We use accepted statistical principles based on the main readouts from each model together with knowledge of the variability those readouts to inform on animal numbers required per type of study to produce statistically useful information.

The number of animals to be used under this licence has been estimated through previous experience and the anticipated requirement, over a five year period, to identify and develop novel therapies/therapeutic agents for the treatment of autoimmune and inflammatory diseases. The animal models used under this licence have been developed and optimised with statistical input to ensure they have sufficiently powered robust and reproducible endpoints, using the minimum number of animals to make informed decisions.

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

All experimental work is planned with the input of statisticians to ensure that experiments provide high quality data using the minimum number of animals. Experimental treatment group size will either be based on existing data, or small trial (pilot) studies will be carried out to establish the variation of the biological system and associated readouts being investigated. Understanding the variation and what constitutes a meaningful biological response to a treatment allows a statistician to calculate treatment group sizes that are used to ensure that statistically meaningful comparisons can be made between treatment and control groups. These design principles aim to reduce the possibility of experiments not generating decision making data, potentially resulting in repeating work and hence using more animals.

In addition to statistical support, all studies conducted under this licence will undergo internal peer review in order to ensure that all aspects of experimental design are suitable for the study being proposed.

Where the biologic effect of a new therapeutic agent or control treatment is being tested, the blood or tissue levels of that substance and the associated biological response will be

measured from the same animal. This allows the direct comparison of treatment levels and treatment effects to be made, and reduces the number of animals required overall. Animals will be randomly assigned to experimental groups using a random number generation system. This will reduce bias in a design that could compromise the value of generated data and potentially lead to more animals having to be used if work were to be repeated. Additionally, for subjective readouts that require a person to make a judgement, bias in data interpretation will be avoided by using experimental blinding. Thus, those involved in making those assessments will not be influenced in the interpretation of readouts by knowing which treatment had been given to animals.

These robust study design measures will maximise the likelihood of generating non-biased experimental results, and limit the number of animals required to generate high quality decision making data.

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

Before performing any work under this project, cell assays (e.g. in vitro) will be performed to determine the activity of the test agent on the relevant mechanisms. Where a new model is required or an additional endpoint is to be implemented within an existing model, initial pilot studies will be undertaken to optimise the conditions and measures will be taken to ensure the maximum level of information (e.g. via serial sampling) is obtained using the least number of animals. Model performance will be continuously monitored and opportunities to reduce variability will be implemented with help from a statistician. To reduce variation in biological readouts we will ensure that our facilities provide a constant optimal environment suitable for the species, and the number of personnel involved in making any subjective experimental measures will be minimised. By controlling variation in biological readouts, we aim to ensure that the minimum possible animal group sizes are used to achieve the scientific objectives of the study.

A retrospective assessment of reduction will be due by 08 January 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 take a staged approach to studies, minimising the risks to scientific objectives and animals: Blood and tissue supply (Protocol 1)
Aspects related to understanding particular mechanisms and/or process involved in inflammatory and immunological processes are evaluated initially through cell assays

performed in tissues provided from non-recovery studies (Protocol 1). Additionally, materials (e.g. T cells) will also be provided for other in vivo models under this licence (e.g. adoptive T cell transfer model of IBD).

Acute challenge models (Protocol 2)

Following confirmation and validation of a mechanism and/or target in cell assays, initial in vivo validation will be performed in an appropriate challenge model (Protocol 2). These models display certain features of human autoimmune and inflammatory diseases, however they do not develop disease-like symptoms and have a short duration, causing less harm than the disease models. They are used to provide further understand of the specific effects of new targets and/or mechanisms in vivo, thus limiting the number of compounds tested in the disease models.

Immune response models (Protocol 3)

Once initial validation of the mechanism and/or target has been determined, further evaluation may be performed in immune response models (Protocol 3) to assess their effects on specific immune responses related to a particular disease, without the need to induce a chronic disease state and the associated harms to animals. The selected models will allow us to explore how altering certain processes and/or functions with effective treatments can modify the immune responses being produced. The information provided form these models will provide further understanding into the mechanism of action of the target, and will provide evidence to support progression into more complex, disease-like models.

Arthritis models (Protocols 4 and 5)

The selected arthritis models (Protocols 4 and 5) represent many of the features of rheumatoid arthritis (RA) and are extremely useful for investigating mechanisms and processes involved. However, no single model reproduces the human disease exactly. Therefore, prior knowledge of the targets and mechanisms being investigated are essential for model selection to assess effective treatments. The mouse CAIA model (Protocol 4) will be the preferred option due to the quick onset of disease symptoms, produced over a duration of no more than 12 days, causing the least harm to the animals. Only in specific instances where the testing of a particular mechanism and/or target is necessary, will the 21 day rat model be considered. This approach will provide all the required data while preventing prolonged harm to the animals.

Mouse colitis models (Protocol 6)

The mouse models of colitis represent many of the common characteristics of human inflammatory bowel diseases (IBD). While no single model reproduces the human disease exactly, the selected models are valuable tools for assessing effective treatments on the mechanisms and processes involved. The DSS model resembles key features of ulcerative colitis (UC), has very quick onset of symptoms produced over a short duration of 7 days, that resolves quickly, providing a simple, robust model. The adoptive T cell transfer model assesses long term colitis that requires a longer duration of 6 weeks for the required disease symptoms to develop, however this provides a wider, more detailed disease assessment to test effective therapies on specific targets and/or mechanisms involved. The approach taken will provide all the required data while preventing prolonged harm to the animals.

Mouse SLE models (Protocol 7)

Different mouse models for Spontaneous Lupus Erythematosus (SLE) will be used (Protocol 7), as a single model alone does not represent all the disease characteristics seen in human disease, required to assess effective treatments. The mouse strains we will investigate, develop mild symptoms of lupus spontaneously at approximately 3 and 5 months of age. The disease onsets can be accelerated and aligned to levels of proteinuria similar to the human disease, by using inflammatory accelerators, allowing us to minimise the period of time for disease development, thereby reducing the cumulative harm of disease and treatment period. Prior knowledge of the target and/or mechanism to be tested is essential for appropriate model selection and prevent prolonged harm to the animals.

New models and Biostatistics

When new models and/or modifications to existing models are required, pilot studies will be performed to ensure the animal model is as refined as possible. The methods employed in each animal model will be suitably refined to ensure the animals are exposed to the least harm to achieve the scientific objectives.

Statistical support will be obtained at all stages of running an animal study, to ensure the least number of animals are exposed to harms to provide the required scientific data. Literature will be regularly assessed to ensure the most up to date models, methods and refinements are used.

Harms associated with each disease model have been minimised by reducing the duration of the model and/or disease and by setting clear humane and experimental endpoints. The humane endpoints consider all aspects of the physical signs or symptoms associated with the particular model and/or disease.

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

Autoimmune and inflammatory diseases are extremely complex conditions affecting single or multiple organs of the body, often involving several systems simultaneously. Rodents are a relevant species as they provide many similarities with the mechanisms, systems and processes involved in human diseases that are not known to be active in less sentient species. To undertake the work proposed under this licence, rodents must have an immune system that has matured sufficiently that it consists of all the required functioning components. Therefore, this requires animals to be at least 6 weeks of age, but preferably in excess of 8 weeks of age. Due to the duration of the models to be used, it is impractical and unethical to keep animals anaesthetised for the duration of the required procedures and sample collection periods.

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

The models described within this licence will undergo continual review to ensure they cause minimum harm to animals, while maintaining scientific integrity. For example, the duration of model implementation will be continually reviewed to ensure that the earliest appropriate biological endpoint is used to achieve the scientific objective. Furthermore, the number and frequency of procedures will be kept to the minimum required to answer the scientific question.

For each inflammatory model, animals will be assessed daily and monitoring score sheets will be used to assess the welfare of the animal as the model progresses.

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

The following is a list of the best practice guidance that we routinely follow.

Percie du Sert N et al. The ARRIVE guidelines 2019: updated guidelines for reporting animal research. BioRxiv. 2019: 703181.

Smith A et al (2018). PREPARE: guidelines for planning animal research and testing. Lab Anim; 52(2):135-141.

Prescott MJ, Lidster K (2017) Improving the quality of science through better animal welfare: the NC3Rs strategy. Lab Animal 46(4):152-156.

Review of harm-benefit analysis in the use of animals in research. Report of the Animals in Science Committee Harm-Benefit Analysis Sub-Group chaired by Professor Gail Davies (Nov 2017).

Review of harm-benefit analysis in the use of animals in research - Report of the Animals in Science Committee Harm-Benefit Analysis Sub-Group chaired by Professor Gail Davies Nov 2017

NC3R's - Responsibility in the use of animals in bioscience research: Expectations of the major research council and charitable funding bodies

LASA - Guiding principles on good practice for Animal Welfare and Ethical Review Bodies Sep 2015 Guidance on the operation of the Animals (Scientific Procedures) Act 1986. (Home Office 2014).
Kilkenny C et al (2010). Improving Bioscience Research Reporting: The ARRIVE Guidelines for Reporting Animal Research. PLoS Biol 8(6).

Diehl KH et al., (2001) A Good Practice Guide to the Administration of Substances and Removal of Blood, Including Routes and Volumes: Journal of Applied Toxicology 21, 15–23

Morton DB et al., (1993) Removal of blood from laboratory mammals and birds Laboratory Animals 27, 1-22

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

We will stay informed about advances in the 3Rs through regular referral to the NC3R's website, as well as other published literature. Furthermore, the establishment Named Information Officer (NIO) will facilitate the dissemination information in relation to any such advances. In accordance with any updates, we will review and revise the protocols within this licence to ensure they have been adequately considered, and where applicable, applied.

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

4. Characterizing the function of POPDC genes in health and disease

Project duration

5 years 0 months

Project purpose

- Basic research

Key words

heart, skeletal muscle, cyclic nucleotides, regeneration, disease

Animal types	Life stages
Zebra fish	adult, embryo, neonate, juvenile

Retrospective assessment

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

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Contains severe procedures

Objectives and benefits

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

What's the aim of this project?

The Popeye domain containing (POPDC) genes are important for preserving the structure and function of the heart and skeletal muscle. We are characterizing zebrafishes carrying altered (mutant) forms of POPDC genes to define their role in health and disease.

A retrospective assessment of these aims will be due by 02 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?

Popeye domain containing genes encode a protein family that bind a signalling molecule termed cAMP. cAMP is produced in cells in response to hormonal (for example adrenaline) stimulation. It is vital for the heart, as it regulates the speed and force of contraction and is also required in skeletal muscle to preserve its structure and function. Patients suffering from cardiac and/or skeletal muscle disease have been identified to carry mutations in one of the three POPDC genes. Introducing these mutations in zebrafish and studying the resulting heart and skeletal muscle defects will help to characterize the activities of POPDC proteins and to define their function in health and disease. Recently, we began to explore the function of this family of proteins in cardiac and skeletal muscle regeneration. Defining the reasons for impaired heart and muscle regeneration in zebrafish mutants will lead to a deeper understanding the roles of these proteins and possibly may lead to a novel class of drugs, which might be able to improve myocardial healing after an infarct.

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

This work is expected to provide new information about the role of POPDC genes in cardiac and skeletal muscle regeneration and will help to elucidate the mechanisms of how POPDC proteins are controlling pathways that regulate the response of muscle cells to stress.

- The primary expected benefit is the publication of new scientific knowledge about how POPDC proteins control cardiac and skeletal muscle regeneration.
- New information will also be obtained about the mechanisms how POPDC mutation are causing heart and skeletal muscle disease.
- The results of this work will be presented at scientific meetings and subsequently in publications in peer-reviewed journals to ensure wide dissemination to the appropriate audience.
- Research may in longer term also result in novel therapeutic products as POPDC proteins represent a unique molecular target, which is involved in many vital functions of the heart and skeletal muscle.

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

The scientific research community will benefit from this work. Primary recipients are the research communities working on cyclic nucleotide signalling, which includes approximately 500 researchers worldwide. Another research community that will be interested are researchers that work on cardiac regeneration in zebrafish with an estimated number of about 100 researchers worldwide. Our work will also be of interest to the wider heart and skeletal muscle research communities, which are estimated to be approximately 10,000 and 2,000 researchers, respectively. In the short term we will provide novel insight into the mechanisms of how POPDC proteins are mediating stress signalling in the heart. In the longer term, the research may yield novel pathways, which could be exploited for the development of novel drugs.

The work on cardiac regeneration in zebrafish may help to develop novel therapies to alter the fate of myocardial infarction, which currently is a highly detrimental disease-causing heart failure and death. Understanding how POPDC genes are involved in heart and muscle regeneration in the short term will lead to novel information about the cellular

pathways involved in this process. It is uncertain whether this novel knowledge will indeed have an impact on clinical practise and alter the outcome of myocardial infarction. I estimate it to have a 20% chance that the new knowledge generated might be of benefit in the clinics in the longer term.

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

The results of this work will be presented at scientific meetings, will be published in peer-reviewed journals to ensure wide dissemination to the appropriate audience. We also regularly writing review articles to further foster dissemination to the various research communities that are interested in our work. These information routes will encourage further collaborations and potentially lead to translational opportunities.

Species and numbers of animals expected to be used

- Zebra fish (Danio rerio): 8,100 adults

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 Popeye domain containing gene family consists of three genes, which are present in all vertebrates including zebrafish. The high conservation grade allows to model mutations in zebrafish, which have been discovered in patients. The adult zebrafish has the ability to fully regenerate the heart after injury. In mammals this ability is also present at birth but is lost one week after birth. Overall zebrafish and mammals display very similar processes in response to heart injury. However, while the zebrafish is able to heal its heart, the mammalian heart will substitute cardiac muscle with scar tissue.

The scar tissue will not participate in pumping blood, which in the long run might cause heart failure. The adult zebrafish may serve as a model to unravel mechanisms involved in cardiac regeneration that lie dormant in the adult mammalian heart but potentially could be reactivated. Studying heart regeneration in adult zebrafish lacking POPDC genes therefore may help to identify novel pathways that are involved in this process, which when activated help to recover the human heart from myocardial infarction. We are also interested in skeletal muscle regeneration and membrane healing. Skeletal muscle unlike the heart maintain the ability to regenerate as muscle contains a specialised cell population, the so-called satellite cells that are able to reconstitute muscle after injury. Skeletal muscle injury is commonly present after sport or walking downhill for example. Restoration of muscle structure is impaired in animals lacking POPDC1 and we are interested to identify the reason for this failure. Knowledge gained in this way may help to understand the function of POPDC proteins in muscle regeneration and this knowledge might help to treat patients carrying mutations in POPDC genes. Patients carrying point mutations in POPDC genes also develop an irregular heartbeat as do zebrafishes carrying mutations found in patients. Characterising the underlying reasons for irregular heartbeat remains challenging but it is hoped that the study of adult zebrafish hearts in this context will help to elucidate the underlying pathology. For this purpose, fishes will be subjected to electrocardiography in order to assess the presence of an irregular heartbeat. Since POPDC genes are involved in stress-signalling, the heart abnormalities will become fully apparent after inducing stress. This is accomplished by injecting substances which trigger a faster heartbeat and it

is in this situation that the mutant heart is expected to show its abnormal response to stress.

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

Adult zebrafishes are anaesthetised and placed ventral side up on a damp sponge. A small incision is made through the chest to access the heart. A small part of the ventricular wall is freeze-injured by applying a cryoprobe precooled in liquid nitrogen. Fishes are returned to water and after overnight recovery from surgery the fishes are taken back to the aquarium facility. At 1-365 days after injury, euthanasia is performed by an overdose of anesthetics.

For skeletal muscle injury, the tail musculature of the anesthetized fish will either be injected with a small volume of a toxin, cryoinjured with a cold probe, or wounded by a needle. These treatments are causing skeletal muscle injury. The healing response will be studied usually in the next four weeks and not longer than 6 months after surgery. Animals will be euthanised at the end of the experiment.

POPDC proteins are important regulators in stress signalling, which in the heart lead among other responses in a faster heart rate. In case POPDC genes are not functioning properly the response to stress is causing an abnormal heart beating. We want to elucidate the underlying cellular and molecular causes of this abnormal stress response. POPDC mutants will be subjected to electrocardiography (ECG) analysis at baseline and after injecting substances, which trigger a molecular stress response. Under these circumstances, the mutant heart will display an abnormal heart beating pattern, which will help us to define the underlying molecular mechanisms. ECG analysis is a non-invasive method. After anaesthesia the animals will be placed ventral side up on a damp sponge and the ECG electrodes will be lowered on the body surface of the fish in order to pick up the electrical signals. Substances will be injected into the abdominal cavity at the appropriate volume and dose and subsequently returned to continue ECG analysis. The procedure will be repeated up to five times in order to study changes in clinical phenotype as a function of age of the animal. Animals will be euthanised at the end of the experiment.

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

Animals that do not recover from cryoinjury normally die in the first 24 hr after surgery. Usually, the mortality of the procedure is around 10%. Successfully operated animals do not display signs of discomfort and are swimming and feeding normally. The animals will be studied usually up to 90 days after injury and in some cases up to one year after surgery.

Animals subjected to muscle injury will show no mortality and only some minor discomfort due to the muscle injury, which however will improve as regeneration will take place. The animals will be studied usually up to 4 weeks after injury and in some cases up to six months after surgery.

Animals subjected to ECG analysis are expected not to show any mortality and the procedures will only transiently cause an abnormal heart beating, which will return to nearly normal heart beating at the end of the procedure.

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)?

- Heart lesoning is a severe procedure and 10% are expected to experience this severity level while 90% will have a moderate outcome.
- Muscle lesioning is a moderate procedure and 100% of the animals are experiencing this severity level.
- ECG phenotyping is a mild procedure and 100% of the animals are expected to show this severity level.

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 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?

Our aim is to model heart and muscle disease found in patients carrying mutations in POPDC genes. These diseases are complex and are only partially modelled using cell culture models. Moreover, we want to study heart and muscle regeneration. Also in this case, cell cultures only can model certain aspects of the wounding response Regeneration of heart and skeletal muscle involves many different cell types. These processes therefore too complex to be investigated in cell culture. While the work in animals will be complemented with experiments in cultured cells, a complete substitution of animal work is currently not possible.

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

We also work with primary cultured cardiac myocytes, frog oocytes and other cell lines. Some of our work involves preparations of the whole heart or part of it, which are maintained in a viable and functional condition outside of the body for several hours, which also helps to limit the number animals used for our research.

Why were they not suitable?

Work in isolated cardiac muscle cells, frog oocytes or cell lines are suitable for certain aspects of our research. However, these cell models do not show the full spectrum of responses that we see in the intact animal and therefore are not sufficient to fully substitute the work in animals.

A retrospective assessment of replacement will be due by 02 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 animal numbers are estimated based on the demand of the previous license.

Protocol 1 Obtaining Zebrafish Gametes (mild procedure): We estimate the need for approximately 100 adult fishes for five years.

Protocol 2 Generation of Founders (F0 Generation) (mild procedure): We estimate the need for approximately 500 adult fishes per 5 years. The production of transgenic animals is a routine procedure. However, depending on the genetic manipulation several attempts might be needed before the right genetic alteration is observed. For the generation of single mutations sometimes only 1% or less of the injected oocytes contain the correct genetic alteration.

Protocol 3 Breeding and Maintenance of Genetically Altered Zebrafish (mild procedure): The number of fishes required for the maintenance of the various lines (breeding as well as the crossings to generate homozygous animals or crossing in any reporter gene or transgenes) will amount to 5,000 adult fishes for five years.

Protocol 4 Heart Lesioning (severe procedure): We estimate a total of 1,500 adult fishes for five years to allow for a detailed analysis of the regeneration defect in the mutants. For some of the examinations, the small size of the fish heart will require the use of several animals per time point to obtain sufficient amount of tissue.

Protocol 5 Skeletal Muscle Lesioning (moderate procedure): We estimate a total of 500 adult fishes for five years

Protocol 6 Phenotyping (ECG) mild procedure: We estimate a total of 500 adult fishes for five years.

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

During the experimental design phase, we will utilize online tools such as the NC3R Experimental Design Assistant in order to reduce the number of animals used in this project. We minimise live animal use by capturing as much ex vivo and in vitro data as possible from each animal. A large part of the experimental work in my group is carried out ex vivo. The PREPARE guidelines will be utilised to optimally plan a new set of

experiments and to avoid any duplication of efforts. The ARRIVE guidelines will be followed and in particular the ARRIVAL Essential 10 in order to standardise the reporting of animal research and improve the reproducibility of the research outcome.

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 trying to optimise the breeding and genotyping of the animals. We will also do pilot studies if a new experimental protocol will be implemented. In order to estimate the required numbers of animals to find significant differences between experimental and control group. If possible tissue will be used for different experiments

A retrospective assessment of reduction will be due by 02 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.

Zebrafish is an established model for biomedical research and the genetics and working protocols are very well developed. We breed the least number of animals for line maintenance and for the various experimental protocols. Injury triggers heart regeneration in zebrafish and the molecular response is very similar to those observed in higher vertebrates including the human heart. Cryoinjury is a less invasive procedure than surgical resection, which is causing bleeding and therefore may cause a higher mortality. Importantly, cryoinjury triggers transient scar formation and therefore models the situation after myocardial infarction in the human heart quite well.

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

For the purpose of studying POPDC gene function, most of the scientific research we are planning requires the use of adult stages. This is for example true to model heart and muscle disease in patients or to study the role of POPDC genes in cardiac regeneration. While regeneration could in principle be also studied in the immature larval stages, at this developmental stage many of the cell types present in the adult heart are not yet present. Observations made at this stage will therefore not be directly applicable to the adult heart. `Similarly patients develop muscle and heart disease in response to POPDC mutations only as young adults at the earliest, some of the mutations trigger a late onset of the disease and only appear when patients are in their forties or even older. Therefore, it is mandatory to study the role of POPDC genes in the adult zebrafish.

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

We have noted and incorporated a number of refinements to each protocol since we started to use zebrafishes more than 15 years ago. We continue to monitor animals closely, and with NVS constantly assess possible improvements in post-operative care, pain management, and animal environments.

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

We follow the latest guidance of publications by the zebrafish community such as: Aleström P, D'Angelo L, Midtlyng PJ, et al. Zebrafish: Housing and husbandry recommendations Lab Anim. 2019;23677219869037. doi:10.1177/0023677219869037. We follow PREPARE guidelines, plan and conduct studies according to the ARRIVE guidelines and use NC3R guidelines to ensure our animal experiments are as robust and reproducible as possible.

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

We are regulalry attending scientific meetings and talk to colleagues using the zebrafish model. We read the monthly updates from the NC3Rs on their events and publications and their e-Learning resources all of which detail advances in 3Rs technologies and best practice and provide information how to put these in place.

A retrospective assessment of refinement will be due by 02 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?

5. Decision Making From Synapses To Circuits

Project duration

5 years 0 months

Project purpose

Basic research

Key words

Decision making, value calculation, hippocampus, physiology, behaviour

Animal types	Life stages
Mice	adult, 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.

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 are working to understand how neurons in the brain communicate with each other to allow them to encode emotional behaviours and make decisions.

A retrospective assessment of these aims will be due by 24 March 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 it's aims and if not, why not?

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

Why is it important to undertake this work?

Problems with the communication between neurons in the brain underlie the vast majority of neurodegenerative and neuropsychiatric diseases, and so our aim is to find novel ways to combat these diseases by gaining a greater understanding of the processes that they destroy.

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

The project comprises basic scientific research that will increase our understanding of how the brain supports decision-making behaviour. The outputs of this project will be new scientific findings, describing how neural activity in the hippocampus (and connected brain regions) supports value- and memory-based decision-making. The outputs will primarily take the form of publications in peer reviewed journals, but will also be disseminated at academic conferences and to the lay public via popular science initiatives. Materials, data and methods may, where appropriate, be disseminated online.

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

In the short timescale, the primary beneficiaries will be other scientists working in the field of learning and decision-making research. Our research will inform our fundamental understanding of how neural networks work in healthy adults, and how this is altered by experience during adolescence.

In the medium term, the basic science knowledge gained will likely inform broader fields of scientific enquiry, some of which have the potential for clinical translation. For example, understanding how memory networks malfunction after adolescent social isolation may inform strategies for developing new pharmaceutical or therapeutic interventions.
In the long-term, a detailed understanding of the neural-network level mechanisms for learning and memory will be an invaluable aid to designing interventions for mental health disorders of all types. For example, the neural circuit changes that promote the transition to mental illness are increasingly viewed to be extremely specific: effecting only specific cell types and the connections between them. Our research aims to uncover these specific alterations, and how they relate to specific behavioural phenotypes and symptoms. We hope that this will provide new druggable targets that are both potent and specific, and will allow for better, more personalised treatment, and the minimisation of off-target side effects.

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

We will disseminate our research by publishing results in peer reviewed journals. We aim to publish all results, including those that do not confirm our hypotheses.
We will also present our work to academic peers at scientific conferences (national and international), and engage with the popular science media in order to disseminate our results to the general public.

Raw data and analysis methods will be shared with the scientific community (following peer-reviewed publication), to allow other groups to gain insights from our experiments, and reduce replication of work.

Species and numbers of animals expected to be used

- Mice: 7500

Predicted harms

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

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

This project will use adult mice. We know a lot about the anatomy and physiology of the mouse brain, and in particular of the parts of the brain to be studied in this project. Mice also offer unmatched access to genetic tools, allowing us to use genetic techniques to record from and manipulate functionally/genetically/anatomically defined ensembles of cells. Together with the ability of mice to rapidly and flexibly carry out value and memory guided decisions, this means that we are well-placed to fill in substantial missing section of our knowledge: how patterns of neural activity influences decisionmaking.

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

In the most typical experiment, mice will undergo a surgical procedure under general anaesthesia, to carry out chronic (long-term) attachment of devices for monitoring neural activity to the skull of the animal. Analgesia will be provided during the surgery and during recovery. Following recovery, the attached devices do not, in themselves, cause any pain or distress to the animal.

Animals will then undergo experiments in which neural activity is monitored simultaneously with behavioural testing. Mice will learn to play a 'game' where there are correct and incorrect answers - mice will be rewarded with sugar water when it chooses the correct answer.

Animals will typically be motivated to learn using appetitive (desirable) rewards such as sweet liquids such as strawberry milkshake, and minimal levels of water restriction. In a smaller number of experiments, mice may also learn using negative reinforcers such as air-puff to the face or mild static shock, or from psychostimulant rewards such as cocaine or amphetamine.

Neural recording and behavioural testing experiments typically continue for weeks, or even possibly months.

At the end of the experiment, animals will be euthanised, using an overdose of an anaesthetic agent.

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

Some animals may feel pain or discomfort during the recovery from surgery (1-2 days). To mitigate this, analgesia will be given to the animal.
Some animals may experience more than average weight loss following food or water restriction. In these cases, animals will immediately be removed from the experiment and provided with freely accessible food and/or water.

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)?

30% of animals will experience the severity category 'Mild'.
70% of animals will experience the severity category 'Moderate'.

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

- Killed

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

During this project we will investigate how the brain is connected to carry out specific functions, and how this wiring is altered by experience. Crucially we will also monitor how these circuits encode and respond to the environment during complex decision-making behaviours. In order to investigate this problem we have to study these neuronal circuits in animals as the animals carry out these behaviours.

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

Computational modelling, in vitro cell culture, human research, research on less sentient animals (e.g. fruit flies / nematode worms)

Why were they not suitable?

There are no computer models or equivalent that can accurately and effectively model these phenomena, and so experiments on living tissue are required. The data obtained during this project will allow for more accurate and precise modelling in the future.
No cultured cell lines are available to study the mechanisms that control synaptic connectivity, and so acute tissue must be used.

Although implantation of chronic electrodes is possible in humans, it is only permissible in a small numbers of clinical situations, and therefore not practical to answer the questions proposed. While increasingly human brain slices are becoming feasible, again these are strictly limited, and most often from diseased tissue. In addition, the specificity required to understand the functioning of these circuits is beyond current human-based techniques. Less sentient species do not have the homologous circuitry to the mammalian decision-making circuitry investigated in this proposal.

A retrospective assessment of replacement will be due by 24 March 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 numbers of experimental animals have been estimated using a) power analyses, b) experience from previous published studies of effect sizes, and group sizes necessary to test effects.

A pure power analysis approach is not always appropriate for in vivo neural recording experiments, as the number of animals required will depend on the success rate of neural recording (numbers of neurons per animal). It is therefore necessary also use estimates based on previous experience of similar experiments.

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

We will seek advice from the University's applied statistics advisors.
We will follow the ARRIVE and PREPARE guidelines and use 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?

Pilot studies, allowing us to explore whether hypothesised experimental effects may be present, before committing larger number of animals.

Technical developments which enable us to monitor the activities of larger numbers of brain cells in each animal.

Use of computational models that enable us to make highly specific testable predictions about the role of hippocampus and other structures during behaviour, minimising the number of experiments required to reach a conclusion.

Most procedures involve long-term experimentation with the same animals, which significantly reduces the number of animals needed to reach statistically significant conclusions.

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

The model animal used will be mice.

The major methods used will be:

Stereotaxic surgery. This is necessary in order to inject and implant substances to identify, record from and manipulate defined neuronal circuitry both in vitro and in vivo. Appropriate anaesthetic/analgesic regimens will be used to minimise pain. e.g. delivery of pre-operative analgesia, as discussed with the NVS and Animal Facility Staff.

Maximum injection / infusion parameters will be strictly adhered to. In the very unlikely case that this is not possible due to extenuating circumstances, any changes will be discussed with the NVS.

Behavioural training. This is necessary to assess cognitive capabilities in animals. The large majority of these tests will use only appetitive (rewarding) stimuli, hence the only harm is mild food or water deprivation.

In vivo neural manipulation and recording. This is necessary in order to be able to draw direct functional links between neural activity and behaviour. Neural recording implants do not cause suffering and distress in themselves, hence the potential for pain and suffering is confined to the post-surgical period (in which analgesia will be provided, see below for details).

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

Mice will be used for this project as they represent the least sentient species appropriate for this type of work.

Decades of research has also resulted in highly advanced and efficient techniques developed for the mouse as opposed to other species. For example, there are excellent stereotaxic maps of the mouse brain, allowing accurate targeting of injections to specific brain regions. Coupled with my expertise in stereotaxic surgery, this results in very high success rate in our experiments (greater than 80% success rate in targeting of brain regions).

The mouse is also high genetically tractable, allowing transgenic identification of specific cell types crucial to the fulfilment of the project
.
Finally, as the proposal aims to investigate how neurons are utilised during behaviour, terminally anaesthetised mice would not be appropriate.

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

Almost all of our behavioural tests will use appetitive (rewarding) stimuli, the only aversive tests used will involve, for example, bright lights/loud noises or mild air puffs. Animals will be motivated to seek reward using mild food (~20% of restricted animals) or water (~80% of restricted animals) deprivation. We will only use the minimum levels of deprivation necessary to achieve uniform consistent behavioural results.

Appropriate anaesthetic/analgesic regimens will be used to minimise pain. e.g. delivery of preoperative analgesia, as discussed with the NVS and Animal Facility Staff.
Maximum injection / infusion parameters will be strictly adhered to. In the very unlikely case that this is not possible due to extenuating circumstances, any changes will be discussed with the NVS.

Housing cages will be spacious and enriched with e.g. rodent toys, chewable materials such as wood, running wheels, shelter, unless these interfere with the experimental design. In addition, where possible animals will be group housed post surgery using strategies devised in collaboration with our local NVS and NACWO.

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

Local NVS and NACWO
Resources hosted on the NC3Rs website, in particular:
ARRIVE guidelines on experimental design and reporting results.
'Procedures with Care': 'Aseptic Technique in Rodent Surgery'.
Rodent housing and husbandry
Rat and Mouse Grimace scales

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

We will keep in constant contact with local NVS and NACWO to ensure we are maintaining best practice.

We will liaise with in-house NC3Rs representative to ensure we are up to date with current best practice.

We will use the resources published on the NC3Rs website to ensure that the group undergoes continuous training and professional development with respect to the 3Rs.

We will follow technological advances in the published scientific literature, allowing more efficient recording techniques (yielding more neuronal data per animal), miniaturising recording equipment (leading to a refined animal experience) or allowing recording in more naturalistic settings (for example, wireless recording).

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

6. Defining the role of G protein coupled receptors in the brain and the therapeutic potential of targeting these receptors in neurological 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

Alzheimer's disease, G protein coupled receptors, Schizophrenia, Pharmacology, Neurological behaviours

Animal types	Life stages
Mice	juvenile, adult, neonate
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.

Objectives and benefits

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

What's the aim of this project?

This project licence is aimed at continuing studies on the role of G protein coupled receptors (GPCRs) in the regulation of the nervous system including brain and neuronal function and how we might target this class of cell surface receptors in the treatment of neurological disease. The GPCR family consist of many hundreds of different receptors and are the most successful drug targets known to man. Despite this the full potential of the GPCR family has yet to be realised. The reason for this is that we lack fundamental understanding of the biology of many of these receptors and how best to target them in disease. In this licence we will conduct experiments focused on the biology and therapeutic potential of GPCRs in the nervous system.

A retrospective assessment of these aims will be due by 20 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 it's aims and if not, why not?

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

Why is it important to undertake this work?

GPCRs are involved in key neuronal processes such as learning and memory and that selective stimulation of certain types of GPCRs can relieve symptoms and slow progression of neurological diseases including neurodegenerative disease. Hence studying them can lead to better understanding of how the brain works and the design of drugs that can impact on diseases such as Alzheimer's disease.

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

In the neuroscience project we aim to establish the role of GPCRs in the regulation of neurological responses such as learning and memory, anxiety-like behaviours and locomotion. We also expect to determine the impact of GPCRs particularly the muscarinic receptor family in the symptoms and progression of neurodegenerative disease, schizophrenia and possibly other neurological diseases.

These discoveries will be disseminated in the following ways;
Peer review literature
Scientific meetings in the form of talks and poster presentations
To the general public in the form of press releases, public seminars and social media

We also expect these discoveries to result in further grant applications and both charitable and government grants.

In addition, we have the anticipation that these studies would change the direction of drug company research opening up new clinical trials and impact on drug discovery strategy.

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

Academic Community - will benefit from an understanding of fundamental biology of GPCRs and the understanding of the best ways to target GPCRs to regulate pathophysiological responses.

Pharmaceutical/drug discovery community – will benefit from the validation of new GPCR targets in human disease and an appreciation of the pharmacological principles that can be applied to drug design.

General public – will benefit from the prospect that new methods will be developed to apply to drug discovery against some of the currently most intractable diseases including Alzheimer’s disease.

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

In terms of publications in the scientific literature and presenting in research meetings we are very experienced in these areas with strong relationships with editors of the top

journals as well as being well connected with organisers of major research meetings. Hence we anticipate that we will continue to have strong outputs through these routes. We have also been developing our outputs via social media with both institutional and personal social media outlets being developed. Finally we are improving our public out-reach with visits to local prisons, schools and presenting to politicians where we present our research and discuss animal research ethics.

Species and numbers of animals expected to be used

- Mice: 20,000
- Rats: 500

Predicted harms

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

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

The overarching purpose of this protocol is to gain greater understanding of the role and therapeutic potential of GPCRs, or GPCR-regulated pathways, in later-stage neurodegenerative disease. The aim is to determine if by activating (or deactivating) GPCRs, or GPCR-regulated pathways, we can alter the electrical brain activity and biochemical processes in the brain to influence progression of neurodegenerative disease. As such we are planning to test rodents models because we and others have established that these models closely resemble human systems and we can mimic human neurological disease. Hence by determining the activity of our drugs/substances and effects of our genetic modifications in rodent systems we are able to determine the role and therapeutic value of GPCRs. We can best do this in adult animals but we can also investigate how our receptors work by conducting studies on primary neuronal cultures derived from new born animals. Hence this study is best suited to adult and new born animals.

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

Animals (mainly mice but occasionally rats) will be inoculated with brain preparations made from animals or humans that have had neuroidegenerative disease e.g. brain preparation from prion diseased mice or brain preparations from humans that have died from Alzheimer's disease.

Animals (e.g. mice or rats that are either genetically modified and/or have neurodegenerative disease) can then undergo behavioural or neurological testing and/or live animal imaging and/or blood sampling and/or monitoring of body temperature and locomotor activity and/or monitoring electrical activity and brain signalling. These steps can be conducted both with or without administration of drugs/substances, that are either delivered minutes/hours before biochemical/electrical/behavioural testings (i.e. acutely) or days before testing (i.e. chronically).

The experience described in point 2 above will also be that of genetically modified mice expressing variant genes coding for GPCR receptor proteins and signalling pathways activated by GPCRs.

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

Mice are inoculated with misfolded protein such as prions/tau/alpha synuclein in order to develop progressive and terminal neurodegenerative disease. Although some of the animals in this protocol will be humanely killed before they reach late stages of disease a proportion of the animals (approximately 8%) will be maintained until late stages of disease, where they will likely develop physical symptoms, we propose a severe category. It is anticipated that some of the mice that enter late-stage disease may die before they arrive at a humane end point.

Furthermore, from our previous experience when testing for adverse responses to GPCR drugs approximately 2% of animals develop physical symptoms that can include pain, weight loss, seizures, or abnormal behaviour.

Other than this the drug/substances administration, behavioural, imaging, surgical and drug administration procedures described here will fall into mild category as described in the above protocol expect for an occasional (approximately 1%) a moderate.

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

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

Approximately 8% of mice/rats will be maintained until late stages of disease, where they will likely develop physical symptoms that result in a severe category.

Approximately 2% of animals develop physical symptoms associated with drug/substance administration that result in a severe category.

All other animals will fall into a mild category with occasional (approximately 5%) moderate .

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

- Killed

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

We are using a number of sophisticated approaches to mimic brain responses thereby offering the opportunity to replace the use of animals in neuroscience research. This

includes working with optic physicists to develop an "optical brain" that mimics brain activity using light and chemists who are similarly developing a "chemical brain" that responds to chemicals in the same way as a brain. However, currently none of these approaches come close to accurately mimicking brain activity. Hence to investigate the three primary areas of this project namely the processes that regulate brain activity via a group of proteins called G protein coupled receptors (GPCRs), ii) how we might change brain activity through drugs that act on GPCRs and iii) how we might cure the symptoms, and slow the progress, of neurodegenerative disease - we need to conduct experiments on animals.

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

We will be using human brain tissue (both normal and disease) obtained from registered human tissue banks for the neuroscience projects described here.

Why were they not suitable?

Human tissue is preferable to mouse tissue however it is not possible to employ genetics to validate the receptor targets in human tissue. It is also not possible to trial drugs that target our receptors in humans - rather we can only test the response to our drug treatment in resected tissue or from post mortem samples. Hence we aim to combine the animal studies with human tissue studies to probe the function of GPCRs in human disease. The ultimate aim will be to subsequently develop drugs based on our findings to trial in human clinical studies.

A retrospective assessment of replacement will be due by 20 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?

We have a great deal of experience in many of the behavioural and physiological experiments that the mice will be used in and have also a great deal of experience in the breeding and maintenance of our lines. Based on previous experience we have been able to apply power calculations in collaboration with statisticians. This has allowed us to estimate how many animals we need for the experiments described in this protocol.

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

We are constantly making attempts to reduce the number of animals in the following ways;

By conducting biochemical experiments, such as determining the changes in the proteins in the brain, we are looking for early markers of neurological disease that can give reliable indications of drug efficacy thereby reducing the number of animals and the time they are exposed to disease. This is particularly the case in the neurodegeneration studies but also in our inflammatory models.

We are using human tissue with increasing frequency to reduce the number of mice used. This tissue includes analysis of normal and diseased post mortem brain tissue that can be used for the determination of GPCR expression levels and testing the activity of GPCR drugs in a disease context.

We are using well described protocols such as elevated plus maze, novel object recognition and pavlovian fear conditioning that we are highly experienced in and therefore requires little to no training and few pilot studies.

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

We have excellent management systems and data-bases in place to ensure efficient breeding and husbandry of the mice.

Where possible we also share tissue amongst users and importantly co-ordinate studies to most efficiently use mice. We also have a large tissue archive which is well indexed and stored.

These approaches will reduce the prospect of overbreeding.

A retrospective assessment of reduction will be due by 20 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.

GPCR mutant mice
These will be used to directly determine the role of GPCRs in normal physiology and in disease. In particular we have developed mutant receptors that cannot be activated by the chemicals produced naturally by the body but instead be activated by drugs. In this way we can not only test the role of the target receptors in normal biology but importantly test the action of drugs that work by activating these receptors. Also we have developed GPCR mutant mice where the receptor is restricted in the number of signalling pathways that can

be activated. Such receptor mutants can be used to determine the biochemical and signalling pathways used by receptors to mediate clinically important effects and distinguish these from pathways that lead to adverse responses.

These models are designed to directly test the modes of action of GPCR drugs. Without these models wild type animals would be used. The issue is that wild type animals will display an array of responses a number of which are adverse such as pain, seizures and adnormal behaviour. These adverse responses are often due to off-target drug activity.

The animal models used here reduce off-target activity and therefore reduce adverse responses.

Animals will be monitored and adverse responses measured using the criteria set out in the section keeping animals alive.

Neurodegeneration models
These strains will be used to determine the impact of GPCRs in the progression of disease and disease symptoms and whether targeting these receptors can modify disease.
Animals will be monitored and adverse responses measured using the criteria set out in the section keeping animals alive.

We will also use a range of genetically modified mice to investigate how GPCRs work in the brain and how these receptors relieve symptoms of neurodegeneration and slow disease progression.

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

Where possible we will use early life stages (e.g. mouse embryos and neonates) to generate neuronal cultures and terminally anaesthetised animals for histology (e.g. perfusion fixation). However the neuroscience projects require models that most closely resemble human physiology and be models that can be genetically manipulated in this case mice are the most appropriate. Also we wish to test the action of receptors and drugs that might lead to new drugs for human use. The receptors and receptor system need therefore to closely relate to humans and therefore mammalian systems such as mice are the most appropriate.

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

We are always looking for further refinement of procedures. For example we have been undertaking a study to determine biomarkers of neurodegeneration that can be used to establish early on in disease if targeting our receptors of interest impacts on disease before clinical signs appear. Hence, we will pilot experiments, reading the literature and discussions with collaborators be looking to minimise welfare costs.
Specially, if biomarkers can be identified then we will significantly reduce the number of prion infected animals that progress terminal stage of disease. Currently this step 1 is classified as severe since a proportion of these animals will be progressed to terminal end points. The biomarkers would predict terminal end points before the animals reached a severe category. Hence determination of disease biomarkers would reduce the number of animals progressing to a severe category.

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

Our primary published source of guidance on 3Rs is via the national centre for replacement, refinement and reduction in animal research (NC3Rs). This organisation publishes regularly on guidance for researchers. The European Medicines Agency also publish excellent practical guidance on 3Rs. We also pay particular attention to the peer reviewed scientific literature for further methods to refine our protocols.

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

We have regular up-date sessions and training in new approaches run locally and nationally. We also keep abreast of the published literature and share good practice locally. Importantly, we also have expert collaborators that share good practice and we are always looking at new methods to improve our 3Rs.

A retrospective assessment of refinement will be due by 20 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?

7. Detection of genotoxic substances

Project duration

5 years 0 months

Project purpose

- Translational or applied research with one of the following aims:
- 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

No answer provided

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.

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?

Genotoxicity studies are necessary for hazard assessment and are legally obligatory. The overall aim is to ensure that both new and existing chemicals do not present a potential carcinogenic, inheritable or otherwise toxic hazard to the public, patients and the environment.

This project will aim to evaluate the effects of chemicals, human and veterinary drugs, medical devices, food additives, biocides and plant protection products to see if they damage cells in animals. If substances damage cells it can lead to the development of diseases like cancer. The tests carried out in this project will identify genotoxic levels of these substances, to enable decisions about how hazardous they are, and so people can be better protected. These tests are legally required before products are exposed to the public by Governments around the World.

A retrospective assessment of these aims will be due by 03 January 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 it's aims and if not, why not?

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

Why is it important to undertake this work?

A key benefit of this programme of work is the provision of safety data to facilitate sound regulatory decisions when assessing the risks to which humans when the test substances are produced, transported or used.

This work is vital to the development of safe substances (chemicals, human and veterinary drugs, medical devices, food additives, biocides and plant protection products) that people will come into contact with.

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

The output of this project will be the provision of safety data to facilitate sound regulatory decisions when assessing the risks to which humans, animals, plants or the environment are exposed when substances are produced, transported or used.

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

The overall benefit is to ensure that both new and existing chemicals do not present a potential carcinogenic, inheritable or otherwise toxic hazard to the public, patients and the environment.

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

Development and validation of new tests or modifications to existing assays will lead to an improved battery of tests for hazard and risk assessment. In addition, many new tests or modifications may allow more thorough assessment of genetic hazard in one step, thus eliminating the need for extensive further testing and reducing overall animal usage. Wherever possible, data from multiple end points will be obtained from the same animal.

Species and numbers of animals expected to be used

- Mice: 5700
- Rats: 20000

Predicted harms

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

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

Adult rats and mice are the species specified in the regulatory test guidelines.

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

Test substance will be administered by oral, intravenous or parenteral routes of administration. Surgical procedures will be used if administration is by continuous intravenous infusion. The duration of dosing is typically 1-3 days, but may be up to 1 month to meet the scientific aim of the study; some studies may require a longer dosing period in order for adequate characterisation of dose response. Dosing may include wash out periods or interrupted dosing intervals.

Animals may be housed in specific cages for the collection of urine and faeces.
Blood samples may be taken by insertion of a hypodermic needle or from cannulae surgically implanted into blood vessels.

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

In the majority of animals, there will be minimal adverse effects. Some animals are expected to exhibit some toxicity, but in these instances effects such as signs of pain or discomfort, or abnormal behaviour will mostly be short-lived, lasting for a few hours after dosing.

In range finding studies, some animals may experience severe toxicity, which could include mortality, in those instances where the effects of the substance are not yet known. Any animals showing severe signs will not be maintained and will be humanely killed at the earliest possible point. Most range finding studies will result in no more than moderate adverse effects that are tolerated for the duration of the study.

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

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

Based on previous experience, it is expected that approximately 80% of animals will be in the mild severity category, approximately 15% will be in the moderate severity category and approximately 5% will be in the severe severity category.

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

- Killed

A retrospective assessment of these predicted harms will be due by 03 January 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?

Regulatory guidelines require the use of animals to investigate genotoxicity. The *in vivo* tests are conducted because some agents are mutagenic *in vivo* but not *in vitro*. The *in vivo* tests also include additional relevant parameters such as absorption, distribution, metabolism and excretion, which may modulate the genotoxic effects of a test substance.

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

The ECVAM database and other literature searches were conducted to determine if any non-animal alternatives were available. However, the animal study is preceded by an *in vitro* assay, the results of which are used to optimise the design of the animal study. All studies will be assessed to verify that there is a need to conduct the study and that there is no other data or approach that could avoid *in vivo* tests.

Why were they not suitable?

No non-animal alternatives are accepted alone by Regulatory authorities. However, if there is data or previous test results available that mean *in vivo* tests are not required, procedures will not be conducted for that purpose.

A retrospective assessment of replacement will be due by 03 January 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?

These are based on the numbers used during the last five years and the expected demand for this service.

The requirements of the various tests and regulators will be followed so that only the required number of animals are used in each study.

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

Standard study protocols are ethically reviewed against the known guidelines/recommendations, AWERB policies and any recent 3R's refinements. The reviews are undertaken by a committee comprising the Project Licence holder, NVS, NACWO and a lay person as well as other interested parties. Any study requested by a Sponsor that deviates from the approved standard design or involves a new animal related procedure or methodology is subjected to a specific ethical review by the AWERB to

ensure all ethical considerations have been taken into account. Statistical advice from in-house experts may also be requested.

ICH promotes the assessment of genotoxic effects by including the relevant end-points into other toxicity studies that are required for regulatory submission. This has clear advantages in terms of animal reduction, however, the study designs must meet specific requirements, so that they are acceptable to regulators and that further animals studies can be avoided.

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

Regulatory guidelines define the minimum testing requirements for adequate data/statistical analyses for the majority of assays and the study plans used generally adhere to these guidelines/recommendations. Where no guideline recommendations exist, animal numbers are selected on the basis of published literature and/or internal validation data that identify the minimum number of animals required for adequate statistical power. We will always seek to minimise the use of control groups and multiple dose levels, where this is appropriate.

A retrospective assessment of reduction will be due by 03 January 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.

Rodents, specifically rats and mice, are the species of choice for these assays, as these species have the largest historical database available and have been routinely used in Genetic Toxicology testing for several decades.

Genetically altered animals may also be used. Only animals without an adverse phenotype will be used.

The methods used (dosing, blood sampling techniques, handling and restraint, use of analgesia) will be reviewed during the life time of the licence and any refinements developed will be implemented.

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

The species used is generally the same as the rodent species used for the general toxicology studies. The toxicology and/or toxicokinetics from the general toxicology studies can then be used to inform dose selection, sample times etc for the genotoxicity tests, thus reducing animal usage. Species selection may also be driven by known absorption, distribution, metabolism or excretion (ADME) differences between rodent species.

The Regulatory test guidelines require the use of young adult animals.

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

Under current regulatory requirements, it is necessary to perform genetic toxicology studies at doses that produce a degree of toxicity, usually in terms of presence of clinical signs and body weight change. Industry guidance and best practice about dose setting are followed, to avoid unnecessary toxicity. All procedures are kept to the minimum commensurate with the study objective. Best practice guidelines for all animal care and use are followed.

Dose range finding studies may be needed to establish suitable dose levels for the genetic toxicology study. Once signs indicate that a dose is unsuitable for genotoxicity assessment, further dosing of the sex/group is usually halted and after any necessary observations have been performed, humane endpoints are applied. Additional observations are included as necessary to closely monitor the condition of the animals. Industry guidance and best practice about dose setting will be followed, to avoid unnecessary toxicity and we will use the results of other studies and data wherever possible, to avoid having to run range finding studies.

Wherever possible, we will use doses that do not produce toxicity e.g. where it is known that a limit dose can be used, or that higher doses are not required. Signs of toxicity are typically seen in mid/high dose animals. Usually these are mild/moderate, however, animals may show significant adverse effects and in this case action will be taken to alleviate signs, such as temporary or permanent withdrawal of the animal from dose, reducing the dose if appropriate or via the application of humane endpoints.

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

We will monitor for updates and other refinements and guidance through the life of the project and apply wherever possible.

Experiments will be conducted in compliance with OECD Test Guidelines and ICH S2(R1). Dose setting will be determined by the requirements of these test guidelines.
For any surgical interventions, then the LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery (2017) will be followed.

For blood sampling and dosing then the following guidelines/literature will be followed:
First report of the BVA/FRAME/RSPCA/UFAW joint working group on refinement, Laboratory Animals, 27, 1-22 (1993).

A Good Practice Guide to the Administration of Substances and Removal of Blood, Including Routes and Volumes, Journal of Applied Toxicology, 21, 15-23 (2001).

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

Through the regular review of non-animal alternative developments / resources, attendance of scientific conferences and animal welfare forums and reviews of scientific literature.

A retrospective assessment of refinement will be due by 03 January 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?

8. Developing safe and efficacious cell-based therapies for kidney disease

Project duration

5 years 0 months

Project purpose

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

kidney injury, regenerative cell therapy, preclinical imaging, safety and efficacy, immunomodulation

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

Retrospective assessment

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

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 develop therapies to treat kidney disease, using mouse models of acute kidney injury and chronic kidney disease. For this purpose, we aim to assess efficacy and safety of cells or their products as regenerative medicine therapies

A retrospective assessment of these aims will be due by 02 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 it's aims and if not, why not?

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

Why is it important to undertake this work?

Acute kidney injury (AKI) and Chronic kidney disease (CKD) affect large numbers of patients, particularly adults but also children, and are frequently associated with underlying health problems including cardiovascular disease and diabetes.
AKI is associated with an increased mortality rate and 20% risk of progression to CKD. Treatment of AKI is limited to management of blood pressure, fluid and electrolytes, but renal replacement therapy can be needed. CKD can progress to end stage renal disease which means that dialysis or transplantation are the only remaining treatment options. Regenerative medicine therapies, including cells or their products, have emerged as a promising avenue for the treatment of AKI or CKD, but our understanding of the mechanism of action and the safety of these cells or their products remain limited. This programme of work will address these points in preclinical studies in mice of various ages (including aged animals), and thus contribute further important knowledge towards the development of safe and efficacious regenerative medicine therapies for treatment of patients.

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

This project will provide a number of outputs, including:
A more thorough and detailed understanding of the interactions between RMTs, damaged kidney and the immune system, including their mechanism of action, to promote or enable therapeutic efficacy in mice with kidney injury, and the influence of ageing on these processes. This may include novel insight into molecular and cellular steps that could be clinically exploited for therapeutics.

The development of novel preclinical imaging assessment methods of renal function and tissue integrity to determine efficacy of RMTs in a non-invasive, longitudinal fashion that can be clinically translated. This includes the development of novel dyes/compounds that can be utilised in these assessments.

The development of novel imaging tools and cell labels for preclinical imaging to track cells in order to determine their safety, both in the kidneys but also off-target in other tissues, since cell administration may be systemic and/or can lead to off-target growths. This involves the development of novel models to interpret and analyse preclinical imaging data.

Multiple research publications in peer reviewed journals. We have a strong publication record in our team, with 2-4 publications per year in recent years. We strive to generate publications from our funded studies, not only for the benefit of the young researchers involved, but also to improve general understanding and knowledge in the field.
Scientists with unique training and expertise in various techniques including surgery, colony maintenance of wildtype and genetically altered animals, use of a range of preclinical imaging modalities, data analysis. These skills will be invaluable for the research careers of the staff and students involved in this programme of work.
The long-term aim of this programme is to generate novel therapeutics that can be translated to the clinic for the benefit of patients with kidney injury or kidney disease.

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

Short term beneficiaries:
The renal research community as well as the regenerative medicine research field, including the area of in vivo imaging and image analysis. Our research findings will be communicated to these communities via workshop and conference presentations, which will lead to exchange of ideas and cross-fertilisation of outcomes, ideas and concepts. The research communities will also benefit from research publications generated from our findings, with the potential to stimulate further research.
The research community will directly benefit, in the short to medium term, from access to novel preclinical imaging techniques and analyses that we will develop during this project, including data on novel dyes and their analysis and interpretation.
Medium to long term beneficiaries:
This will include the research team as short-term outcomes will build further expertise of the entire team, leading to continuity and consolidation as well as refinement of techniques.
The research field (both renal, regenerative medicine as well in vivo imaging) will be beneficiaries because accumulation and consistency in outcomes will consolidate the reliability and reproducibility of our work, leading to an improvement in standards.
Clinicians (renal and others) will benefit from access to improved understanding of mechanisms of action of RMTs and their safety. We expect that our findings will be important contributors to the development of clinically relevant RM therapeutics for treatments in renal patients that can be tested in clinical trials, as well as other diseases where RMTs have shown promise.
The public will benefit from interactions amongst the above beneficiaries as well as their disseminations and progress in the development and anticipated validation of RMTs. This includes patients with kidney disease (AKI or CKD, possibly renal transplant patients) who would benefit from the development of RMTs as therapeutics that can be tested in clinical trials.

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

Our outputs are maximised by interactions with colleagues from preclinical and clinical research fields, as well as interactions with patient representatives. We have ongoing international and national collaborations. We are strongly engaged with the UK renal research community and a large number of charities. Through these interactions, we reach a wide audience of specialist preclinical and clinical researchers and practitioners as well as lay people.

We maximise our outputs through attendance at workshops and conferences, and most importantly through publication of our findings in open access journals, including the use of pre-print serves (e.g. bioRxiv). We are keen to publish unsuccessful approaches, and datasets are made available after publication.

We have a range of international and national collaborations which arise from our active engagement with the national and international research field of renal regenerative medicine and preclinical imaging. This involves SMEs that are involved in the improvement of imaging modalities, dyes and analyses.

Species and numbers of animals expected to be used

- Mice: 4450

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 want to develop treatments for kidney disease or injury that could in the future be used in patients. We test cells or cell-derived substances as treatment, and use animals in order to find out which are the most successful cells or substances to reduce the injury in the kidneys. We use mice because their kidneys work very similarly to those in humans, and show similar injury response which we use to determine levels of damage, but also because mice are the simplest animal model with this level of similarity to humans. Therefore, mice are the most appropriate animal to achieve our aims. Because kidney injury can occur in younger and middle aged people, but also in older people, we use adult mice, but also aged mice.

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

Animals in this project may be part of studies that optimise conditions, or be part of a larger study to test and determine the success in treatment of the cells or cell-derived substances. With these studies, we also wish to understand how the successful treatment works, and where the cells that we use as treatment, go in the body.

During either studies, most of the mice will get a kidney injury which has similar clinical signs to kidney injury in patients. This can be a severe acute kidney injury caused by injection of a drug, or by surgical induction under general anaesthesia. During the surgery, the blood flow to one or both kidneys is halted for a pre-determined time period in order to establish the desired level of injury in the organs. The mice will be unwell for a period of 2-7 days, but recover. Some mice will be treated so that they develop chronic kidney disease, which is milder in its onset but can show clinical symptoms for longer; mice may be monitored for this condition for up to 6 months.

Some of the mice in either injury condition will receive cells or cell-derived substances as test treatment and we will monitor and measure clinical signs of progression of the injury response with our without treatment using the most non-invasive approaches possible that have also clinical relevance. These approaches involve injection of small volumes of contrast substances that can be detected in imaging instruments to determine how well the kidneys function. These steps are typically performed under general anaesthesia. These measurements can take place repeatedly over the study period but at the most appropriate times to reduce harm to the mice.

In some of the mice, we will have removed specific cell types of the immune system. We know that the immune system plays a role in the development of kidney injury and the limited natural healing process that can take place, and by removing certain immune cell types we can determine whether they are essential for development of the disease or the treatment process.

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

The experimental conditions may lead to loss of weight in mice that have kidney injury. The treatments which lead to kidney injury, will lead to the most critical loss of weight, but other steps, including imaging assessments under anaesthesia, can also lead to weight loss. Because of the need for clinical signs of kidney injury in order to assess the effect of the treatments, these adverse effects are necessary.

Aged mice may be more frail than healthy adult mice and their treatment regime during the steps of protocols will be adjusted so that they are milder without preventing us from obtaining relevant information on the same question of kidney injury and effectiveness of treatments with cells or cellderived substances.

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)?

Two of the protocols have a severity level of moderate, but most mice in these protocols will experience mild conditions. In the one moderate protocol we may breed genetically modified mice which may show signs of moderate health problems, however, ageing mice will also be generated under this protocol, which can lead to moderate severity in their clinical signs. Overall, we expect that not more than 10% of the mice under this protocol show signs of moderate severity.

In the other moderate protocol we optimise conditions for removing specific immune cells. This can involve the development of clinical signs of moderate levels of severity in the mice, caused by toxicity of some of the agents. After optimisation of the treatment regime with agents used to remove immune cells, no adverse effects have been reported. Therefore, we expect that no more than 10% of mice under this protocol show signs of moderate severity.

Three of the protocols have a severity level of severe. This includes two optimisation protocols where we will optimise conditions to induce kidney injury. We expect that up to 50% of mice in these protocols will experience signs of severe severity. However, we will use small groups of mice for these optimisation protocols so overall numbers of mice experiencing severe severity from these protocols is small.

The experimental protocol with severe levels of severity can also lead to up to 30% of mice experiencing this severity. In this protocol, mice may experience kidney injury which can lead to loss of weight, but treatment with cells or cell-derived substances or modification of the immune system may alleviate the injury level. Some of these mice may experience moderate levels of severity. Also in this protocol included are mice that are not experiencing kidney injury based on experimental design of cell tracking in non-injured animals.

Our careful optimisation approach, combined with monitoring of the wellbeing of the mice throughout, should allow us to minimise adverse events.

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

- Killed

A retrospective assessment of these predicted harms will be due by 02 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?

The conditions that contribute to the development of kidney injury and possible treatment cannot currently be reproduced in the non-animal setting since it involves the effect of blood flow, immune cells and other physiological factors beyond the interactions of cells inside the kidneys. Mammalian physiology and anatomy are complex and other organs may also contribute to the injury and any beneficial treatment response. Any beneficial treatment response observed in a non-animals condition may have to be validated using animals models before treatments can be trialled in patients.

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

In vitro (cell culture) and ex vivo (kidney slice) systems offer some opportunities to replace the use of mice. Cell culture work is being performed to study effects of therapeutic cells on cells of the immune system, but only deliver limited information due to the isolated setting. Kidney slice models require optimisation of set up which we are currently trying to obtain funding for. However, both approaches will be complementary rather than full replacements of animal studies.

Why were they not suitable?

In vitro approaches cannot reproduce the entire disease setting and physiological condition of the animal. These are important aspects that contribute to the injury and treatment response. This includes interactions with other organ systems and cells as well as physiological parameters like blood flow to the kidneys. Although cell-based studies produce important information in their limited format on the response of isolated cells to specific conditions, a whole range of these conditions are not being assessed. In kidney slice models, a more complex system of various cell types and interactions can be replicated, however, they still lack the intricate interaction between various organs and systems in the animal.

Since the aim of our work is to determine the efficacy and mechanism of action of cells or cell-derived substances within the animal to kidney injury, we need to consider that these processes in the patient take place in a complex environment and that the in vitro or ex vivo experiments cannot completely replicate the animal model.

A retrospective assessment of replacement will be due by 02 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?

My experience in this area of research over the last 10 years allows me to estimate with confidence the mouse numbers needed for this programme of work. Animal numbers are determined based on currently funded studies and the experience with previous project licences. These are based on effect sizes (including variances) from previous work, and also considering adverse effects.

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

The studies funded for this programme of work will have undergone peer review which includes providing detailed statistical analysis and power calculations. We have made use of the NC3Rs experimental design assistant but also other online power calculation tools (G star power etc) to design our studies and power our experiments so that we can achieve sound primary outcome measures. We base calculations on group sizes on estimates of effect sizes from our previous work, including preliminary data, and on published studies.

We frequently perform longitudinal studies which for example include multiple measurements of renal function parameters at various time points in the same animal. These longitudinal assessments allow for reduction of animal numbers since it reduces end point analysis at these different time points.

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 experimental design specifically for acute and chronic kidney injury studies where ischaemia reperfusion injury was induced by surgical approach. We recognised that animals undergo larger variability in response unless certain surgical parameters were more tightly controlled (see Refinement). The reduction in variability leads logically to a reduction in animal number as the experimental design calculations will include adjusted effect sizes, since variability in response doesn't need to be addressed in larger group sizes.

We perform pilot studies wherever necessary, especially for crucial experimental conditions like induction of injury and removal of immune cells. Optimal conditions arising from these pilot studies will lead to a reduction in animal number since they reduce variance in the experimental and control groups. This can be further refined after ANOVA-type statistical analysis for follow-on studies.

Post-mortem, tissues and other materials will be used to assess the outcome of the experiments. This may involve re-assessment of data using modelling of outputs using optimised computer programmes, for example by making use of machine learning. We are

currently starting a project where this is trialled with histological data obtained from our experiments.

Analysis and interpretation of other digital data is also being constantly optimised to both drive optimisation of interpretation and also develop novel approaches to data analysis which may have relevance for applications in the clinic.

A retrospective assessment of reduction will be due by 02 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.

In this programme of work, we use mouse models of kidney injury that are very closely replicating clinical symptoms. These injuries are required to achieve our overarching aim of developing therapies for treatment of kidney injury, using cell or cell-based treatments. These injury models are widely accepted as standard for mouse experiments that replicate symptoms of kidney injury in patients.

The methods used to induce kidney injuries either involve the injection of a nephrotoxic substance or surgery to induce an injury to the kidneys. Mice in these studies may be administered analgesics to reduce any pain, and surgery will be performed under anaesthesia.

We need to induce acute kidney injury in a subset of the experimental animals as we can follow the generally quick injury response over a short period time; in mice where we want to model chronic kidney disease, a less distressing injury is induced and the animal's response followed over a longer time period.

Some of the mice in either experimental treatment group will receive versions of cells or cell-derived substances with the aim to reach an reduction of the level of injury, which should correlate with the level of suffering and distress.
Pilot studies will be performed to optimise conditions of treatment to induce kidney injury, and also removal of immune cells. In order to understand the mechanisms of action that underpin any therapeutic response of the cells or cell-derived substances, we will study the response of the immune system which is intimately involved in injury and healing responses.

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

In this programme of work, we study effects of cells or cell-based substances as therapeutic treatment in mouse models of kidney injury, mimicking variances of the disease found in patients. The mouse is the simplest model organism that can replicate this disease scenario because it has a very similar anatomy and physiology to the human. Adult or even aged mice are needed to perform these studies because patients typically fall within this bracket. It is necessary to have fully functional organ systems available in the animal for the studies to yield relevant information, and early embryonic stages would not be providing the required data. It is not possible to perform the studies in terminal anaesthetised animals as various outcome measures wouldn't be able to be assessed, making this an impractical approach.

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

I have worked with mouse models of kidney injury for 10 years now, and during this time we have developed several models (Adriamycin glomerulosclerosis model, ischaemia reperfusion injury model). We have also established multi-modal imaging in healthy and injured mice during this time.

I have >20 years of experience in mouse models of various diseases, and have used genetic lineage tracing and ablation models under a different project licence. During this time, I have established expertise in assessing and scoring animal wellbeing, which could present stages of disease progression and injury. In order to implement the kidney injury models and other procedures within this programme of work, mice are closely monitored during the critical phases after injury induction in order to maintain the severity levels and determine when humane end points are reached. One of the main predictors is animal weight, and so animals are weighed regularly and their overall appearance monitored every few hours on the day of surgery or other induction of injury, and daily afterwards. This may involve close monitoring of wellbeing during critical phases of injury response by night visits in order to avoid unnecessary suffering of the mice.

We constantly strive to refine procedures to minimise suffering and improve welfare of the animals. This includes regular reflection of steps that could be improved. For example, we have optimised the experimental design for surgical induction of acute and chronic kidney injury, since we recognised that animal groups undergo larger variability in response unless certain surgical parameters were more tightly controlled. These observations and refinements have been published on the bioRxiv preprint server and are currently in revision at one of the leading renal physiology journals. This refinement subsequently leads to a reduction in animal number as the experimental design calculations will include adjusted effect sizes, since variability in response doesn't need to be addressed in larger group sizes.

Previously, we established the use of the ultrasound imaging instrument to refine the administration of cells into the left ventricle in order to avoid misinjections and unnecessary suffering.

We are currently working on a protocol to establish physiological and functional renal parameters (including GFR) as predictors of overall animal wellbeing. We plan to incorporate expertise by BSU staff and grimace scoring into an overall score system that allows us to monitor closely the wellbeing of the mice in an unbiased way.

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

We will follow LASA guidelines on dosing and administration of agents, and withdrawal of blood.
For the scoring of ageing mice, we follow the guidance by Wilkinson and colleagues (Wilkinson et al., 2020, Lab Anim. 54(3), 225-238. DOI: 10.1177/0023677219865291), and also the internal score sheet that has been set up in collaboration with colleagues at the University with longstanding expertise in research using ageing mice.
We have sent PILs/researchers on international courses to obtain specific training in the models used in this project, including the surgically induced kidney injury model. We have taken guidance from the publication by Skrypnyk and colleagues (Skrypnyk et al., 2013, J Vis Exp (78):50495. doi:
10.3791/50495) and others.
We follow the ARRIVE guidelines for which an update (2.0) was published this year (Percie du Sert et al., 2020, PLoS Biol 18(7), e3000410. doi:
10.1371/journal.pbio.3000410).
We have sent researchers to other laboratories nationally and internationally to learn and improve on techniques that are relevant for this programme of work, for example with regards best practice in using in vivo imaging instruments. As part of the EU Marie Curie training network, that is contributing funding to our work, we are in constant exchange with other researchers in the Netherlands, Germany, Italy and Ireland to discuss and improve our experimental designs and approaches.

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

Our Biomedical Services team is very active in announcing and organising NC3Rs meetings, workshops and conferences. I have attended several local NC3R workshops, and have presented at an international NC3R conference on severe suffering. I encourage members of the team to use the NC3Rs website, communicate information that becomes available, and attend conferences. The animal unit at our establishment is proactive in displaying important publications, posters and other information leaflets that are important for work in the NC3Rs area. The NC3Rs regional manager for the establishment is actively engaging with the animal researchers, and happy to provide support where required.

In our work, we constantly aim to apply the 3Rs principles; this includes continuous reflection on studies and subsequent optimisation so that refinements can be introduced to limit suffering, and also to reduce animal numbers. We are also keen to explore alternative model systems (ex vivo etc) in the laboratory.

A retrospective assessment of refinement will be due by 02 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?

9. Development, functions and programming of embryonic and adult immune cells

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

No answer provided

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.

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 investigates (i) the development and functions of embryonic and adult immune cells as well as (ii) the effects of early life adversity on immune development and function and the consequences of this "programming" on later life health and disease.

A retrospective assessment of these aims will be due by 03 March 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 it's aims and if not, why not?

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

Why is it important to undertake this work?

We currently have a **limited understanding** of what functions embryonic immune cells exert during gestation. Likewise, it remains controversial if their functions are distinct from those of immune cells generated at later stages. This is of particular interest for conditions where embryo-derived cells persist and co-exist with adult immune cells, like female reproductive organs and tumours. Similarly, we do not know if and how adverse early life environments change the normal developmental sequence and functions of immune cells, and if this predisposes for later life disease. This work is important to fill these **critical gaps**. In the future, it might help stratify individuals at risk for chronic inflammatory diseases like rheumatoid arthritis, and identify new avenues for preventive or early therapeutic intervention. It might also guide novel therapeutic approaches for pregnancy-related complications such as preterm birth as well as tumour immuno-therapy. Although fundamental in nature, this project thus has immediate **translational relevance**.

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

This project will primarily generate **new information** related to several scientific questions that are fundamental in nature, but nonetheless of immediate biomedical relevance: Our work will further improve our understanding of how the immune system normally develops, what functions the very first immune cells have in the embryo and in pregnancy, and if these functions are "hijacked" in tumours. We will also generate new information concerning the role of immune cells in maternal tissue remodelling in the context of pregnancy. Lastly, we hope to generate new insights in how adversity experienced early in life changes the normal course of immune development, and if and how this in later life increases the susceptibility to tumours and chronic inflammatory disease like rheumatoid arthritis. We thus hope to provide a mechanistic understanding of cause-consequence relationships underlying observations made in human epidemiological data. These findings will be **published** in scientific journals, but may also be disseminated to the general public. Moreover, while the project will not directly generate products for therapeutic use, it might inform **new strategies** for medical purposes.

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

In the first instance, this project will benefit the **scientific community** by filling critical gaps in our understanding of immune development. It will also benefit the research field by generating new models with which to address questions that have remained unanswered or controversial. It might also benefit **society** by increasing **awareness** of how early life adversities can have long-lasting impact on the immune system. In the **future**, this work might also help **meet clinical needs**. Specifically, it might aid stratification of individuals at risk for chronic inflammatory diseases like rheumatoid arthritis, and identify new avenues for preventive or early therapeutic intervention. It might also guide novel therapeutic approaches for pregnancy-related complications such as preterm birth, as well as tumour immunotherapy.

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

We aim to maximise the output of this project in several ways:

This work will be highly **collaborative**. It will involve existing and new collaborations, both locally and internationally, and regular exchange on technical aspects and progress of the project.

We plan to **disseminate** the data and knowledge generated within the **research community** through participation in expert **congresses** in relevant fields (e.g. Immunology, Developmental and Reproductive Biology, Rheumatology), **seminars** at other research institutions and **publications** in peer-reviewed journals. We also plan on disseminating our findings to the larger public through in science communication efforts and **public outreach** activities. These are strongly supported by our Centre and the University. We also intend to share within the scientific community any **unsuccessful approaches**, with the aims of troubleshooting these and preventing unnecessary unknowing duplication efforts. Where possible will we also publish negative data in scientific journals. Although unfortunately rather uncommon, there has been a recent surge in publications reporting the absence of phenotypes in studies aimed at understanding mast cell functions, or studies refuting functions previously attributed to these cells using different models. This example illustrates that the field is growing increasingly susceptible for sharing negative findings.

Where appropriate, we also aim to interact with and share our efforts and findings with more specific public bodies such as **patient initiatives** (e.g. for rheumatoid arthritis) and **charities.**

Species and numbers of animals expected to be used

- Mice: 12.000 + 30.000 additional offspring

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.

Central questions underlying this project are how the immune system develops, and what its functions are at different life stages. Immune development is initiated early in gestation. We are therefore interested in animals across the entire lifespan, ranging from embryos to newborn, juvenile and adult animals as well as pregnant females. In addition to our interest in embryos that necessitates pregnant adults, we are also interested in them in their own right where the role of maternal immune cells in pregnancy-related tissue remodelling are concerned.

While we are using and analysing animals of all life stages, experimental procedures will only be directly performed on adult animals, with very few exceptions in which substances or cells are delivered directly to embryos or newborn mice.

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

Experiments are aimed at studying either (1) the origins and functions of immune cells in development and pregnancy or (2) the effects of prenatal adversity on immune development and disease susceptibility.

Origins and functions of foetal or maternal immune cells in development and pregnancy. (1.1) Successfully mated female mice will proceed through pregnancy with or without an inflammatory challenge. Where inflammation is induced, this models acute inflammatory episodes or infections and in most cases will induce partial loss of foetuses

or preterm birth. Inflammatory agents will be delivered typically by injection or orally. (1.2) Pregnant mice will undergo usually one additional treatment aimed at modulation of inflammation, the immune response or gene expression and again consisting of one or several injections or oral deliveries. Exceptionally, this will be replaced by so-called "minipumps" that deliver substances more continuously and that are surgically implanted, usually before pregnancy. In some cases, blood will be drawn on up to two occasions. Other experimental readouts will be non-invasive and include ultrasound imaging and monitoring by cameras. (1.3) Animals will be terminated without additional treatments and tissues analysed either before or after giving birth.

Effects of prenatal adversity on immune development and disease susceptibility. (2.1) To generate mice that have prenatally experienced adversity, inflammation or arthritis will be induced during pregnancy. Where inflammation is induced, these models chronic low-grade inflammation and does not normally affect pregnancy success. To do so, substances will be delivered to pregnant mice either orally or by injection, typically on one or several occasions at different stages of pregnancy. In some instances, embryos will be injected directly using an ultrasound-guided method. In addition to injections, blood will be drawn from some pregnant mice in order to measure inflammation and disease parameters. A very limited number of mice will undergo surgery before pregnancy, with the aim of placing pumps that will continuously deliver substances to these animals. (2.2) The offspring of these pregnancies will be allowed to grow adult. (2.3) These offspring mice will then be subjected to treatments that either label immune cells according to their developmental origins, induce tumour growth or arthritis as well as additional treatments that modulate the immune response, disease or gene expression. Typically, animals will undergo maximally two procedures that consist of one or several injections. In a small minority of mice, surgery will be performed instead of injections to place minipumps that deliver substances continuously. In addition, blood will be analysed from some of these animals. In most cases, additional analyses will be non-invasive, such as clinical scoring of joint swelling or tumour size. (2.4) Experiments will be terminated and tissue analysed at different disease stages.

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

Adverse effects on animals may be caused either by genetic modifications or by experimental procedures and disease models.

Genetic effects: We will be using genetically modified animals, some of which may show preterm birth or foetal death during pregnancy. Although we are also using animals that are genetically predisposed to developing diabetes, we will normally only use these prior to disease onset for breeding.

Experimental effects:

- **Substance administration**: The nature and delivery regimens of most substances administered in this Project are not expected to cause persistent adverse effects. In rare exceptions, irritation may occur. Some substances used to modify gene expression may interfere with the ability of pregnant animals to give birth. In such cases, mothers will be humanely terminated, their pups delivered and transferred to foster mothers.
- **Surgical complications**: Surgical and post-surgical complications such as infections are extremely uncommon. Nonetheless, we strive to use refined methods circumventing the need for surgery and expect to perform only a very restricted number of surgeries.

Disease models:

- **Acute inflammation**: will be induced in pregnant animals at different time points of gestation. While inflammation may be accompanied by symptoms of discomfort, these are usually of only transient nature.
- **Rheumatoid arthritis**: is a chronic inflammatory disease characterised by joint swelling. Further symptoms may include weakness, loss of body weight, lameness and a hunched appearance. In many cases however, experiments will be terminated before animals experience these symptoms. We are using models that usually result in a self-limited disease.
- **Tumour growth**: will be induced in some animals and followed for up to several weeks. While tumours can in rare instances be associated with additional clinical symptoms like skin necrosis, hypothermia, weakness, diarrhoea and body weight loss, we will normally terminate experiments before.

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)?

Sub-threshold: The vast majority of all mice (more than 85%) is not expected to experience any pain, suffering, distress or harm that are more than transient or mild.
Mild: A minority of animals (approx. 3.5%) will suffer mild harm.
Moderate: In a further minority of all animals (approx. 8%), we expect to observe moderate severity, usually associated with experimentally induced inflammation, arthritis or tumours.
Severe: We expect only a small fraction (no more than 1%) of all animals to experience severe symptoms, likely associated with advanced rheumatoid arthritis.

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 03 March 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 studies complex interactions between immune cells and their environment in both physiological and pathological conditions like pregnancy, tumours and inflamed joints. We aim for a mechanistic understanding of these interactions, their cause-consequence

relationships and long-term effects, such as the impact of early exposure to adverse environments on health and disease later in life. Even refined in vitro or ex vivo studies cannot replicate the complexity of the cell-cell communication underlying these processes, or their long-term consequences in such intricate biological systems. Therefore, the need to study these biological responses using in vivo models remains.

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

We have considered the use of cell lines, primary cell culture (i.e. ex vivo culture of cells derived from human or animal tissues), more complex co-culture systems, in which for example immune cells and non-immune cells are cultured together, as well as organ explant cultures and organoids (i.e. "miniorgans" grown in culture that contain multiple cell types and may model some organ functionality).

Why were they not suitable?

Neither relatively simple approaches like cell lines and ex vivo culture of primary cells, nor more sophisticated ones like organ explants and organoids can fully replicate the complexity of the cell-cell communication underlying immune functions in pregnancy and embryonic development, tumour growth and chronic inflammatory disease. There are two main reasons for that: One, these interactions are not simply binary e.g. between one specific immune and one epithelial cell type. Second, they occur over an extended period of time, e.g. spanning all of gestation, and have long-term consequences on multiple organ systems. Co-culture systems for example are limited to binary or a restricted number of cell-cell interactions, and even though organoids can be cultured for extended periods and contain multiple organ-specific cell types, these are of epithelial nature in the classical models, and immune cells have to be added exogenously in a co-culture manner. Therefore, these non-animal alternatives are not suitable for addressing our scientific questions. However, they may complement in vivo studies and represent promising reductionist models in which to address e.g. the involvement of a specific inflammatory mediator produced by immune cells at the maternal-foetal interface. Moreover, animal experiments will be complemented with in silico approaches wherever possible. Gene expression analyses will maximally exploit published datasets and publicly available databases of relevant immune cell types, tissues and developmental stages, e.g. the ImmGen consortium or recently published single cell atlases for mouse gastrulation and organogenesis, as well as comparable resources for human data.

A retrospective assessment of replacement will be due by 03 March 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 number of animals we estimate to be used throughout this project is based on statistical and experimental design considerations as well as project-specific ones:
Our target is the smallest number per experimental group that still has the capacity to generated statistically powerful data. For the sake of this estimation, this number is based on experience, but we will initially perform a formal sample size analysis and pilot experiments wherever this has not previously been done.

This project generates and uses genetically modified animals. In many breeding schemes, not all offspring animals can be used in experiments, because they do not carry the right genetic modification. These animals are, however, accounted for in the total estimation.

This also applies to experiments in which pregnant animals are undergoing experimental procedures. In some cases, our scientific interest is in maternal processes rather than the offspring. However, our estimation does take into account any offspring that is produced.

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

For many of the experiments in this project, we or our collaborators have previously established treatment regimens and in some instances have obtained preliminary data that have guided experimental design. In many other cases, the scientific literature has been consulted, in which similar experimental approaches have been reported. For further refinement, we will be closely working with the local veterinary services team and routinely consult the NC3R's website.

An experimental design consideration that is of particular importance for this project is that of the experimental unit. In accordance with its definition as "the smallest division of the experimental material such that any two experimental units can receive different treatments", the experimental unit in experiments addressing the effects of maternal treatments on the offspring is a given pregnancy or an entire offspring litter, and not individual offspring animals. To reduce the overall use of experimental animals with this aspect in mind, we will therefore use the offspring of a given litter for different readouts wherever possible. Conversely, for a given experimental readout, we aim to use individual mice from different litters.

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

Efficient breeding: In many of our experiments, we will use genetically modified animals. Wherever possible will we follow breeding strategies in which most if not all offspring animals can be used for experimental purposes, thereby reducing production of surplus animals.
For most experimental procedures, treatment regimens have previously been optimised by ourselves, our collaborators or published in the literature. As a starting point, we will closely adhere to these protocols.

We will also share tissues from experimental animals wherever possible, either for different projects within the group or with other researchers interested in and authorised for studying immune development.

A retrospective assessment of reduction will be due by 03 March 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.

This project uses in vivo mouse models of embryonic development, pregnancy and adult disease. Specifically, we will study pregnancy in normal and conditions challenged by inflammation, chronic inflammatory disease that resembles rheumatoid arthritis in humans as well as tumour development. Although clinical symptoms cannot be avoided altogether, the precise models and methods we will use are designed to minimise pain, suffering, distress and lasting harm to the treated animals.

Substance delivery, prenatal inflammation: For the vast majority of substances that we will deliver, treatment regimens (i.e. dosage, timing and duration, delivery route) have previously been optimised by ourselves and our collaborators, or published in the scientific literature. Where there are several options available we will always opt for the less invasive or more refined method. For example, where substances shall be administered orally and in a non-punctual manner, we will do so e.g. via the food or drinking water. Where substances shall be delivered directly to embryos during pregnancy, we will opt for an ultrasound-guided rather than the classical surgical technique. Treatment regimens will also be tailored to the specific scientific question. For example, we will elicit inflammation during pregnancy, either to study the involvement of foetal and maternal immune cells in preventing preterm birth, which requires stronger inflammation, or in order to study its long-term consequences on offspring immune development and disease susceptibility, for which lower-grade inflammation will be induced. Finally, wherever possible, substances will be provided in sterile solutions.

Immune development: We and others have previously established refined models that allow pinpointing the developmental origins and kinetics of immune cells across the lifespan. For example, instead of using whole-body irradiation, which is known to induce systemic inflammation and often tissue damage or even radiation sickness, we will use refined models in which cells are transferred into unconditioned mice or animals that have undergone only partial irradiation, leaving the rest of the body protected.

Arthritis models: We will use several well-established arthritis models in pregnant and non-pregnant adult mice. These models have distinct advantages for our questions. For example, arthritis induced by administration of serum from already arthritic mice or antibodies generally causes rapid, self-sustained disease are ideally suited to model maternal arthritis with minimal suffering for the pregnant animals. Arthritis induced by immunisation with collagen, on the other hand, represents a valuable model to test if animals that have prenatally been exposed to adversity are more likely to develop arthritis,

since the mice we use are normally rather resistant to this model. In all instances we will minimise suffering by environmental enrichment e.g. with soft bedding, which maximises comfort for arthritic animals.

Tumour development: We will use a model in which tumour cells are engrafted into unconditioned, immunocompetent mice. This significantly reduces suffering associated with many other tumour models. The humane endpoints for this model are well defined and take into consideration tumour growth and other clinical symptoms such as substantial weight loss and general weakness, however, these are rarely observed in this particular model.

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

Our aim is to establish a mechanistic understanding of how immune cells contribute to embryonic development, pregnancy, arthritis and tumour development and establish cause-consequence relationships between e.g. deleting a particular cell type and an observed biological effect. We therefore depend on genetic tools, for which mice remain the species of choice. Moreover, stages of immune development are relatively conserved between mammals, and much of our knowledge has been established in mice. They thus represent the best model organism for our scientific questions.

Owing to our interest in immune development, we will study animals at an immature life stage (e.g. embryos or newborns) in many of our experiments. However, we also study long-term consequences of experimental treatments, such as the effects of prenatal exposure to adversity on offspring immunity or disease susceptibility. In these cases, we therefore cannot terminate the experiment at such early stages. Moreover, where we study rheumatoid disease or tumour development, we are often required to monitor disease longitudinally. However, we will always use the earliest possible experimental time point that produces meaningful scientific data, and have implemented well-defined humane endpoints as well as refinements.

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

The following measures will be applied throughout the project:

Monitoring: In general, all animals will be regularly observed by trained staff and animal caretakers. Experimental animals will be additionally monitored at intervals and by means tailored to the experimental treatment. Immediately following treatment, e.g. substance administration by injection, animals will be inspected for any signs of distress. Animals that have been subjected to disease models will be monitored and clinically scored e.g. every 1-2 days for arthritic and tumour bearing animals. Wherever possible will we use non-invasive means of monitoring that also enable longitudinal analyses. For example, camera monitoring and ultrasound imaging will be used to monitor preterm birth or other pregnancy complications. Ultrasound or e.g. bioluminescent imaging approaches may also be used to follow tumour growth.

Surgery and post-operative care: Although post-surgical infections and death resulting from anaesthesia or surgical complications are very uncommon, in the few cases where surgery is performed, great attention will be paid to post-operative care: Anaesthetics will be used at correct doses in accordance with local and HO guidelines, and body temperature will be maintained post surgery e.g. via heat pads. Pain will be controlled during surgery by general anaesthesia and post surgery by pain killers, unless these

interfere with the scientific purpose. Risk of post-surgical infection will be minimised by good surgical and aseptic techniques. Surgical sites will be monitored for signs of inflammation and infection. Post-surgical infection is unlikely and antibiotic cover may be given under the advice of local veterinary services.

Pain management: In both the arthritis and tumour models, mice will be provided pain relief at defined clinical scores, unless these interfere with the scientific question, e.g. where the inflammatory response shall be studied, but is impaired by administration of non-steroidal anti-inflammatory drugs.

Animal handling, environment: Mice will be handled gently and via tunnel handling at all times, and we will also provide an environment enriched e.g. with nesting material. Tumour-bearing and arthritic as well as pregnant animals will be handled with particular care and may be provided additionally e.g. with soft bedding to maximise comfort. Where experimental regimens require repeated treatments, animals may be trained to the procedures prior to initiating the experiment to minimise stress.

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

In addition to the aforementioned resources (webpages of local veterinary services, NC3R), which include ARRIVE and PREPARE guidelines, we will specifically follow published guidelines for the welfare and use of animals in cancer research (*Workman et al. British Journal of Cancer 2010, DOI: 10.1038/sj.bjc.6605642*), as well as refinement in arthritis research (*Hawkins et al. Inflammopharmacol 2015, DOI 10.1007/s10787-015-0241-4*), as well as their updates and similar guidelines published in the meantime.

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

We will regularly consult with local veterinary services and stay informed via the NC3R website as well as other resources concerned with animal welfare in biomedical research). We will also regularly participate in HO "road show" events and those organised by our local AWERB committee.

A retrospective assessment of refinement will be due by 03 March 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?

10. Ecotoxicity Studies with 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

Endocrine, Disruptor, Xenopus, XETA, Chemicals

Animal types	Life stages
Xenopus	adult, pregnant, neonate, embryo

Retrospective assessment

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

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Contains severe procedures

Objectives and benefits

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

What's the aim of this project?

The overall aim of the project is to assess the interactions of a range of chemical types (industrial chemicals, agrochemicals, pharmaceuticals, biocides and microbial pesticides) with the endocrine systems of Xenopus tadpoles, Eleutheroembryos or transgenic Eleutheroembryos, such that the hazardous properties of these substances with respect to their ecotoxicological properties can be assessed. These properties are a fundamental requirement of the risk assessment process for such substances.

A retrospective assessment of these aims will be due by 21 January 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 it's aims and if not, why not?

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

Why is it important to undertake this work?

The data obtained in these tests are submitted to regulatory authorities to inform decision-making processes and, where appropriate, satisfy the governmental regulatory requirements that are necessary to gain product registration or to assess the risk and impact posed to the natural environment or human health by the use of chemicals, agrochemicals or pharmaceuticals. Ecotoxicology studies in general are designed to assess the likely impact on populations of plants and animals, and to identify ecologically benign concentrations.

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

The assessment of the risk posed by new and existing chemicals and waste materials to the natural environment continues to be an important international issue for industry, governments and the public alike. Synthetic chemical substances will inevitably enter the natural environment as a result of their use and disposal in industrial and domestic environments. Ecotoxicology studies are designed to assess their likely impact on natural populations of plants and animals, and to identify ecologically benign concentrations. The main aim of the studies conducted within this project is to identify substances that may interfere with the normal function of the hypothalamic-pituitary-thyroid (HPT) axis in amphibians, specifically *Xenopus laevis*.

This is an important assay as amphibian metamorphosis is a thyroid dependent process, which is well studied and is the only assay that detects thyroid activity in an animal undergoing morphological development.

The studies are designed to evaluate a number of endpoints, including survival, developmental stage and associated morphological features, body wet weight and thyroid histology.

The use of Xenopus Eleutheroembryonic Thyroid Assay (XETA) will allow detection of potential modulations of thyroid activity induced by a range of test chemicals.

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

There are many concerns that environmental levels of specific chemicals interacting with the endocrine system (the oestrogen, androgen or thyroid hormone) may cause adverse effects in both humans and wildlife populations and have wider implications for both human and ecosystem health. Aquatic organisms have been identified as the most convincing examples of evidence for the impact that potential endocrine disrupting chemicals can have on animal health. These concerns have led to the revision and development of guidelines in order to facilitate screening and testing of these potential endocrine disruptors.

The data obtained in these tests are submitted to regulatory authorities to inform decision-making processes and, where appropriate, satisfy the governmental regulatory requirements that are necessary to gain product registration or to assess the risk and impact posed to the natural environment or human health by the use of chemicals,

agrochemicals or pharmaceuticals. Ecotoxicology studies in general are designed to assess the likely impact on populations of plants and animals, and to identify ecologically benign concentrations.

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

All of the studies conducted in this facility with aquatic species are bound by confidentiality agreements and unless contracted to provide support through the registration process, the testing facility does not normally receive information regarding the progression of a substance through to marketing authorization. It is not possible therefore to identify the number of substances tested in the facility that have gained marketing authorization or product registrations. Success in this programme of work is not only associated with the successful registration of a substance but also the refinement of risk assessments, and the derivation of risk-reduction and toxicity mitigation strategies. Another important role of studies in this programme is the identification of substances with unacceptable effects or safety margins.

Species and numbers of animals expected to be used

- Xenopus: 50 (Adults), 13500 (tadpoles), 36,000(eleutheroembryos)

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 *Xenopus laevis* are required in order to produce eggs for the acute and definitive tests for the 21 day metamorphosis assay.

For this assay to be assessed correctly it is essential that a life stage is used that is going through the process of metamorphosis so that developmental end points can be assessed. Amphibian metamorphosis is a thyroid dependent process and is the only assay that detects thyroid activity in an animal undergoing morphological development.
Adult Xenopus laevis may also be used in order to produce eggs/ Eleutheroembryos for the XETA range-finding assay. Eleutheroembryos or transgenic Eleutheroembryos at stage NF45 are used for the XETA range-finder or definitive test respectively as this assay requires embryos at a specific developmental stage in order for this assay to work successfully. The definitive test requires the use of transgenic Eleutheroembryos as the fluorescence of each organism needs to be quantified at the end of the test.

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

21 day metamorphosis assay:
In order to conduct the aquatic toxicity tests using tadpoles at a specific morphological stage (Stage 51), the production of eggs from breeding adult *Xenopus laevis* held in the laboratory must be induced. All tadpoles exposed in the metamorphosis study must originate from the same spawning event, and therefore sufficient numbers of eggs are required. Approximately 12 hours before the required egg collection, injections of hCG (human Chorionic Gonadotrophin) are given, and the breeding pairs are then allowed to perform natural mating and egg laying procedures.

In order to conduct the assay Animals will be transferred from holding tanks to test vessels, transfer to fresh test media (where applicable) in all animals.
The developmental stage of the animals is determined using a binocular dissection microscope to ensure that stage 51 tadpoles are utilized during the test. A selection of animals (approximately 20) may also be measured and a mean whole body length for this group of animals determined, minimum and maximum limits for the whole body length of experimental animals can be set by allowing a range of the mean value ± 3 mm.
There is a minimum of three test concentrations and a clean water control (and vehicle control if necessary). Separate groups (typically 80 tadpoles over 4 replicates per concentration) of stage 51 Tadpoles will be exposed concurrently to varying concentrations of the test item for a period of up to 21 days under semi-static or continuous flow test conditions.

Animals are observed at least daily. Where signs of toxicity are seen, the frequency of observations will be increased. At each observation time, animals that are considered likely to die or are showing symptoms of exposure that represent a significant departure from the animal's normal state of health or well-being will be identified and humanely killed by an appropriate non-schedule 1 method at the designated establishment. The tadpoles will be given an overdose of anaesthetic (eg MS222) for a period of at least 2 hours. The absence of response to a physical stimulus will be confirmed followed by permanent removal from the aqueous environment prior to immersing them in a suitable fixative (Davidsons solution or a suitable alternative). All humane kills are recorded.
Food will provided throughout the test, the type and amount of feeding will be dependent on the life stage of the larvae, whilst minimising the surplus that will be removed throughout the study.

A selection of tadpoles will be humanely killed and sampled once during the study, this will be conducted (e.g. on Day 7) by a non-schedule 1 method at the designated establishment. The tadpoles will be given an overdose of anaesthetic (e.g. MS222) for a period of at least 2 hours. The absence of response to a physical stimulus will be confirmed followed by permanent removal from the aqueous environment prior to immersing them in a suitable fixative (Davidsons solution or a suitable alternative).
At the end of the exposure period (e.g. Day 21) surviving tadpoles will be humanely killed by a nonschedule 1 method at the designated establishment. The tadpoles will be given an overdose of anaesthetic (eg MS222) for a period of at least 2 hours. The absence of response to a physical stimulus will be confirmed followed by permanent removal from the aqueous environment prior to immersing them in a suitable fixative (Davidsons solution or a suitable alternative) and all appropriate end-points determined.

Xenopus Eleutheroembryonic Thyroid Assay (XETA):
In order to conduct the assay Eleutheroembryos will be transferred from holding tanks to test vessels and transferred to fresh test media (where applicable).
The developmental stage of the Eleutheroembryos is determined using a binocular dissection microscope to ensure that stage 45 Eleutheroembryos are utilized during the test.

Eleutheroembryos between developmental stages NF45 (beginning of the test) and NF47 (end of the test) are used for this test. They are not fed before or during the test as yolk is still present in the intestine from stage NF45 to stage NF47 and is used as the source of energy for the development of the eleutheroembryo (Nieuwkoop and Faber, 1994).
There is a minimum of three test concentrations (+/- triiodothyronine (T3) 3.25 µg/L)) plus relevant control groups (Test media, Triiodothyronine (T3) and Thyroxine (T4)) (and vehicle control if necessary). Separate groups (typically 20 Eleutheroembryos over 2

replicates per concentration) of stage 45 Eleutheroembryos will be exposed concurrently to varying concentrations of the test item for a period of approximately 72 hours under semi-static or continuous flow test conditions. Three runs will be conducted per test. Eleutheroembryos are observed at least daily. Where signs of toxicity are seen, the frequency of observations will be increased. At each observation time, Eleutheroembryos that are considered likely to die or are showing symptoms of exposure that represent a significant departure from the normal state of health or well-being will be identified and humanely killed by an appropriate non-schedule 1 method at the designated establishment. When both the acute and XETA studies have been completed, all remaining Eleutheroembryos are humanely killed by a non-schedule 1 method at the designated establishment. The Eleutheroembryos will be given an overdose of anaesthetic (eg MS222) for a period of up to 45 minutes (as specified in the test guideline) followed by permanent removal from the aqueous environment. All Eleutheroembryos will then be frozen (-18˚C) prior to destruction by incineration. (AC).
The fluorescence of each organism is quantified after 72 ± 2 h of exposure.

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

Transfer of animals from holding tanks and transfer to fresh test media (where applicable) will cause mild stress in all animals. The discomfort will be transient in the majority of animals (>90%) and no additional action will be required.

It is anticipated that only transient minor discomfort should occur in the adult animals during injection procedures. Reactions to the gonadotrophin injected are not expected. Due to the inherent nature of LC_{50} testing deaths and adverse clinical signs (e.g. abnormal swimming behaviour, loss of orientation, lack of surfacing activity, muscular spasms or lethargy) will be noted in the tadpoles especially at higher test concentrations, no adverse effect are to be expected at the lower levels. It can be assumed that cumulative signs of toxicity may be seen as the study progresses.

It is not the intention of the definitive test protocols to cause mortality or significant signs of toxicity. However, typically, the highest concentration tested in the metamorphosis assay will be near the maximum tolerated test concentration, or may be approximately one-third of the LC_{50}, depending on the dose response. As such, at the higher concentrations being tested there may be accumulation of toxicity noted during the exposure period which may result in adverse clinical signs (e.g. abnormal swimming behaviour, loss of orientation, lack of surfacing activity, muscular spasms or lethargy) being apparent (but not to the same degree as in the acute protocol) for a period of time resulting in cumulative toxicity/severity.

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)?

21 day metamorphosis assay:
All adult animals which are subject to gonadotrophin injections will feel mild discomfort at the site of injection for a short period of time.

In the acute toxicity protocol it is anticipated that approximately 50% of the individuals will suffer mortality or adverse effects such as abnormal swimming behaviour, loss of orientation, lack of surfacing activity, muscular spasms or lethargy.

It is anticipated that approximately 15% of the individuals may suffer moderate effects such as abnormal swimming behaviour, loss of orientation, lack of surfacing activity or lethargy. These effects/clinical signs should not be experienced by the tadpoles to the same degree as in the acute toxicity protocol but due to the length of time that they may persist, cumulative severity may result in a moderate classification. Malformations may also be seen on occasion which will be assessed for severity.

Typically, the concentration range in the definitive test will be based on the results of acute toxicity tests or metamorphosis assays, and therefore it is not the intention to cause mortality. It is anticipated that no adverse effect will be observed at the lower levels.
All animals at some point will require moving to fresh test media, this is considered to cause mild stress in all animals. The discomfort will be transient in the majority of animals (>90%) and no additional action will be required.

Xenopus Eleutheroembryonic Thyroid Assay (XETA):
In the acute toxicity protocol it is anticipated that approximately 50% of the individuals will suffer mortality or mild to moderate effects such as abnormal swimming behaviour, loss of orientation or lethargy. Due to the low neurophysiological sensitivity of the life-stage of the tadpoles being used, death is classed as a moderate effect.

Typically, the concentration range will be based on the results of acute toxicity tests and therefore it is not the intention to cause mortality. It is anticipated that no adverse effect will be observed at the lower levels.

All animals at some point will require moving to fresh test media, this is considered to cause mild stress in all animals. The discomfort will be transient in the majority of animals (>90%) and no additional action will be required.

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

- Kept alive
- Killed

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

Currently there are no validated non-animal alternatives to replace the whole animal toxicity tests that are mandatory for the evaluation of new and existing chemicals to assess their safety and efficacy. To assess morphological development due to effects on the thyroid axis, the use of a live animal is a necessity.

Xenopus laevis is proposed as the test organism as it is known to be widely available, obtainable throughout the year and relatively easy to maintain. It is representative of the more sensitive species and a large literature base is available to provide background information.

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

Currently there are no validated non-animal alternatives to replace the whole animal toxicity tests that are mandatory for the evaluation of new and existing chemicals to assess their safety and efficacy.

Why were they not suitable?

Currently there are no validated non-animal alternatives to replace the whole animal toxicity tests that are mandatory for the evaluation of new and existing chemicals to assess their safety and efficacy.

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

Amphibian metamorphosis Assay:
Numbers have been calculated based on running six LC_{50} tests and six definitive tests per year over the five year duration of this project licence.
Xenopus Embryonic Thyroid Assay
Numbers have been calculated based on running twelve LC_{50} tests and twelve definitive tests per year over the five year duration of this project licence.

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

Regulatory guidelines dictate numbers of organisms exposed per concentration and the spacing factor employed for definitive stage testing. Statisticians are consulted to ensure studies are designed to maximise the strength of replication in order to use the minimum number of organisms per vessel.

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

The use of a range finding phase of testing where existing information of the toxicity of a chemical is not available reduces the risk for repetition of the full metamorphosis or Xenopus Embryonic Thyroid Assays using high numbers of tadpoles or eleutheroembryos respectively, as a suitable range will be identified which aims to ensure the study endpoints are achievable.

Whenever possible, the maximum spacing factor will be employed when setting concentration ranges.

The priming and induction procedures utilised during the production and collection of eggs, in preference to relying on natural mating procedures, ensure that the minimum number of adult Xenopus laevis are used (e.g. 3 tanks of 3 pairs per spawning event), in order to achieve the maximum fecundity levels.

It is aimed that approximately 5000 eggs will be produced from the spawning event, from which the tadpoles from the spawn with the highest hatching success are selected and a minimum number (approximately 800) of these individuals are raised to the independently feeding stage.

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

Adult frogs used to produce eggs will suffer mild pain at the site of injection for a very small amount of time. As eggs are required for these protocols there are no other options available other than using adult animals.
Amphibians represent vertebrates and one of the highest trophic levels in the aquatic ecosystem and are of particular importance in the testing scheme because of their developmental life-history and sensitivity to chemical substances, making them important ecological indicators. The species and stage of tadpole development employed are considered to represent the most suitable model to identify effects of exposure in the environment. The objectives and protocols are aimed at assessing the impact of a substance on the survival and development of Xenopus tadpoles, and therefore on the potential for further effects on the ecosystem, using mandatory tests that follow

internationally accepted test designs. Due to the low neurophysiological sensitivity of the life-stage used during XETA, death is classed as a moderate effect and as such this life stage (tadpole) will be the one that suffers the least during this type of testing.

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

As eggs are required for these protocols there are no other options available other than using adult animals.

As the objectives are aimed at assessing the impact of a substance on the survival and development of Xenopus tadpoles, it is essential that living animals are used such that the development of these animals can be assessed. These animals are considered to be the least sentient species at this life stage used in this project licence where a meaningful assessment can be made on their development. The life stages that are required to be used are specified in the relevant OECD test guidelines.

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

Any refinements identified during the conduct of tests under this licence, as experience with these species is gained, will be assessed and put into common practice where possible. Methods for handling and identification have been designed within the field of amphibian laboratory use in order to minimise the chance of causing stress and pain in the adult Xenopus.

Appropriate handling of the adult organisms will ensure restraint procedures are effective for injection purposes and therefore ensure time taken for completion of injection procedures is minimised.

When necessary, any identification of the adult stock animals will take place using non-invasive methods e.g. photographic records in preference to toe clipping, tagging or microchip implant. Tank labels will be used to identify groups of animals.
The minimisation of excessive stress and rapid changes to environmental conditions, especially during movement, cleaning of vessels and manipulation of larvae, has been identified as a key factor in the optimisation of using Xenopus laevis. Husbandry and exposure conditions, and the associated techniques, will be well defined to ensure noise, vibration and activity levels within the laboratory are kept to a minimum. Environmental conditions e.g. temperature, pH, dissolved oxygen, light levels and water flow rates will be controlled and maintained.

Regulatory systems that require specific test requirements may allow little discretion in the species used. However, the most appropriate species, particularly in terms of species sensitivity and availability of background data, as well as the species having the lowest neurophysiological sensitivity, are chosen. Thus the most appropriate species is chosen on scientific grounds rather than custom and practice.

All scientific procedures using animals are performed in accordance with UK Good Laboratory Practice regulations. Standard Operating Procedures define animal welfare practices and experimental procedures. Licencees are fully trained and competent in the appropriate procedures and are familiar with the signs of pain, discomfort or distress in the species with which they are working. Training records are maintained to document training levels, retraining and competence. Staff are encouraged to identify and encourage

improved methods particularly with regard to procedural methods, housing and handling of animals.

Animal tests are designed and conducted in every case so that any actual or potential pain, discomfort, or distress to the animals is minimised or alleviated by choosing the earliest endpoint that is consistent with the scientific objectives. The term “endpoint” is defined as the point at which an experimental animal’s pain and/or distress is terminated, minimised or reduced, by taking actions such as killing the animal humanely or giving treatment to relieve pain and/or distress.

The ultimate purpose of the application of humane endpoints to ecotoxicology studies is to be able to accurately predict severe pain, severe distress, suffering, or impending death, before the animal experiences these effects. However, the science of ecotoxicology is not yet at the point where such accurate predictions can be made prior to the onset of severe pain and distress. It is possible at this time to identify pain, distress and suffering, very early after their onset by careful observations of animals on test. This test facility is fully committed to the implementation of the recognition, assessment and use of clinical signs as humane endpoints for test animals.

Before starting a safety evaluation study, consideration is given to relevant background data or information supplied with the test material, together with databases for similar chemicals or substances or previous experience with other sponsor products. Thus, the type and severity of the abnormal signs of toxicity, particularly in terms of time of occurrence in relation to time of dosing may be anticipated. Technical and animal welfare staff will be alerted to what signs to look for.

Studies likely to cause significant acute adverse effects are scheduled to start as early in the working day as possible. This ensures that the critical period for the animals occurs during normal working hours when the frequency of observations can be maximised or increased depending on the potential for increasing pain or distress.

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

The proposed work will be conducted according to standardised test guidelines. These guidelines incorporate the scientific rationale for national regulatory requirements and are peer reviewed by scientific and industry experts to reflect current scientific knowledge and ethical standards in animal experimentation. Completed studies are peer reviewed by Competent Authorities, the company Registration Department and Sponsor representatives, and the comments received from post study peer reviews are used to further refine subsequent testing. Animal studies are not initiated until written confirmation is received from the sponsoring Company that they are needed to satisfy a regulatory authority and that funding to cover the full cost of the studies will be provided. The company Project Management Department (PMD) co-ordinates major notification projects and liaises with the Study Directors in charge of each study to ensure that unavoidable animal studies do not commence until the results of related non-animal studies are known.

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

All staff involved in animal testing at maintain an up to date outlook on animal welfare considerations via the attendance of relevant meetings, review of publications in journals and other sources, and face to-face meetings with other individuals working in the area of

ecotoxicology or aquatic husbandry. This ongoing knowledge building allows for any refinements that are identified to be introduced into standard procedures.

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

11. Efficacy evaluation of products to support the health and welfare of farmed fish

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 animalsreared 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

aquaculture, product development, vaccine efficacy, therapeutic efficacy, feed additives

Animal types	Life stages
Atlantic salmon (Salmo salar)	juvenile, neonate, embryo

Retrospective assessment

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

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Contains severe procedures

Objectives and benefits

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

What's the aim of this project?

This project aims to evaluate the efficacy of products, including veterinary vaccines, therapeutics, feed materials, feed additives, breeding technology, veterinary and other devices and biocides, intended for use in the production of farmed fish. The project will provide a service to commercial clients and research providers seeking to develop

products and technologies for fish. The principal objectives are to provide data on the effectiveness of the products and technology in relation to prevention and treatment of infectious disease and other pathologies, and impacts on performance and welfare.

A retrospective assessment of these aims will be due by 17 March 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?

These studies are necessary to enable assessments of the effectiveness of the products and technologies for use in farmed fish, including regulatory assessment for the purposes of product licensing, to identify the preferred treatment procedure and to understand the modes of action of the products so that the products can be used safely and effectively.

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

The expected benefits of this project will be to:
Establish a platform which clients can access to develop products for aquaculture. A shortage of capacity at present is restricting the availability of new products for farmed fish.

Support the evaluation, development and licensing of new products and technologies to improve the health and welfare and performance of farmed fish. These will be used by fish farmers to reduce losses due to salmon lice and other diseases and to improve production efficiency.

Generate high quality data to ensure that new licensed products are demonstrably effective. These data will be produced to the internationally recognised quality standards required by relevant regulatory authorities.

Support the production of fish as food that is safe, healthy and nutritious, economically sustainable, environmentally acceptable and produced to the highest animal welfare standards.

Tackle biological challenges which threaten the sustainability of an industry which supports jobs and economic activity in remote areas.

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

The benefits will be realised by:

Farmed fish which will benefit from improvements in health and welfare.

Husbandry and veterinary staff who will benefit from access to new tools to maintain and improve the health and welfare of animals in their care.

Aquaculture producers, processing companies and retailers who can expect marketing and price advantages based on reduced losses and more efficient production of fish with higher health and welfare standards.

Supply chain companies who will benefit from opportunities to develop new products and services.

The consumer who will benefit from access to food produced using products which have been developed according to established and assured safety and welfare standards.

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

We will advertise our capabilities and expertise within our target market so that a wide range of clients can take advantage of these.

We will encourage our sponsors to publish study findings, including negative findings, where appropriate.

We will offer experimental models to research groups for testing novel products and technologies. Knowledge of these models will be shared and may be published.

Species and numbers of animals expected to be used

- Other fish: No answer provided

Predicted harms

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

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

The fish species and life stages used in this project are representative of the farmed fish species and life stages for which the products are being developed.
The developers of the products and Licensing Authorities responsible for approval of new products require data from these 'target' species for decision-making and formal regulatory assessment.

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

A test product may be administered to experimental fish, for example by voluntary feeding daily for up to 12 weeks, by immersion or by oral gavage or intra-peritoneal or intra-muscular injection under temporary anaesthesia, at a dose which has previously been shown to be safe.

In studies to determine the efficacy of the product against pathogen challenge, fish may be challenged with a pathogen under controlled conditions. Pathogen challenge may be before or after administration of a test product. Fish will be held in tanks and may be anaesthetised or euthanased and sampled during the study. Samples will typically be collected in order to assess infection status, for example to count the number of attached sea lice at a certain time point or to collect samples of blood and mucus for diagnostic tests.

In fish performance studies, fish will be held in tanks and may be anaesthetised or euthanased and sampled in order to evaluate key performance indicators including growth and feed conversion rate, nutrient retention and physiological function.

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

The expected impacts are those associated with disturbance and handling of animals for dosing and controlled infection with the pathogen of interest. Short-term effects are expected to include increased ventilation rate, skin darkening and inappetance with full recovery within 24 h.

In the case of sea lice challenge and sea lice product efficacy studies, sea lice infestation may cause irritation leading to increased jumping behaviour and inflammation of the skin in approximately 20% of fish which will resolve within a few days. The other 80% of fish are expected to experience no adverse effects.

In microbial pathogen challenge, multifactorial pathogen challenge and microbial pathogen product efficacy studies, experimental infections may lead to the development of clinical signs and occasionally death in the absence of other clinical signs.

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 fish in sea lice challenge and sea lice product efficacy studies will experience Mild severity. Approximately 20% of fish in each study are expected to experience Moderate severity.

All animals used in fish performance studies are expected to experience Mild severity. In fish with chronic disease due to microbial or multifactorial pathogen challenge, no more than 15% (typically less than 10%) of animals will experience severe harm. In fish with acute disease, particularly small fish where clinical signs are difficult to identify and progression is rapid, severe harm (ie direct mortality in the absence of other clinical signs) may occur in up to 50% of the experimental animals.

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 17 March 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?

The project will test the effectiveness of products and technologies administered to the intended target species which will be a species of farmed fish. Due to the complex nature of the interaction between a product and the animal (and pathogen where used) the effectiveness of the products cannot be evaluated adequately using non-animal alternatives.

In studies conducted for regulatory purposes, the use of relevant animal models is a requirement of the regulatory authorities in order to properly assess the product.

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

Computer simulation models. *In vitro* bioassays.

Why were they not suitable?

These non-animal models are either not available or not well-enough developed to provide the required high level of confidence in the results.
In vitro bioassays will be used for initial product screening.

A retrospective assessment of replacement will be due by 17 March 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?

For each type of study, fish numbers are based on the typical study design requirements (based on regulatory guidelines) and on published data and/or past experience of appropriate sample sizes.

The estimated total number of animals is based on expected demand and capacity for approximately 40 studies using standard study designs during the course of this project.

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

The study designs use prior data to estimate the likely magnitude of variation in response due to random effects and the level of treatment effect which is practically valuable. The discrimination of the study design (i.e. its ability to distinguish treatment effects from random variation) is maximised by minimising the effects of random variation by the practice of using fish of similar age, source, size range and history, similar experimental tanks and consistent environmental conditions across each study.

When designing new challenge models, guidance on study design will be sought from a biostatistician. Study designs for individual experiments will be evaluated by a biostatistician as part of the experimental and ethical review procedure.

Group sizes for voluntary feeding studies are restricted by the requirement for fish to show a uniform feeding response. A minimum group size of 20 fish is used in these studies since this is considered to be the minimum necessary to overcome social hierarchy effects and provide an acceptable feeding response in the majority of individuals in the population.

Smaller numbers of fish may be used where dosing is by immersion, oral gavage, injection or topical administration.

In time series studies, for example to determine the duration of efficacy, where a group of fish may be treated with an experimental product and sub-sets challenged and sampled at intervals, numbers of fish reflect the number of sample points and the number of individuals required for sampling at each point. In studies which are required to generate quantitative data, numbers of fish used at each point are those necessary to provide an accurate and precise measure of the magnitude of response. This is determined separately for each study using sample size calculation methodology but is typically in the order of 10-20 fish per time point.

In studies to assess genetic differences in disease susceptibility, 10-20 fish from each family group are typically used. Since breeding populations contain multiple family groups and it is important to evaluate each of these, the studies may be relatively large using 1000-2000 individuals.

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 may be used to determine the magnitude of effect of treatment and thereby the number of animals/samples necessary in pivotal regulatory studies.

A retrospective assessment of reduction will be due by 17 March 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.

The sea lice challenge procedure uses the number of fish necessary to support the required numbers of parasites without over-infestation of fish and without the use of too many fish. Individual animals may be used for a series of challenges when the harm caused by the additional challenge is less than the harm caused by acclimatising, challenging and habituating new fish to attachment of the parasites. The experimental challenge procedure is well established and refined so that settlement rates are predictable.

A sea lice product efficacy test aims to minimise random variation (noise) in sea lice data so that it is possible to detect effects due to the test product, statistically, with use of the minimum number of fish.

A fish performance study aims to evaluate the performance of fish receiving the product under practical conditions of use. High welfare standards are necessary to achieve commercially realistic growth and feed conversion rates and experimental procedures are minimal in order to limit any risk to welfare and reduction in performance.

The microbial pathogen challenge and multifactorial pathogen challenge involves (1) manipulation of the pathogen(s), host and environment in order to understand key factors which influence the outcome of the experimental disease model and thus minimize numbers of animals required, and (2) assessment of early clinical signs, in order to minimize pain, suffering and distress in individual animals.

A microbial pathogen product efficacy test uses these refined models to assess the efficacy of products including vaccines, therapeutics, and zootechnical feed additives.

Products used in these studies will be used at dose rates or inclusion levels which have been shown elsewhere to be within the safe range.

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

The species and life stages used are those for which the products are being developed. Reliable data is important to ensure the safety of farmed fish and the consumer. Less sentient animals have not been shown to provide data which can be reliably extrapolated to the target animals.

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

Frequent monitoring for adverse effects using established criteria as described in the relevant protocols.

Refinement of the procedure used for identifying, describing and recording clinical signs, for example identification and specific focus on relevant new operational welfare indicators identified in future publications, increased use of video monitoring to avoid disturbing fish and refinement of terminology used to describe clinical signs.

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

NC3Rs PREPARE and ARRIVE guidelines
Festing, M.F.W., Overend, P., Borja, M.C. and Berdoy, M. (2016). The design of animal experiments. 2nd Edition. Laboratory Animals Handbook No. 14.
Noble, C., Gismervik, K., Iversen, M. H., Kolarevic, J., Nilsson, J., Stien, L. H. & Turnbull, J. F. (Eds.) (2018). Welfare Indicators for farmed Atlantic salmon: tools for assessing fish welfare. 351 pp.

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

NC3Rs Newsletter and Website.
Norecopa fish as research animals website
Local 3Rs Group.
Relevant training courses.
Communication with sponsors and colleagues working in the field.

A retrospective assessment of refinement will be due by 17 March 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?

12. Embryogenesis, stem cells and cell fate decisions

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

No answer provided

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.

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 cells choose and maintain specific fates during development of the mammalian embryo and in the adult animal, how this leads to disease when the processes go wrong.

A retrospective assessment of these aims will be due by 22 January 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 it's aims and if not, why not?

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

During the development of an animal, cells have to undergo decisions of cell fate, choosing which path to follow. Similar decisions are made throughout life by stem cells and progenitors in many tissues. These decisions rely on intrinsic factors, such as transcription factors, and extrinsic signals, which together establish gene regulatory networks that define specific cell states. These have to be coordinated in time and space to generate functional tissues, organs and the animal, where little in the latter is static: cells are constantly having to be replaced due to normal wear and tear and to cope with changing physiological states, trauma, and disease. The main purpose of the work to be conducted under this Project Licence is to provide fundamental knowledge on cell fate decisions in specific biological systems, notably the gonads, CNS, pituitary, gut, and sensory systems, which is relevant to how these develop, mature, and age normally. However, there are many situations where decisions of cell fate are aberrant or discordant. This can lead to infertility or embryo failure, to congenital disorders, disorders affecting maturation, health span or aging, or to cancer. There can also be aberrant responses to environmental factors. Improved understanding of underlying mechanisms can lead to improved diagnosis and/or novel forms of treatment.

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

Improved understanding of gene regulatory networks in the early gonad will not only inform clinical cases of Disorders of Sex Differentiation (DSDs), but be of broad significance in studies of organ formation.

Improved understanding of gene regulation during gonadal development will also inform clinical cases of Disorders of Sex Differentiation (DSDs), as it is become apparent that many are due to regulatory mutations. We also anticipate that this work will provide general insights into both temporal and spatial control of gene activity, especially for genes such as *Sox9* which are active and have critical roles in many tissues.
Improved understanding of gonadal sex maintenance and reversal will lead to new insights into cellfate reprogramming and organogenesis, both of which could be of potential importance for regenerative medicine. More directly, this could be of clinical benefit for some cases of DSDs, giving new options for treating patients, such as in cases where ovotestes are present it may be possible to turn the whole gonad into a testis or ovary, or to convert the entire gonad, either to match chromosomal sex or, perhaps, gender identity.
Improved understanding of female reproductive function, fertility and premature ovarian failure, could help inform new strategies to manage or maintain fertility in women.
Developing methods to obtain gonadogenesis and gametogenesis in vitro, could provide information relevant to DSDs and to causes and potential treatments for infertility. It is very difficult to study the etiology of DSDs given that the phenotypes develop in the embryo in utero, and they are generally recognised at birth or, often, at puberty. The only alternative at present is to make and study an animal model, which, while useful, may not always accurately reflect the human situation. (N.B. Our work deriving Sertoli-like cells in vitro from pluripotent stem cells has already contributed to one study (yet to be published), where patient derived cells were unable to give rise to Sertoli cells, unlike controls, showing that the defect was in primary sex determination). Current efforts to derive sperm or eggs in vitro from pluripotent stem cells reveal that co-culture with somatic cell types from the gonad is essential for primordial germ cell-like cells to progress into later stages of spermatogenesis or oogenesis. Our culture systems, if validated in animal studies, will help these endeavours, which are important both to allow detailed study of human germ cell development and, in the long term, to provide ways to treat infertility or even as a route to correcting deleterious gene variants in subsequent generations.
The use of animal models to provide improved understanding of the mechanisms underlying sex bias in human diseases will be of clinical benefit in terms of improved

diagnosis and perhaps options for treatments. Our current work on Hirschsprung's Disease provides an example, where two mechanisms that could contribute to the distinct sex bias have been identified. Work on sex differences in the biology of neural stem cells is of potential relevance to understanding and perhaps eventually treatment of a range of CNS disorders that affect one sex more than the other, such as depression.

In addition to providing novel fundamental insights, our studies on intrinsic versus extrinsic control of CNS development and disease could be of benefit in providing new options for avoiding or treating diseases that have previously thought to have their origins within the CNS.

Our work on the role of SoxB1 and SoxE genes in development, from the early embryo to neural stem cells in the adult, has already led both to new fundamental knowledge and to clinically relevant findings. These range from Sox2 being recognised as a gene essential for pluripotency and hence to the development of iPS cells, to studies on the decline of neural stem cell populations in ageing. We expect our further studies on these genes to continue to give new knowledge.

Improved understanding of neural stem cell niches will contribute to basic knowledge, but also potentially to the development of new clinical options for treatments of CNS defects and trauma. Neural stem cells have already been used in attempts to treat a range of diseases as well as conditions such as stroke, but with limited effectiveness. Providing other niche components, whether factors produced by cells or the cells themselves, may be beneficial.

Gliomas and glioblastomas have proved to be very hard to treat. Our work on this topic is designed to reveal more about the origins of these aggressive tumours, and the knowledge gained will potentially provide new ideas for treatment options.
Our work on the pituitary and hypothalamus, and stem and progenitor cells in both, has already led to new insights. Ultimately, we hope this work will lead to better, more physiological options to treat a range of clinical disorders that involve deficiencies in pituitary hormones and/or in hypothalamic function. These can be congenital or occur after trauma, disease (including cancer), or may be due to current treatment regimes.
Pituitary tumours are relatively common, but not well understood. Our work may provide new treatment options, especially as surgery on the organ, which is centrally located below the brain and highly vascularised, is often very challenging. For example, via the use of drugs to disrupt specific cell signalling molecules that our current work suggests are required for tumour growth or that might reduce *Sox2* expression, which is also associated with tumour growth.

Our work on the development and function of endocrine organs and sensory systems is mostly to provide fundamental knowledge of the role of specific genes. However, this knowledge will be relevant to diagnosis and management of patients and may eventually provide new treatment options.

We will publish all of our findings in open access journals, with data in a reusable form. Moreover, any genetically altered mice produced as part of this PPL, which should be of benefit to other researchers, will be made freely available, as we have done so in the past.

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

There are likely to be multiple beneficiaries from the outputs above. These will first include other scientists and clinicians involved in similar studies or who are interested in the

systems we are exploring. Benefits to patients may come within a few years, perhaps some within the time frame of this PPL, from improved diagnosis and from better management of disorders or trauma. In the longer term, perhaps in 5 to 15 years, we would expect our work to lead to new treatment options.

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

Alongside the publication of primary research papers in open access journals, we will present our work at meetings, ranging from small focussed workshops to large international conferences. Critically, we will communicate negative results and approaches as well as those that are positive.

We collaborate widely, both internally within the host establishment, and externally with scientists and clinicians based in the UK and in several other countries (including currently France, Spain, Israel, the USA and Canada, and Australia). We share details of approaches and data generated with our collaborators, which allows for improved development of methods and better synthesis of findings.
Where we develop new transformative methods, we will publish the protocols as papers and post them on bioRxiv.

Species and numbers of animals expected to be used

- Mice: 95,700

Predicted harms

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

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

We will use mice for all the projects covered in this PPL. Mice are chosen because of the powerful techniques and knowledge available for this species in terms of genetics, cell biology, embryology, physiology, reproductive biology, and behaviour, but also as they have relevance to the human situation.

Evolutionary comparisons can be very informative. A separate PPL will cover our work with the chick. In addition, our mouse work will include a small number of chimeric animals that will be generated by admixing mouse embryos with cells (such as embryonic stem cells) from another mouse or small mammal, such as the rat. We will use this strategy to replace the elements of, for example, the developing brain and spinal cord so we can observe some aspects of how these systems develop, and how much is determined by the cells of the system and how much by the surrounding tissues. This approach can be used to study aspects of physiology or disease that affect other mammals where it would be difficult to conduct the experiments in these mammals. This could include humans, although this is not part of our current programme of work.

With respect to life stages, our work ranges from preimplantation embryos all the way to ageing adults. This range reflects, in part, the focus of the lab on certain genes, for example *Sox2*, that function in cell fate decisions throughout many or all these stages, but also the importance of understanding the changes that take place during organ development and maturation and altered physiological circumstances, including ageing.

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

Because we often use genetic approaches, much of our work involves breeding, including GA animals, and harvesting embryos or tissues from postnatal animals after they have been killed (using a schedule 1 procedure or fixation/perfusion) for detailed analysis of phenotypes. We will use hormone injections to control aspects of reproduction, e.g. superovulation when making genetically altered animals. Quite often we make use of substances, such as tamoxifen, to induce a genetic alteration, e.g. a conditional loss- or gain-of-function of a specific gene, as well as to follow cell fates or isolate specific cell types (e.g. by activation of a fluorescent reporter gene).

For a smaller number of animals, we will use some surgical techniques, always employing appropriate anaesthesia and analgesia, and in consultation with the NVS. These include methods associated with the production of genetically altered mice, including chimeras, and studies on reproductive biology, such as embryo transfer, vasectomy, ovariectomy and orchidectomy. Some of these methods are also used for routine maintenance or preservation, as frozen embryos or sperm, of specific genetically altered mouse lines. We also use surgery in a small number of animals to explore the consequences of removing target organs (such as the adrenal gland) of the pituitary on the hypothalamic-pituitary axis.

Our work has shown that this leads to activation and differentiation of stem cells within the pituitary. Occasionally more than one organ will be removed (e.g. adrenals and testes) for the same reason, and to explore if the effects are additive or affect distinct cell populations. As part of this PPL, due to having derived a new genetically altered mouse line that permits cell fate mapping without the use of tamoxifen (which disturbs reproductive function and the hypothalamic-pituitary axis) we will investigate the effects of changing physiology, such as puberty, pregnancy, lactation, on the pituitary stem cells. This will not necessarily involve surgery, but may involve additional injection of relevant substances including into pregnant mice, where the main object is to study the mothers rather than the embryos.

We will also use substances, such as EdU or BrdU, to look at cell proliferation in tissues after harvesting. These substances are introduced by injection or gavage, into pregnant females or live-born animals, and may be carried out multiple times over a few days, followed by a variable period prior to the animal being killed and the tissues analysed. This can be to follow cell fates over this period, or to examine the consequences of an induced mutation at different life stages, or to carry out specific assays, such as a ‘label-retaining’ assay, often used to identify quiescent stem cells.

Some projects, involving few animals, make use of conditional or inducible genetic systems or drugs to deliberately kill specific cell types. This can be to study the consequences of their loss on the organ system under study, such as the pituitary or stem cell niches in the brain. And a new project will explore the effects of radiation or anti-mitotic chemicals on the hypothalamic-pituitary axis, which is known to be compromised after radiotherapy or chemotherapy. Most of these experiments are short term, lasting days to a few weeks, before the consequences are analysed. Alternatively, these methods may be for studies using blastocyst complementation, when specific cells, such as neurectoderm progenitors, are deleted in the developing host embryo, but replaced by cells differentiating from pluripotent stem cells introduced at blastocyst stages. In this case, the effects are assessed in a stepwise manner, looking at embryos less than two thirds through gestation, then at embryos shortly before term, before allowing any chimeras to be born. If these are

viable, the animals will be kept longer for subsequent phenotype analysis, but with careful monitoring.

For some projects, but again involving small numbers of mice, we need to introduce substances and/or cells into the brain, which will also involve surgical procedures, including implantation of canulae and osmotic minipumps, injection needles, and, electrophysiology. Substances and/or cells may also be introduced into the developing brains of embryos in utero, which also requires procedures to be performed on the pregnant female. Imaging methods, such as ultrasound, may be used to guide positioning of needles. For monitoring changes in physiology or hormone levels, we may implant a cannula into a blood vessel to sample blood.

A small number of control and genetically altered mice, some after surgery, may be subject to (mild) learning and memory or other behavioural tests.
Some projects are also concerned with cancer and/or ageing. In the majority of cases, those mice expected to develop tumours will do so as a result of a particular breeding protocol involving certain genetically altered strains, such as with null or conditional mutations in *p27*. In other situations, the tumours will develop through injection of cells that are known to lead to tumours, either from an original tumour, such as a gliobastoma, or from pluripotent stem cells that can give rise to teratomas or teratocarcinomas. In all these cases we monitor tumour development, using imaging methods where possible, as detailed in the relevant protocol. Extensive post-mortem tissue analysis will be performed to maximise the information obtained from each animal. For certain types of tumours, such as those developing in the pituitary due to mutations in *p27*, these generally only become apparent in older animals. The effects of ageing on stem and progenitor cells in the CNS and pituitary, and on gonadal sex reversal, also require some animals to be kept for more than a year.

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

For the majority of our experiments, wild type and genetically altered liveborn mice should experience no more than mild effects. For strains that carry harmful mutations, the lines are maintained as heterozygotes, which themselves tend to have mild or no apparent phenotypes. However, animals may be crossed to generate homozygotes or compound mutations, where stronger phenotypes occur, including embryonic or postnatal lethality, or reduced lifespan. It will be necessary to study embryonic stages and to keep some animals with harmful mutations until the phenotypes develop, in order to study how they arise. We will kill animals before end points for the relevant severity band are reached.
The types of phenotype range, according to the specific gene being altered and the type of alteration, from complete or partial sex reversal and/or infertility, craniofacial defects, loss of hearing or vision, CNS defects, abnormal behaviour and/or defects in learning and memory, epilepsy (although this can be managed by careful handling), hypopituitarism, tumours, to shortened healthspan or lifespan. With mutations affecting some genes, there may be phenotypes outside the tissues we study, which can lead to lethality. An example would be kidney defects in addition to sex reversal with mutations in *Wt1* or in addition to enteric nervous system defects with mutations in *Ret*. In these cases, the animals are killed prior to the kidney defects becoming deleterious shortly after birth. In other cases, we are interested in the origins of a deleterious phenotype, such as the failure of the enteric nervous system to colonise the distal gut in mouse models of Hirschsprung's disease, and not the problems this leads to. We therefore kill any newborn animals with the relevant genotype before symptoms of the disease, such as 'megacolon', become evident.

The frequency, type and severity of any adverse event depends on the procedures being used, together sometimes with genetic status, including the genetic background of the strain. We endeavour to minimise the chances of these occurring, but the cause is sometimes unknown, such as the occasional death after administering tamoxifen. Depending on its severity, the duration of an impact or adverse effect may range from a small number of days to a small number of weeks, or even for much longer if fertility or behaviour are affected, with the animal being killed prior to it reaching the relevant end points as defined in the protocols.

For animals undergoing surgical procedures, most should only experience transient discomfort with pain being managed by anaesthesia and analgesia. They would be expected to recover fully within a few days. For some, the outcome may be more severe, notably after adrenalectomies there can be significant weight loss associated with 'salt wasting'. This can be managed by adding salt to their drinking water, but this fails to rescue the mice after 4-8 weeks. We therefore kill all such mice at a maximum of 3 weeks post adrenalectomy. If combined with tamoxifen treatment, animals that have been subject to adrenalectomy have a significantly increased risk of death about three days afterwards, which can be up to 50% (with no clear reason). For this reason, animals treated under this combined protocol (No. 11), which is used for a small number of animals, are maintained for no more than 7 days, and the protocol is classified as severe.

With aged animals, the chance of an adverse event, including unexplained death, is also increased. Such animals are therefore also maintained under a severe protocol (No. 9), although the majority do not exhibit severe effects.

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

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

The expected severities for the mouse experiments are:
Mild; about 75% of the animals.
Moderate; about 23% of the animals.
Severe; less than 2% of the animals

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

- Used in other projects

A retrospective assessment of these predicted harms will be due by 22 January 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?

Our work is very much embedded in mouse genetics, and this requires breeding animals. Furthermore, most, if not all cell fate decisions in the embryo and adult animal take place within a complex environment, where events intrinsic to the cells are influenced by a variety of extrinsic signals, whether these are from neighbouring cells, involve molecules, such as growth factors, cytokines and hormones (which can act over considerable distances), are commensal with the animal, such as gut microbiota, or are part of the environment, i.e. are external to the organism. Moreover, most tissues develop in a complex way in three dimensions over time in a carefully orchestrated manner. Therefore, although some aspects of certain cell fate decisions can be studied *in vitro*, and we both use and develop such approaches, it is generally essential to study them *in vivo* (as a minimum to judge the suitability of *in vitro* systems to give meaningful information). This is best illustrated with a few examples relating to our work:

(i). Several distinct cell lineages give rise to the developing gonads and their continued interactions are required for appropriate gene activity leading to the development of either testes or ovaries. For example, in the mouse, the supporting cells arise from the coelomic epithelium overlying the genital ridge, the steroidogenic cells arise from mesonephric mesenchyme at early stages, and the germ cells are specified amongst extraembryonic mesoderm in the base of the allantois during gastrulation and then migrate back into the embryo and eventually to the gonad. Some connective tissue cells arise from unspecified mesenchyme in the early gonad, whereas endothelial cells that are critical to establish an arterial blood flow in the testis migrate from the mesonephros after Sry activity has triggered Sertoli cell differentiation. Early Sertoli cells also influence germ cells to enter mitotic arrest, and actively prevent them from early entry into meiosis, which is typical of the ovary. It is currently not possible to mimic all of these cell-cell interactions using cells maintained *in vitro*. We can culture the intact early gonad for periods of two to three days, which does allow us to follow some events in real time (and reduce animal numbers), but this still requires breeding to produce the animals.

(ii). To study postnatal gonadal sex reversal, such as when *Foxl2* is conditionally deleted, cannot meaningfully be studied in any in current *in vitro* system. While it is possible to culture isolated granulosa cells for a limited time, they tend to lose expression of critical genes and their normal phenotype. The same is true of Sertoli cells. Without a robust and reliable culture system that could maintain both of these cellular phenotypes, it would be impossible to address the consequences of deleting *Foxl2* in meaningful way. Moreover, it would not be possible to investigate how other testicular cell types differentiate, nor the morphological changes that accompany the changes from ovary to testicular-like structures.

(iii). The pituitary develops through a complex series of reciprocal inductive events between the oral ectoderm and the overlying ventral diencephalon. Some progress appeared to have been made several year ago to mimic aspects of this *in vitro*, beginning with ES or iPS cells; however, the structures reported failed to reflect the cellular organisation of the pituitary, or appropriate production of hormones. Moreover, these experiments have proved difficult to replicate. We can also grow stem cells from the pituitary for a limited time *in vitro* and, by changing conditions they can differentiate into each of the hormone producing cell types typical of the anterior lobe and we use this *in vitro* model to ask some questions about factors influencing stem cell self-renewal and differentiation. However, it has not been possible to reintroduce these to the pituitary *in vivo* to test if they retain relevant functional properties. Nor are these ‘pituispheres’ likely to

have sufficient complexity to model the real organ or to be useful to address issues of the interactions of the pituitary with the hypothalamus and its target organs, which requires whole animal studies.

Over the last few years we have determined that the pituitary stem cells respond to changing physiological conditions. For example, the stem cell population is mobilised by estrogen treatment of males or by gonadectomy or adrenalectomy, but it is not known how a systemic signal affects the stem cells, whether this is via other cell types in the pituitary, or the hypothalamus, etc. We have also recently discovered that the stem cell population is itself complex. To answer these questions requires *in vivo* experiments. Moreover, as we move to explore how the stem cells respond to normal life events such as puberty, pregnancy, lactation, etc, these again have to be carried out *in vivo*.

(iv). To explore the role of specific *Sox* genes in CNS development and their association with the origin of specific tumour types or with ageing, similarly cannot be adequately replicated *in vitro*. For example, there is no *in vitro* model of hippocampus development. Addressing the consequences of abnormal CNS development on behaviour or learning and memory also requires *in vivo* experiments.

(v). There is now increasing evidence that many aspects of anatomy, physiology, behaviour, pathologies, and responses to treatment, differ between the sexes; and even when these appear similar, the underlying mechanisms may be different. These differences are likely to be due to direct effects of X and Y linked genes, to sex hormones made by ovaries or testes, or both. Moreover, these effects can be organisational (i.e. they are established during development, perhaps prior to any obvious difference), or activational (require constant input). Experiments to understand the mechanisms involved, the importance of which have been widely recognised in recent years, cannot be conducted *in vitro*.

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

We complement our *in vivo* analyses with tissue culture models and organoids. These include the pituispheres mentioned above, as well as neurospheres and NS (neural stem) cell cultures for the CNS. We also make use of cell types obtained from mouse or human pluripotent stem cells (ES or iPS cells) via processes of directed differentiation *in vitro*. These can give rise to cell types typical of the CNS and, from our work, to the early gonad. We have also, with collaborators, attempted to use tissue engineering to construct 3-D models of neural stem cell niches, and plan to use similar methods to assemble gonad-like structures.

Why were they not suitable?

We can gain a certain amount of information from these *in vitro* systems, but there are serious limitations. It is not currently possible to replicate the complexity of mammalian tissue structures in culture models. In addition, while some molecular assays (e.g. RNAseq), suggest that the various cell types we have derived from pluripotent stem cells are similar to the endogenous cell types we wish to study, they are not identical. Moreover, the proof that we can derive and propagate relevant cell types, will depend on their ability to function when reintroduced into the relevant organ *in vivo*, which can be challenging and still requires animals. Finally, such cell or organoid cultures cannot permit research on aspects of biology such as reproduction, brain function, or physiology.

A retrospective assessment of replacement will be due by 22 January 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?

This estimate is based on several factors. It is based on our past experience, particularly over the past 5 years. We have also taken into account the number of researchers within the group who perform mouse experiments (currently 10, and it is likely to remain around this number over the next five years, with MSc students usually boosting this (currently two) for 3 to 6 months during each spring/early summer). We also continually re-evaluate the numbers of mice required for each experiment using power calculations. For this we access help from an in-house statistician when necessary. This will allow us to determine the number of animals required per experiment to give statistically valid results. Numbers of mice used for breeding are based on best practice, experience with each strain or combination of strains, and factoring in the likely proportion of the desired genotypes, including controls.

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

When designing specific experiments within the overall project we estimate the minimum number of animals required to give robust answers. Most often this can be based on our prior experience or on published data. Often it is not necessary to use statistics (e.g. three transgenic lines giving the same pattern of expression shows that this is correct), however, we perform statistical analysis whenever necessary. Where experiments involve physiological manipulation, or result in phenotypic and/or physiological consequences, for which we have little or no prior information, usually around 5 or 6 animals per treatment group (which will include sex as a variable when relevant and possible) are sufficient to obtain robust results. The design of quantitative experiments generally follows the ARRIVE guidelines and sample sizes may be set using power analysis. Any exceptions are where there is a degree of variability beyond our control (for example, where minor fluctuations in conditions together with threshold effects require more animals to be examined in order to have statistically significant results). We generally use a significance level of 5% and a power of 80%, estimating standard deviation from pilot experiments. We include advice taken from local statisticians as well as make use of online tools, such as the NC3Rs' Experimental Design Assistant.

For some important questions that we wish to address there can be a choice between using a mild procedure but many animals because the measurable effect is weak, or a moderately severe procedure with few animals because the effect is robust. Our choice will depend on the specific question and available resources, but it will most often be to use fewer animals.

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

We try to keep as few mice as possible by careful monitoring our mouse colony and good practice. Whenever possible and when there are no harmful phenotypes (or infertility) we maintain genetically altered mouse lines as homozygotes to reduce the numbers of animals required and to reduce the need to genotype. These may be crossed to another genetically altered strain or to wild type mice prior to beginning an experiment followed by intercrossing the heterozygote offspring when wild type and/or heterozygous animals are required as controls for the homozygotes. We also make use of fluorescence reporters that can also (in some circumstances) avoid the need for biopsies, especially for genotyping. To minimise breeding, lines under sporadic use are maintained at lower levels. We also use cryopreservation, such as of embryos and sperm, whenever a strain of mice is not in current use, to preserve unique alleles or allele combinations, and also to permit efficient export or rederivation of animals.

Whenever possible, we prescreen substances (including molecules to induce gene expression, cell death, mutagens, etc) and agents such as viruses *in vitro* to determine approximate doses required *in vivo*. When possible, we also test genome editing components *in vitro* (e.g. with ES cells), prior to the generation of genetically altered animals.

To maximise information gained from single animals, we use *in vivo* imaging when feasible, obtain data on as many tests of behaviour and learning and memory as possible on single animals, and obtain relevant tissue samples from multiple sites after killing. Where more than one project involves the study of an animal with a particular genotype, for example *Sox9* is relevant to studies on the CNS, pituitary and gonads, we often collect multiple tissues from single animals. Similarly, when designing new genetic tools, and maintaining animals derived with these, we will, wherever possible, do so in a way to allow them to be shared amongst as many people as possible, including making use of the host establishment sharing platform. This efficient use of animals minimizes the number used.

A retrospective assessment of reduction will be due by 22 January 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 choose well-established protocols, known to have minimal harmful effects, whenever possible.

Although it is not always possible to predict the nature or severity of any defect that arises from a newlygenerated genetic alteration, we take steps to minimise unwanted phenotypes and/or the number of animals exhibiting these. For example, we make use of tissue-specific regulatory elements and whenever practical, we prefer to make genetic alterations that are inducible, so that the animals do not show a phenotype until expression of the candidate gene or a deletion is induced. Animals exhibiting unexpected or detrimental phenotypes will be killed by a Schedule 1 method, or in the case of new lines or individual animals with phenotypes that may be of particular scientific interest advice will be sought from the Home Office Inspector.

When the experiment is predicted to lead to harmful effects outside the body system under study, we will provide treatments designed to alleviate these – for example, high salt will be given after adrenalectomy, and calcium lactate will be after removal of thyroid and parathyroid organs, or if tumour formation is not a desired outcome, then it may be possible to give anticancer agents (or growth inhibitors). By introducing substances, including viruses and cells, into specific tissues or cavities (such as the lateral ventricles of the brain) we minimise suffering because other body systems are not affected.
To minimise stress during breeding and maintenance we follow best practice guidelines, institute refinements and, for some strains, our own specific procedures of husbandry. These include cage enrichment, sufficient nesting material, and, for particularly sensitive strains and animals subject to specific procedures, minimum disturbance. In the case of any new strain of animal or application of any new procedure or refinement we pay special attention by increased observation and monitoring until we have become familiar with the phenotype and/or the consequences. If welfare implications are identified they will be acted upon and refinements considered in consultation with the NVS, NACWO and animal technicians.

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

A significant fraction of our research involves studies on mouse embryos prior to two-thirds through gestation. We also make of chick embryos (covered under a separate PPL), for some projects. This is partly for evolutionary comparisons, and in some respects the chick and human may be closer than the mouse is to human (in morphology of the foetal ovary or as a model for the craniofacial abnormalities associated with mutations in *Foxl2*), and partly because certain embryological techniques are feasible *in ovo*, but not in utero. However, in most cases the mouse is a better model for the human situation, and the wide range of methods and tools that have been developed for the mouse makes this a more tractable model to study. In addition, many of the systems we study including sex determination, reproductive biology, the pituitary, and the CNS have aspects that are specific to mammals.

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

For all manipulations, we will adhere to the relevant guidelines that aim to minimize suffering. We examine the animals for signs of pain and discomfort (such as grimacing), providing additional analgesia if appropriate, and monitor body condition, killing the animals if the distress is likely to be more than temporary. Many of the genetic and physiological manipulations, as well as the administration of substances, including gene inducers and repressors, viruses, cells and grafting of tissues, are standard and previous

refinements from the literature will be used and added to if possible. For novel types of manipulation, or where insufficient information is available, small-scale pilot experiments are conducted in order to determine the best conditions to obtain a sufficiently robust and meaningful response from the minimum dose, exposure time or treatment. These pilot experiments help to minimize any potential suffering.

In all surgery, analgesia will be provided according to best current practice and with advice from the NVS/NACWO. Appropriate aseptic surgical techniques, heat, and fluid therapy, will be applied as necessary. For studies involving tumours, we will check the animals every day and kill any that exhibit signs of significant illness. Where possible, we will also use imaging methods to monitor the growth of tumours.

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

These will include publications from the NC3Rs and the Institute for Animal Technology, but also relevant articles in scientific journals. In the case of cancer models, we will follow the guidelines in Workman et al, British Journal of Cancer (2010), 102, 1555-1577 (PMID: 20502460); or any subsequent updates as appropriate.

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

We stay up to date via regularly communication with animal facility staff at the host establishment, other scientists in our fields, via e-mail and other updates and publications from, and occasional attendance at meetings held by, the NC3Rs, the Institute for Animal Technology, and the International Society for Transgenic Technology, and through regular visits to their websites: https://www.nc3rs.org.uk/3rs-resources https://www.transtechsociety.org/ https://www.iat.org.uk/

A retrospective assessment of refinement will be due by 22 January 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?

13. Energetics and homoarginine in heart & metabolic diseases

Project duration

5 years 0 months

Project purpose

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

Key words

Heart failure, Ischaemia, Diabetes, Metabolism, Therapy

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

Retrospective assessment

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

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 further our understanding of energetic pathways and the interplay between creatine and homoarginine in the heart and other metabolic tissues. This includes generating proof-of-principle evidence in rodent models in support of new therapeutic approaches for ischaemia and chronic heart failure.

A retrospective assessment of these aims will be due by 26 February 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 it's aims and if not, why not?

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

Why is it important to undertake this work?

In the UK alone there are ~100,000 heart attacks per year and 800,000 individuals living with heart failure. Even with optimal treatment, 59% of men and 45% of women diagnosed with heart failure will be dead within 5 years, a clear indication that new and better treatments are urgently needed.

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

The major output of this programme of work will be the generation of new information that will advance scientific knowledge, in particular, how creatine and homoarginine interact and affect whole body metabolism, ischaemic disease, diabetic cardiomyopathy, and chronic heart failure (Objective 1). We will also provide proof-of-principle evidence for therapeutic potential using appropriate disease models, with the aim of moving closer towards clinical translation to humans (Objective 2).

The outputs will be evidenced by publication in international peer-reviewed scientific journals and by presentation at scientific conferences. Other outputs may include the characterisation of drug-like molecules that we would consider patenting and could be used by others as a starting point for the development of new medicines.

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

The immediate beneficiaries will be the scientific community, who within the time-frame of this PPL, will gain new information that can be used to guide future work and will lead to the gradual accumulation of scientific knowledge in this area. This knowledge is also likely to be useful to the medical community and pharmaceutical industry since our research aims to answer key scientific unknowns that will bring us closer towards clinical translation to humans.

By way of example: -

Our finding that homoarginine preserves contractile reserve in mice with heart failure already has potential for direct translation to benefit patients, particularly since hArg is cheap, can be taken orally and is safe in humans. Within the next few years we will seek funding for a clinical trial in heart failure patients. However, the work in this PPL will remove barriers to translation by addressing unknowns such as optimal dosing and a detailed understanding of mechanism in the heart and other organs, thereby paving the way for these trials. In the long-term (7-10 years), as an add-on to standard therapy, homoarginine has the potential to improve the quality of life for patients with chronic heart failure.

We have identified augmentation of creatine kinase activity or myocardial creatine levels via activation of the creatine transporter as a potentially beneficial strategy in diseases of ischaemic origin. The largest obstacle to translation is a lack of pharmacological tools to test this strategy in other disease models and as lead compounds for drug development, hence we are currently collaborating with the pharmaceutical industry to remedy this. By testing new compounds, or equivalent genetic modifications, in relevant disease models we will provide proof-of-principle evidence for future translational studies. By including co-morbid models of diabetes, we will ensure these benefits are also realised in the diabetic heart. In the longer term (10 years), this will move us towards the realisation of clinical benefit, e.g. for cardiac protection during heart surgery, treatment of angina and peripheral vascular disease, and diabetic cardiomyopathy.

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

We aim to disseminate our findings as widely as possible to the scientific and medical communities via publication and presentation at conferences. Outputs are maximised by ensuring our research is conducted and reported with openness and rigour, with the aim of creating a legacy of trusted and reproducible new knowledge. This includes the publication of unsuccessful approaches, so that others can learn and concentrate resources on what works best without unnecessary duplication. We will actively engage in informed debate by authoring review articles, by giving seminars, and by public engagement.

We will seek opportunities to collaborate with other scientists and medical professionals in order to identify areas of synergy that will further our objectives, expand our findings into new therapeutic areas, or open new areas for investigation. For example, collaboration with colleagues that are expert in the design of clinical trials or drug development will be necessary to realise the long-term benefits of this project.

Species and numbers of animals expected to be used

- Mice: 16400
- 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.

Adult mice will be used for the vast majority of this project because this species provides a unique combination of easy genetic manipulation with a cardiovascular system and disease models sufficiently similar to humans. Occasionally we will use rats, for example, because it is possible to induce coronary vasospasm under terminal anaesthesia as a refined model of angina in this species, or where a larger heart is required to make scientific measurements. We may also use rats to demonstrate that a new drug works the same way in different species, which would be necessary to justify more complex experiments in larger species such as pigs.

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

We will use surgical models that mimic the major causes of human heart failure since these are the most translational and therefore considered the gold standard in our field. A typical experiment might compare whether mice with a specific genetic alteration in the heart are better than control mice at responding to a heart attack. To do this, we perform surgery under general anaesthesia to stop blood flow in a coronary artery, thereby inducing a heart attack. The animal is given painkillers during recovery and we follow how the heart responds over the course of 6-8 weeks. This involves similar techniques to those found in a cardiology clinic, for example, ultrasound or MRI imaging on one or two occasions to measure heart function and structure, and placing a catheter in the heart to directly measure pressure generation. All of these are performed under general anaesthesia and to control cumulative suffering the maximum number of anaesthetics in a lifetime is limited to 8. At the end of the experiment the mice are killed humanely and the heart removed to study the molecular and cellular changes.

Other variations of this experiment may use mice or rats that are being treated with a novel drug that we hope will be beneficial in heart failure. In this case we will give the drug by the oral route whenever possible, but if not, this may require daily injections. We will perform similar experiments using other models of heart failure, e.g. due to narrowing of the aorta or secondary to diabetes.

Mice will be made diabetic by one of the following methods: daily injections for 5 days with a compound that disrupts insulin secretion; feeding a Western-style diet to make them obese; or they will harbour genetic mutations that lead to diabetes. Experiments will last several months to allow for development of diabetes and cardiac complications during which time they will have urine and blood samples taken regularly to monitor glucose levels. At the end of the experiment we will measure heart function by ultrasound and cannulation for pressure measurements. Some of these animals will be given a heart attack under general anaesthesia as a final step, after which they will be killed humanely. Approaches that are beneficial in the heart will also be tried in a model of skeletal muscle ischaemia, where blood flow to one of the legs is restricted to mimic peripheral vascular disease in humans. This involves surgery to occlude one of the main arteries supplying a hind-limb (the other limb acts as a normal control). An experiment may last up to 3 weeks post-surgery during which mice may receive test compounds and housed singly with access to a voluntary running wheel to measure exercise capacity. They will be anaesthetised on 1 or 2 occasions to non-invasively measure blood flow in both legs before being killed humanely to collect tissues for further analysis.

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

We will use surgical models of heart failure and many of the adverse effects are related to recovery from the surgical procedures used to create the disease models, e.g. pain, weight loss, poor body condition. However, these can mostly be controlled via good practice, for example, surgery takes place using aseptic technique within a clean air environment, animals are closely monitored during recovery and provided with pain killers, fluids, heat support and access to softened food. Most recover well within a couple of days and will remain free of adverse effects for the duration of the protocol. However, over subsequent weeks, approximately 10% may develop shortness of breath or laboured breathing, which is indicative of congestive heart failure. We monitor mice frequently to identify these symptoms and humanely kill affected animals at the earliest opportunity. Nevertheless, some animals will die of heart failure since these symptoms can develop rapidly.
In some models of diabetes the mice will lose weight, while in others the diabetes is secondary to obesity. In both cases, the mice may show poor body condition, will feel thirsty, and urinate more. Absorbent bedding, extra water and cage changes are utilised to prevent the potential for developing skin sores.

Although mice will develop measurable heart disease, this will not be severe enough to cause clinical symptoms.

The model of peripheral vascular disease utilises the same good surgical practice outlined above. Mice are given painkillers during recovery and will typically limp for a few days while their leg adapts. They will always have one good leg and are therefore still mobile and able to feed for themselves. Strength will progressively return to the affected limb over the course of the experiment. Use of a running wheel necessitates single housing, which may be stressful to the mouse, however it is partly mitigated since mice enjoy running on a wheel.

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 mice will not experience adverse effects because they will be used for breeding purposes in order to establish and maintain animals with specific genetic alterations. Approximately 15% of these lines may have a harmful phenotype of moderate severity, e.g. they will be underweight with muscle weakness or obese with early signs of diabetes. Animals that have surgery but otherwise recover well are also considered moderate severity, since the adverse effects are well controlled and of short duration. However, the <10% of animals that develop congestive heart failure might have a severe experience, since they may have difficulty breathing for several hours before being found dead or euthanased. There is also potential for cumulative suffering leading to a severe severity, e.g. where the same animal receives multiple procedures as part of a single protocol. To mitigate against this, animals are allowed to fully recover before the next step in a protocol and we have limits on the total number of general anaesthetics administered in a lifetime.

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

- Killed

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

We are studying complex diseases such as chronic heart failure, which is a progressive disease that develops over many weeks. This represents a level of complexity that can only be fully represented by the intact animal since there is dynamic interplay between mechanical stress, haemodynamic loading, and the nervous, vascular, endocrine and inflammatory systems.

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

We use non-animal alternatives whenever possible and consider these as complementary to the animal work, i.e. they may reduce the number of animals required, but do not completely replace the need for subsequent animal work. For example, we are running an *in vitro* screen to discover new drugs using a cell culture assay, however, any positive hits from this will ultimately need to be tested *in vivo*. We routinely perform many *in vitro*

experiments using cell culture, since these are particularly useful in determining the consequences of altering gene expression at a molecular and cellular level.
We have an active collaboration with clinical colleagues to study energy metabolism in human heart disease. This includes access to small amounts of human myocardial samples and we also have access to a biobank of human adipose tissue that will allow us to validate our findings and show that they are relevant to human disease.

Why were they not suitable?

Human tissue is a valuable and useful resource, but the quantities obtained are very small, the samples exhibit a lot of variability, and it represents a snap-shot of disease. Taken together this greatly limits the types of experiment it can be used for.
We have previously used computer modelling for certain aspects of our research. However, when we tried modelling the effect of altered creatine levels on heart metabolism, we found that the computer model could not predict the widespread and varied metabolic response we have observed in animal experiments.
Homoarginine is our other metabolite of interest, but it has not yet been incorporated into any computer models, since it is unknown what proteins and pathways it interacts with. This project aims to address this gap in our scientific knowledge.

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

Breeding will make use of the majority of animals in this project and we have based our numbers on usage over the previous 5 years, since we anticipate the overall workload to be similar. We also know from experience that a fully controlled study in our heart failure models requires ~100 mice and will take ~1 year to complete, so 500 mice over a 5 year period is the maximum we anticipate using. In practice, the protocols we use will be guided by our scientific findings, so it is highly unlikely that we will use the maximum numbers across all protocols.

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

We routinely use power calculations to guide our experimental design. This is a statistical technique that provides an estimate of how many animals are required in each experimental group based on the anticipated effect size and the variability observed in previous experiments.

We plan to make good use of non-invasive imaging techniques, such as ultrasound and MRI, which allow repeated measurements to be made in the same mouse at multiple time-points. This significantly reduces the number of mice required for longitudinal studies.

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

By careful advance planning, we aim to closely match our experimental requirements with breeding output, and thereby avoid wastage. Our institution has a dedicated mouse colony expert who provides advice on efficient breeding. We freeze embryos or sperm from mouse lines that are not in routine use, since this avoids the need to breed mice simply to maintain a live colony. Wherever possible we will share spare tissue with other groups that can use it. For example, we do not study the brain, but our collaborators in Germany do, so we freeze organs from our experiments that may be of use to others.
We make use of pilot studies when working with new drugs or a new mouse line. Initial study in a small number of animals provides information on adverse effects and the natural progression in our disease models. We can then adjust the monitoring, experimental and humane end-points accordingly.

A retrospective assessment of reduction will be due by 26 February 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 use mostly mice for this project because of the ease of genetic manipulation and the similarity of the cardiovascular system to humans. We will use surgical models of heart failure, because these recapitulate the complexity of human disease and the discomfort associated with surgery can be mitigated (as detailed below). Alternatives include infusing drugs to cause heart failure, but the overstimulation of a single pathway does not reflect any common cause in patients, and mice are still at risk of developing congestive heart failure, which is the single biggest welfare concern. We therefore believe that the heart failure models used in this project are the most clinically relevant that also cause the least suffering to the animals.

We will use mouse models of diabetes in order to study diabetic cardiomyopathy and the early response to heart attack. These include injection of streptozotocin or high-fat diet or genetic models of obesity to reflect different types of diabetes. We need these models to develop heart dysfunction when measured by ultrasound, but they are less likely to develop clinical symptoms of shortness of breath compared to the surgical models of

congestive heart failure. The major clinical symptoms are therefore related to changes in body weight and elevated blood glucose.

We will generate a model of peripheral artery disease (hind limb ischaemia) by surgically tying-off an artery in one leg. The other leg is left unaffected, so although mobility is impaired in the first few days, animals will always be able to move around the cage and feed normally. The alternative model removes the entire artery and surrounding vessels and is associated with a high level of foot necrosis (up to 25% compared).
We will use a range of non-invasive methods to measure the effect on the heart and other organs, e.g. electrocardiogram, ultrasound, MRI, laser Doppler, relaxometry (body composition), blood sampling, and voluntary wheel running. These are all particularly benign and produce a wealth of scientific data without causing distress or lasting harm to the animals.

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

We are studying how the heart responds over time to injury and the complex interplay of haemodynamic forces, energy requirements and regulatory systems. The human heart, in common with other mammals, has four chambers and generates normal pressures of 100-120 mmHg. Fish can be used to study acute heart injury and healing, but they only have two chambers and generate 2-3 mmHg in pressure, while frogs have a 3-chamber heart and generate 30 mmHg. Mice are therefore the least sentient species that have a cardiovascular system and response to disease that is sufficiently similar to humans. We need to use adults, because most heart disease affects adults.

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

When genetically-altered mice exhibit a harmful phenotype it is usually possible to conclude our experiments at an earlier time-point in order to minimise potential suffering or distress.

We will use disease models that have direct correlates to the major causes of human heart failure, e.g. ischaemic (following a heart attack), pressure overload (aortic stenosis), and diabetic cardiomyopathy. The first two of these are surgical models with the potential to cause pain and suffering during recovery, however, this will be mitigated by using aseptic technique and giving pain killers, fluids, softened food, and heat support during the recovery period. The majority of mice make a full recovery within days and will not experience further adverse effects. In the ischaemic model there is a risk of sudden death due to scar tissue rupturing, but this affects mostly males, so we typically use females for these experiments. Around 10% of mice will develop shortness of breath or laboured breathing during the following 6 weeks, which is indicative of congestive heart failure. Suffering is minimised by increased monitoring and using this as an immediate humane end-point.
For diabetic mice, we will regularly monitor blood glucose levels and humanely kill animals if they become dangerously high. We expect the mice to be thirsty and urinate more, so we will make use of absorbent bedding and keep fewer mice in each cage.
For mice with hind limb ischaemia, the same controls as above will mitigate against pain and discomfort caused by the surgery, and after a brief spell of reduced mobility, mice usually recover well.

Regular monitoring will identify animals that do not recover mobility or that develop early signs (e.g. skin discolouration or nail injury) and these will be humanely killed.

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

We will follow Home Office guidance on the "Code of practice for the housing and care of animals bred, supplied or used for scientific purposes". Guidance for aseptic surgery will be taken from "Guiding Principles for Preparing for and Undertaking Aseptic Surgery" (LASA 2017). At the experimental planning stage we will refer to the PREPARE guidelines checklist ("Planning Research and Experimental Procedures on Animals: Recommendations for Excellence") and to ensure our experiments are reported effectively we will adhere to the ARRIVE guidelines ("Animal Research: Reporting of *In Vivo* Experiments").

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

At the start of each experiment we will check 3Rs websites such as NC3Rs (https://www.nc3rs.org.uk/3rs-resources) and Norecopa (https://norecopa.no/3r-guide). We also receive regular newsletters from these and other organisations, our department holds animal welfare meetings three times a year where progress on the 3Rs is discussed, and there is a regular institution-wide newsletter on the 3Rs.

To keep abreast of new applications we actively scan the scientific literature for alternatives and talk to other researchers that are performing similar techniques to establish best practice. Hands-on advice from the vet is always available in implementing advances effectively.

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

14. Experimental investigation of microplastic ingestion bias

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

Key words

Microplastic, Behaviour, Ingestion, Health, Welfare

Animal types	Life stages
Zebra fish	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.

Objectives and benefits

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

What's the aim of this project?

The aim of this project is to investigate whether certain characteristics, including size, concentration, density and composition, alter the tendency of zebrafish to ingest microplastic. We will determine the anatomical position of microplastic accumulation in the body, and investigate whether feeding behaviour influences ingestion.

A retrospective assessment of these aims will be due by 25 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 it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these

could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

In recent years plastic items have been found accumulating in the guts of marine and freshwater animals due to the increasing quantities of plastic dispersed into the environment. Fish are especially susceptible to ingestion of microplastic because such particles can be mistaken for food, with fish that eat plankton being particularly affected. Many fish species are consumed by higher trophic groups and there is also potential for plastic to be concentrated in the food chain. This research will demonstrate which types of microplastic are most detrimental to fish, with the potential to help improve their welfare in the future.

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

This project will generate new information regarding microplastic ingestion by zebrafish, exploring the extent to which microplastic size, concentration, density and composition biases the likelihood of ingestion; whether microplastic becomes trapped in zebrafish tissues; and if not, what their residence time is in the gastrointestinal tract.
We will publish our findings in scientific journals. We will aim for top-tier open access peer-reviewed journals with a broad readership in order to disseminate our findings to as many people as possible. We will also share our findings with the general public via press releases and social media.

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

Scientists interested in fish welfare will benefit from a better understanding of the correlation between microplastic type and harm caused to fish health. This may help inform future policy regarding pollution of the oceans and the types of plastic to use in manufacture.

Members of the public are increasingly aware of the need to protect the environment, including reducing or removing plastic waste. High quality scientific data showing the impact of microplastics on fish health and welfare will further raise awareness of this important issue.

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

We will maximise the outputs of this work through collaboration, publication and dissemination. This project represents the first step in a new collaboration. The results obtained here will form the basis of joint grant applications. We will publish our findings in open access journals as described above. We will further disseminate our results though our lab Twitter accounts and press releases, approaches that we have used successfully before.

Species and numbers of animals expected to be used

- Zebra fish: 90

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 zebrafish are the ideal animals to use in these experiments. They are small, easy to maintain in the laboratory and we know a lot about their behaviour, which can be used to assess changes to health during the experiment. They are also a model for other fish , allowing the information here to be used to understand other species including wild fish.

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

Animals will be fed one of two types of microplastic (polypropylene and polyethylene terephthalate) on the first day of the experiment. We will compare two different concentrations and sizes. The animals will then eat their normal diet for the rest of the experiment. Animals will be sacrificed at three time points (48h, 168h and 336h) using a standard method. We will then collect their organs and assess both the amount of microplastic eaten and its accumulation in different tissues of the body.

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

The experiments proposed here will be used to generate pilot data for a larger set of experiments. While we do not expect adverse impacts during the course of these experiments, there is a possibility that the fish's health is affected by microplastic ingestion. This could cause a change in behaviour, (such as a alteration of locomotion or feeding, freezing, a loss of balance or increased time spent at the surface of the tank water) or even mortality.

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 changes to health caused by microplastic ingestion in this pilot study could be severe. Previous research suggests that microplastic ingestion can be lethal to fish meaning that their behaviour and welfare needs to be monitored very closely. If we do not observe such mortality here we will apply to amend the severity rating for these experiments before carrying out further research.

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 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?

The research described in this proposal investigates microplastic ingestion by zebrafish. Since we will analyse behavioural preference for different types of microplastic, accumulation in organs and the transit time through the body it is impossible to use cell lines or organ cultures for this research. I have looked at the FRAME website and the NC3Rs website for possible replacement protocols but have not found suitable alternatives.

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

We considered using organ cultures for this research.

Why were they not suitable?

Organ cultures are not suitable, because we will investigate the feeding behaviour for different types of plastic (e.g. that sinks or floats) and the accumulation of microplastic in many different organs of the body.

A retrospective assessment of replacement will be due by 25 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?

We have estimated these numbers based upon data collected during pilot experiments.

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

In general, we use the NC3R's Experimental Design Assistant tool when designing experiments in our laboratory. This allows us to check the statistical analyses and number of animals to use.

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

We will use pilot studies to refine the size and quantity of microplastic to use in our main experiment. We will collect data from multiple tissues in each animal, reducing the total number of animals needed overall.

A retrospective assessment of reduction will be due by 25 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 have chosen to use zebrafish for this research because of the combination of well-established behavioural protocols and ease of maintenance in the laboratory. Adult wild-type zebrafish (AB/AB strain) will be housed in the best possible conditions in our aquarium. This aquarium has constantly circulating water which is regularly monitored for quality. Fish are maintained at low stocking density in specially designed tanks.
Fish will be minimally handled during the project. Pilot experiments will be used to calculate the concentration and size of microplastic to use in our experiments. In the case of unexpected adverse effects caused by feeding plastic (e.g. a reduction of swimming, freezing, loss of balance or surfacing), the experiment will be terminated. The Named Veterinary Surgeon and Named Animal Care and Welfare Officer will be contacted for advice before feeding microplastic to fish again.

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

The goal of this research is to characterise the fate of microplastic in fish following ingestion. Species that are less sentient or that have been terminally anaesthetised may not eat any microplastic, making it impossible to carry out these experiments.

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

We will refine the procedures that we use by monitoring the behaviour of fish during the course of this research during routing health checks. We will look for signs of distress, including changes to swimming, freezing behaviour, or an increase in opercular beat rate or tail beat frequency, indicators of pain in zebrafish. In case of unexpected change to behaviour we will terminate the experiment and contact the Named Veterinary Surgeon and Named Animal Care and Welfare Officer for advice before carrying on.

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

We will follow the ARRIVE and PREPARE guidelines, both when designing our experiments and publishing our findings.

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

We will stay informed about advances in the 3Rs by reading scientific literature as it is published and by monitoring the NC3Rs website. We will use this information to update the protocols that we use when possible.

A retrospective assessment of refinement will be due by 25 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?

15. Exploring novel strategies for cardioprotection

Project duration

5 years 0 months

Project purpose

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

Key words

Cardiovascular disease, cardioprotection, disease models, cardiotoxicity, cardiorenal protection

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

Objectives and benefits

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

What's the aim of this project?

The aim of this project is to identify new ways of protecting the heart, improve current therapies, and possibly re-purpose some of the treatments already available for other diseases, if there is an indication that these can help improve heart disease. These new strategies will be tested on appropriate preclinical animal models with the view to translating into patients with different degrees of heart disease and with co-existing complex diseases such as diabetes, obesity, chronic kidney disease (CKD) and cancer; and corresponding medications.

A retrospective assessment of these aims will be due by 05 April 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 it's aims and if not, why not?

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

Why is it important to undertake this work?

Ischaemic heart disease is associated with narrowing or blockage of one or more blood vessels supplying the heart muscles resulting in deterioration of heart pump function. This is the largest cause for mortality as per the global estimates for 2016 published by World Health Organization (WHO). Projections for year 2030 suggest that if measures for reducing the prevalence of this disease are not in place, it will continue to be the single largest cause for death globally.

Coronary artery disease, the most common type of heart disease, causes blockage of one or more blood vessels supplying blood to the heart muscles leading to heart attack. When this occurs, the heart does not get enough oxygen and a part of the heart muscle wall that is supplied by the affected blood vessel becomes damaged. A large number of cells in the affected area die and since new cells cannot replace the dead cells in the heart, the damaged area will not be able to function normally. This increases the workload on the rest of the heart, which will further worsen the heart function with time leading to what is called heart failure and eventually death. The first obvious step to reduce the extent of injury to the heart is to restore blood flow by opening the blocked vessels using drugs and surgery. However, this sudden return of blood flow (called reperfusion) is known to make the injury worse. In addition to finding ways to limiting such reperfusion injury, an additional approach to decrease the global heart disease burden would be to reduce the incidence of coronary artery disease itself.

Coronary artery disease results from the presence of several cardiovascular risk factors such as high blood cholesterol, high blood pressure, obesity, diabetes etc. Most of the heart therapies in use are based on treating these risk factors and also implementing life-style modifications. Despite improvement in treating these diseases, the number of patients with heart disease continue to rise globally. This suggests that there are other, yet unidentified, harmful effects of co-existing diseases that may point to additional ways of protecting the heart and improving outcomes in these patients. Recent clinical trials have confirmed that in order to get the maximum benefit, it is important to understand how the different organs in human body interact in a state of health and ill-health, which in turn seem to affect how the body responds to treatments including those for cardiac disease. In addition to the diseases, advanced age is a non-modifiable, untreatable factor that has significant effect on cardiac health and efficacy of treatments.
Limiting the pandemic of heart disease is extremely important not only to reduce the global burden of this disease on health services and healthcare costs, but also to improve outcomes in certain other disease states such as diabetes, CKD and cancer. For instance, a vast majority of patients with diabetes and CKD die of cardiovascular complications. Patients with diabetes mellitus (DM) are 2-4 times more likely to develop cardiovascular disease, which is the main cause for death in ~65% of patients with DM. In 2010/11, diabetes cost NHS about £9.8 billion, of which around £3 billion was spent on heart disease and associated complications. With diabetes projected to become the 7th leading cause of death worldwide by 2030, improving efficacy of treatment/management of heart disease in diabetic patients is essential to reduce the healthcare expenses. Another factor that can increase the chances of heart problems in the clinical setting is treatment with anti-cancer drugs such as anthracyclines. These drugs are cardio-toxic – i.e., they can weaken the heart muscle within a span of as little as a couple of weeks to as much as 10

years after treatment. As many cancer patients are over the age of 50, the chances are fairly high that they already have heart disease when diagnosed with cancer; and the cancer treatments can make their hearts more vulnerable. Hence the provision of life-saving chemotherapy is restricted in cancer patients presenting with cardiac risk factors. It is also known that cancer survivors with no history of cardiac diseases may develop heart diseases and heart failure as a result of chemotherapy. For instance, a significant proportion of childhood cancer survivors who received these chemotherapy agents show the development of heart failure in a fairly young age.

It is also extremely important to note that, not only do other diseases and certain drugs used for cancer treatment increase the propensity to developing cardiac disease, but heart disease can also increase the incidence of other diseases. For example, acute kidney injury is a frequent complication of heart attack and can strongly affect the short-term and long-term survival in these patients. Therefore, in order to effectively prevent and treat cardiac disease in young and old patients with/without co-existing pathologies, it is extremely important to understand how effective current heart treatments are in different patient populations.

To obtain data with potential beneficial effect for large patient groups, pre-clinical research involving use of animals mimicking human diseases are essential – more importantly to ensure that the refinements and new strategies do not pose health risk contrary to being beneficial. The insights from these will help refine and personalise current treatments and if needed, devise new treatment modalities.

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

The project detailed in this licence outlines research intended to 1) improve knowledge of mechanisms of cardiac cell death caused by decreased blood flow and other causes of cardiac injury; leading to 2) identification of new therapeutic targets; 3) development of new methods/drugs based on these newly identified targets for protecting the heart; 4) testing these new methods and also refining available treatments in animals harbouring diseases similar to human patients (e.g. diabetes).

Additionally, our project will undertake studies aimed at re-purposing clinically approved non-cardiac medications for application to cardiac diseases and chemotherapy-induced cardiotoxicity, if there is an indication that these may help protect the heart.
The insights gained from the studies carried out under this project will help improve the ways by which heart can be protected in the larger patient population who may present with several other clinical conditions in addition to cardiac disease. The knowledge obtained from this research can also contribute to the scientific understanding of injury caused by both the lack of blood flow and subsequent return of blood flow by drug or surgical interventions, as occurs in other vital organs such as brain (stroke), kidney and liver, and during procedures such as cardiac surgery and transplantation. The new therapies may also provide valuable adjuncts to current clinical interventions.

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

Our work aims to improve current methods/drugs and to find new ways of reducing injury in patients who experience a heart attack. Since those who survive a heart attack frequently develop heart failure due to the damage to the heart, improving available treatment methods and developing new techniques will help patients to survive longer and have a better quality of life. A decrease in the occurrence of heart failure following heart attacks would also provide substantial cost benefits to the public health service. Using

animal models that have disease conditions similar to humans (e.g., animal models with diabetes, chronic kidney disease or cancer models receiving anti-cancer drugs) we also plan to estimate the success of cardiac treatments in patients with other diseases. This is particularly important since different diseases can increase the risk of developing heart disease and can change the way patients respond to treatments.

Short-term outputs from the project will be mostly in the form of new scientific information regarding how the cardiac disease progresses and new ways of targeting cardiac cell injury. These will be presented in the form of peer-reviewed scientific publications. Other research groups studying cardiac diseases and pharmaceutical companies developing therapies will be the immediate beneficiaries of these findings.

Medium-long term outputs expected of the project mainly include information obtained from studies using currently available therapies - where the refinement of current treatment methods is aimed for. The data from these will also be presented in scientific journals and may translate into modifications in clinical practice benefiting patients directly.
A decrease in cardiovascular morbidity and mortality in different disease settings can translate into substantial cost benefits to the NHS. Thus, the major beneficiaries in the long run are the healthcare network who can provide better care at reduced costs and most importantly, the patients who receive optimum personalised treatments positively impacting their quality of life.

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

We will try to maximise outputs from our project by planning the experiments such that maximum amount of data can be obtained while reducing number of animals used - e.g., using non-invasive techniques (such as echocardiography) to obtain important information on cardiac function as well as collecting tissue samples for biochemical analysis at the end of experiments collaborating with other groups who carry out research in similar fields so as to optimise experimental protocols and avoid unnecessary repetition of experiments collaborating with other groups who may be able to obtain data from the same animals - e.g., in experiments involving diabetic animals, other research groups interested in pancreatic cell function may be able to obtain tissue samples of interest in their research publication of all important data (both positive and negative findings) from the research in peer reviewed scientific journals - regardless of the immediate impact on existing scientific knowledge in the field

Species and numbers of animals expected to be used

- Rats: ~5500
- Mice: ~5500

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 planning to use rats and mice in our studies because;
These species exhibit anatomical, physiological and genetic similarity to humans
The small size, ease of maintenance, and short life cycle offers the advantage of studying the different phases of disease processes within much shorter time span

- Heart attack can be induced and the resulting damage to heart muscle can be assessed with accuracy and ease

- Heart function can be maintained by perfusion with crystalloid buffers, allowing examination of factors that are altered within the heart in the absence of external influences

- Reduced collateral blood supply in the heart - reduces the variability in the results between animals and thereby reducing the number of animals used in each experiment

- These animals can be genetically modified to express diseases that are seen in humans (e.g., diabetes kidney disease). This will provide a good model to understand how these diseases affect heart and to improve the use of currently available heart medications in patients who often also suffer from such diseases.

- These animals can be genetically modified to express proteins that are shown to be relevant to the development of heart disease or relevant to rescuing the heart from injurious insults like heart attack. This will provide a good model to test new treatment targets leading way to new drug development.

In experiments, where the immediate effect of a treatment on heart muscle function and injury, young adult animals will be used and they will be used in experiments under anaesthesia and humanely killed while still under anaesthesia - the tissues from these animals will then be used to obtain relevant information.
In experiments where the long-term effects of treatments and/or other diseases are to be studied, young adult animals will be treated with different medications or disease-inducing factors; maintained for duration sufficient to observe the effect of these treatments and then used in experiments. These animals will be monitored at regular pre-specified intervals to ensure that the relevant experiments are carried out at the correct stage of disease and the animals do not suffer unduly.

Aged animals may be used in both the above types of experiments, as a way to understand how and why the aged heart responds differently to heart injury and treatments.

Animals with changes in protein expression or harbouring disease-causing mutations will be obtained from established commercial suppliers and if possible bred locally. These will be checked to ensure the presence of desirable gene changes at the time they are weaned as pups and then let grow normally until they are young adults or aged before being used in experiments as stated before.

The choice between mice and rats will be based on the desirability of gene mutations in the study design. The commercial availability of large number of transgenic mouse models with gene expression changes desirable for studies involving cardiovascular disease makes it a desirable species to work with. Despite this, the relative larger volume of blood and tissues from rats makes them ideal for experiments involving repeated blood sampling for circulating marker measurements. Also, the recent development in inducing gene expression changes in rats may help decide the species chosen for each study protocol.

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

Breeding protocols - Mice will be mated and maintained under ideal conditions of housing. The litter of pups obtained will be checked for presence of the gene mutation and the randomly assigned to experimental groups. In some cases, these mice may receive pre-treatment with drugs that can modify their response to subsequent heart attack.

Tissue studies - Part of our work involves collecting tissues from animals and analysing these for different markers of injury and drug. In these studies, the animals may or may not receive treatment with drugs before or during heart attack; with the corresponding effects observed in tissues collected. The animals will be deeply anaesthetised (terminal anaesthesia) before inducing heart attack for a specific duration and then collecting the tissues. These experiments are particularly important to see any early harmful effects of treatments with respect to how the heart function when subjected to the injurious insult. Only after a beneficial effect or absence of harmful effect at this point is confirmed that the treatment can be tested in a long-term recovery model (as stated below). An additional advantage of this model is that a lot more information can be obtained from the different tissues we collect from each animal.

Whole animal studies - Once the early/immediate phase of injury has been studied, the findings will then need to be extended to the whole animal model which sustain heart attack, survives with some heart injury and then goes on to develop heart-failure. This is particularly important if we want to take any promising intervention to the next step of clinical application in humans. In these experiments, the animals may receive treatments before, during or after the heart attack procedure, which will be done under deep anaesthesia. The animals will be allowed to recover after the heart attack, the pain being managed using pain medications as in humans. The duration of these experiments will range from a few days after the heart attack procedure to up to 4 months. The duration will be decided based on the extend of complications of heart failure we need to see in order to meet the study objective. In some of these studies we will use animals harbouring diseases like diabetes and kidney disease. This is especially important since the human patients who sustain heart attack often present with many more disease and health concerns in addition to the heart disease.

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

Animals will be used in experiments deemed absolutely necessary, based on initial data from nonanimal models.

In a few experiments animals may be treated for a defined period of time with drugs which are already being used in patients, or in preclinical/clinical trials. Hence the safety, dose, and the methods of treating animals with these drugs are well-documented. This will help design experiments taking into account possible non-desirable side effects and effective management of the same. Although most of the drugs used are not expected to cause adverse reactions at the concentrations intended, the animals will be closely monitored and necessary steps taken to ensure their well-being.

The severity limit set for the different procedures ranges from ‘Non-recovery’ to ‘Severe’. All the animals will be observed regularly for food and water intake, general features of discomfort (e.g. starey (puffed-up) coat, hunched position, lethargy, reluctance to move, isolation from the group, self-harm, changed nesting behaviour) and weight loss (maximum 20%). If these symptoms develop, the animals will be humanely killed. Experiments using animals in this project involves administration of drugs and monitoring their effects on body tissues.

These can be broadly classified into two:

Non recovery – experiments carried out under general anaesthesia; deep anaesthesia will be maintained throughout the duration of experiment followed by a final overdose of anaesthetic to kill the animal without waking it up from the experiment. This method is not expected to cause any suffering as the entire protocol is carried out under deep anaesthesia.

Recovery – experiments carried out under general anaesthesia; deep anaesthesia will be maintained throughout the duration of experiment. Upon completion of the experiment, animals will be allowed to recover from anaesthesia. These animals will be under continuous observation until they become fully mobile and start to feed and drink; which normally happens in the first 3hrs after completion of procedure. In animals undergoing heart surgery, wherein the chest cavity is opened, surgery performed and the wounds closed surgically before recovering the animals from anaesthesia, effective pain relief by medication will be provided to ensure that the animals are not in pain while recovering from the procedure and in the days thereafter.

Further, we also undertake studies on animal models with diseases such as diabetes, kidney disease and chemotherapy-induced heart failure. In these animals, the severity is due to the disease severity - a factor we need to include in our experiments so as to make any new findings applicable to the wider human patient population.

The animals will be killed by humane methods after the experiments and tissues of interest collected for further studies, making maximum possible use of the animals. At all points in time either during maintenance of the animals or during experiments or afterwards all possible steps will be taken so that animals do not suffer unnecessarily. Where the pain/suffering is not transient (occurring within the duration of recovery as a result of the procedure undergone) and cannot be resolved by pain-relief medications, humane methods of killing will be used to end 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)?

Mice - **Mild** severity (~20% in breeding experiments, <25% in tissue studies or non-recovery experiments, ~50% in diabetic mice), **Moderate** severity (<5% in kidney disease models, ~5% in diabetic animals), **Severe** effects (~5% in diabetic animals, ~15% in recovery/whole animal models, ~10% in chemotherapy studies).

Rats - **Mild** severity (<25% in tissue studies or non-recovery experiments, ~50% in mutant diabetic rats), **Moderate** severity (<5% in kidney disease models, ~5% in diabetic animals), **Severe** effects (~5% in diabetic animals, ~15% in recovery/whole animal models, ~10% in chemotherapy studies).

All the remaining animals will fall under the category of **Non-recovery** (i.e, no procedure afflicting painful, distressing or harmful effects has been carried out in these). Additionally, the animals used only for breeding purposes will not be used for any other experiments and therefore categorised under **Subthreshold** severity category - unless unexpected

suffering observed, in which case the animal will be treated to minimise suffering and severity category recorded appropriately.

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 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 aims to help heart survive better after a heart attack and to treat heart disease in patients with other co-existing diseases. The overall aim is that the findings will help improve treatment options for all patients regardless of their general health status. Such personalised medicine will improve the quality of life in the long term in these patients. Our initial 'proof of concept' experiments will be carried out on experimental models of non-animal origin. These models include cell lines - cells available commercially and grown in the laboratory, and also cells and tissue samples from human volunteers. Based on this pilot data, experiments that show promising results will have to be confirmed in more complex biological systems similar to the human heart and whole body before the treatment can be tested in patients. Since these studies cannot be carried out in humans until more information on the safety and effectiveness of the treatment is available, experimental models of animal origin have to be used. Among animal-based work, we initially use the isolated heart and cell models (which are of lower severity), and also get relevant additional information from other tissues from each animal. This will help to increase the output from each animal used. The studies will then be expanded to the whole body and recovery models (higher severity) only if positive results are obtained in the initial animal experiments.

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

In vitro non-animal models -
Includes single types of cells, co-culture systems, cardiac tissue slices (organotypic heart slices), microphysiological systems like organ-on-a-chip and organoids - maintained in culture. All of these can be specialised by using cells or tissues obtained from patients with specific disease type. These can be useful in replicating the disease phenotype, validate possible genetic differences while comparing tissues from groups of patients who are more susceptible to disease or adverse reaction to certain drugs compared to other patients. These properties make in vitro models involving cells and tissues from humans ideal in drug toxicity and safety testing and to a certain extent can also help understand the interaction between different cells within the same organ (e.g. organotypic heart slices)

and between limited number of organs (human-on-a-chip, where different compartments containing cells from different organs are maintained).

Ex vivo tissues from humans -
Tissue biopsies can be obtained postmortem or from live human patients (with prior consent) during cardiac surgical procedures. These may be a good alternative to tissues obtained from animals subjected to interventions so that they develop disease phenotype.

Why were they not suitable?

In vitro models - We do use *in vitro* cell cultures, specifically immortalised cell lines from humans or animals in our preliminary experiments. We also use tissues from consenting patients whenever possible, however the usability of these tissues is greatly restricted by the short duration within which the experiments need to be carried out. These tissues are usually obtained from patients with heart diseases and due to lack of blood flow within the tissue after excision, the core of the tissue dies with time rendering it unusable in subsequent experiments that require prolonged incubation with test drugs.

While the cell and tissue biopsy models are ideal to test the 'proof of concept' or 'test the effect' studies, these do not replicate the complexity that is seen within the intact human heart - be it in terms of the structure, the function or how the cells react in the native tissue to injurious insults. In order to achieve this, we will need to test the treatments in intact organs. Although the organoids and organs-on-a-chip seem like an attractive alternative for this purpose, we are not able to use this owing to the following reasons:

Firstly, the technical expertise and facilities required to develop, establish and maintain these are beyond our reach and are prohibitively expensive to set up.

Secondly and more importantly, the premise of our project is to look at how the changes within the whole body affects the heart - in different disease conditions and in response to different treatments, over a period of time recapitulating the duration that it takes in human body.

The human-on-a-chip, at its current state is still limited by the absence of all the neuronal and blood borne factors that keep changing in response to the different bodily and environmental cues - for e.g., disease states, diet types, medications, physiological stress, gender differences, diurnal variations - to name just a few.

So, although we use *in vitro* models to obtain as much pilot data as possible and are an essential part of our overall research programme, we will need to rely on animal models when it comes to confirming our findings in the complicated biological system - that is the working heart complete with the circulatory and nervous systems that conveys signals between the heart and the rest of the body.

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

Animals will be assigned to experimental groups randomly, as appropriate and necessary, and blinding of data prior to analysis will be done so as to prevent bias and ensure best research practice. Appropriate statistical tests will be used as applicable to the study design. The number of animals required in each experiment will be determined by statistical analysis - based on expected effect size of each output/data (pilot data), significance threshold set at 5% and at a statistical power of 85%. The statistical analysis used will be based on the number of factors studied in each animal (single factor, multi-factorial or randomized block designs). We use the Experimental Design Assistant (EDA) available within the NC3R's, to design and arrive at the optimum number of animals required for more complex studies. Based on our previous work, we expect ~80-85% success rate considering all proposed protocols. This has been factored in while estimating the number of animals required for the different protocols. However, a considerable number of animals are expected to be common to one or more protocols. Hence, taking into account the number of different treatments we want to undertake in our research, the different experimental models that will be used in arriving at useful, reproducible, clinically relevant information, we have arrived at the number stated above for a project duration of 5 years.

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

Whenever possible, we undertake small scale pilot studies to estimate the size of change in the measured parameter(s) as an indicator of expected biological effect. If the effect is too minuscule to be of any clinical/biological relevance, then the study using that particular treatment modality will be discontinued. If the results are suggestive of clinical importance, but need further validation, then we plan studies involving animals. In this phase of planning and designing animal experiments, we use different guidelines/tools - For example, the (https://norecopa.no/media/7864/prepare_checklist_english.pdf); the 3Rs resources and the NC3R's Experimental Design Assistant (EDA). The PREPARE checklist provides us a framework to ensure we have closely considered all required factors including available literature in the field and the novelty of our own preliminary findings - so as to avoid unnecessary repetition of research and animal use. EDA helps design the study considering the different treatment groups, interacting/affecting factors and also suggests the number of animals required for obtaining robust, reproducible and statistically significant data. It also suggests the appropriate statistical tools that can be used for subsequent analysis of results.

In addition to the EDA, we plan to enlist support from the institute's statistics experts for calculating the number of animals prior to large-scale animal studies. For simple two group studies, we use online statistical tools or software like G*Power or ClinCalc.

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 animal use we will:

Use alternative methods that do not rely on use of animals wherever possible - e.g. non-animal derived models in preliminary experiments.

Using pilot data from non-animal studies to assess the need of extending the investigations to animal models.

Plan experiments using minimum statistically relevant number of animals.

Use of experimental techniques that will give maximum amount of data from least possible number of animals (e.g. using whole heart, aortic ring, blood and other tissues from same animal)

Use viral vectors for mutagenesis – reduces (by at least half, if not more) the number of mice bred to produce transgenic strains

Careful experimental planning to include the minimum required number of animals in experiments without compromising the quality of results - e.g. repeated cardiac imaging of animals to examine changes in heart structure and function with time

This will reduce the number of animals needed to study all the time points and also will avoid variations that may be present between the animals.

Regular monitoring of the experimental activity in the lab by the project licence holder and experienced senior scientists to ensure high quality of research and optimum use of animals.

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

Rats and mice will be used in this project as it is easy to measure extent of damage to the heart and experiments can be performed in a controlled manner. Rats and mice with diseases similar to human conditions (for e.g. diabetes) will also be used in the project for studying heart disease in a setting similar to human diseases.

The study will use protocols where the animals are terminally anesthetised and tissues collected for further investigations. These methods will cause no pain, suffering, distress or lasting harm and will be the mildest amongst all the procedures in the project. In other experiments, animals will have to be subjected to cardiac surgery under anaesthesia and then recovered for a specific duration of project to study the effects of treatments on the heart. In these studies, unfortunately, occurrence of pain and suffering in the initial duration after surgery is unavoidable. To reduce the pain and suffering, the animals will be provided with pain medications at appropriate dosage and monitored regularly to ensure that there is pain relief. If these steps do not remedy the suffering, then advice will be sought from the Named Veterinary Surgeon on other possible measures, failing which the animals will be euthanized.

As refinement to techniques used during the study, we aim to assess cardiac function/infarction using minimally invasive techniques (MRI, Echocardiography) for studies involving genetically modified animals, we plan to use ways of inducing genetic changes that will greatly reduce the number of animals used (viral vectors for transgene induction) use drug-filled minipumps implanted under the skin for chronic drug administration. The process of implanting minipump itself does not cause any suffering, since this is done under anaesthesia and the region chosen for implantation is such that the process can be achieved with the smallest of openings on the skin and the implanted mini pump will not interfere with any regular activity use microsampling techniques (tail vein prick) for assessing diabetes in animal models. This will be done in animals fed normally, avoiding stress due to overnight fasting and also less harmful compared to cannulation of vein where larger volumes of blood may be lost use drugs for developing obese/non-obese diabetic models with different disease severities in a much shorter duration than genetic models; minimises suffering especially in studies addressing effect of diabetes and age on heart Heart disease affects the long-term survival and quality of life in patients. To help provide better treatments for the varied patient population, it is important to have a better understanding of how the heart disease itself and heart's response to treatments is affected by different disease states. For this purpose, we will need to use animal models with diseases like diabetes followed by induction of heart attack. Unfortunately, no available non-animal alternative is able to replicate all the complex responses that are seen in a whole animal model as this. We will ensure that the minimum necessary level of clinical symptoms are evident in these animals and not to the extent that it causes severe harm.

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

The development of cardiac disease has strong association with the aging process and the presence of cardiovascular risk factors such as high cholesterol. It has been shown that the incidence of these in juvenile/ adolescent or young humans is very less unless the disease is due to a genetic condition. It is also known that the hearts of young humans are more resilient to injurious insults compared to the adults and the aged. We find similar differences between cardiac cells prepared from neonate rats/mice versus that prepared from adult animals. The neonatal cells are different from the adult cells not only in their appearance, but also in their susceptibility to injury and general functional properties. Considering these and also the fact that our research is aimed at translation into the clinical setting where the vast majority of patients are adults with other disease states, we use mostly adult/aged animals in our studies. Where parallels can be drawn to clinical conditions in the young - for e.g. childhood survivors of cancer developing heart failure, we may use juvenile animals to mimic this disease of the young.
In order to keep the animal suffering to a minimum, wherever possible in our studies, we use animals that are terminally anaesthetised.

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

Refining experimental skills: In addition to the training undertaken for obtaining personal licence, new members of the research team will be trained, supervised and guided by more experienced investigators in all aspects of animal research including designing experiments, handling animals, recognising signs of pain, suffering, lasting harm and distress; and sacrificing animals by humane methods when necessary.
Researchers will be assessed for competency before being allowed to carry out experiments on animals.

Where animals are to be subjected to repeated experimental interventions (e.g. blood sampling at regular intervals in diabetic animals), the animals will be conditioned to the repeated handling by the same researcher or team of researchers - this will reduce the stress associated with the unfamiliarity towards the researchers.

The researchers will be encouraged to adhere to the least stressful procedures for the animals, where possible. For example, microsampling of random blood glucose by tail vein prick will be carried out for routine glucose level checks as opposed to overnight fasting (for fasting glucose levels) and invasive blood vessel cannulation.

At all instances special care will be taken to prevent and reduce animal suffering caused by the experiments.

In experiments known to cause some degree of harm, the researcher will work closely with the animal care team ensuring that the general body condition and any signs of stress are recorded and remedial steps taken as soon as possible. To this end, a body condition score sheet will be made available on which all findings are recorded.
We will work closely with the Named Veterinary Surgeon (NVS) and the Biological Services to help us refine our procedures and also for advice while planning new procedures.

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

<u>For general guidance on compliance to best practice in conducting and reporting research on animals:</u>
Guidance on the operation of the Animals (Scientific Procedures) Act 1986
Legislation for the protection of animals used for scientific purposes Directive 2010/63/EU as amended by Regulation (EU) 2019/1010
Planning Research and Experimental
Procedures on Animals: Recommendations for
Excellence (PREPARE) guidelines
Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines

<u>For choosing appropriate models of cardiovascular disease:</u>
We will use the established models available in peer-reviewed publications, as appropriate to answer the questions we are trying to address - with refinements/ updates implemented if it supports the 3Rs.
(E.g., Lindsey ML, Bolli R, Canty JM Jr, et al. Guidelines for experimental models of myocardial ischemia and infarction. Am J Physiol Heart Circ Physiol. 2018;314(4):H812–H838. doi:10.1152/ajpheart.00335.2017)

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

We will stay informed about advances in 3Rs by keeping track of refinements in techniques published in the literature and also by discussions with other research teams undertaking similar techniques and experiments. In our experience, we have found that involving the animal care technicians, the Institute's Animal Welfare Officers and the Named Veterinary Surgeon in the experimental planning phase is extremely helpful in implementing small but significant refinements to our procedures resulting in considerable improvement in animal experience. This may be as small a change as the bedding material to as significant as changing the route of drug administration and improving post-treatment monitoring. Additionally, we will make use of information available in resources such as NC3R's website to identify useful modifications with the view of refining and replacing animal use in research.

A retrospective assessment of refinement will be due by 05 April 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?

16. Gene and cell therapies for Ischaemic disease

Project duration

5 years 0 months

Project purpose

- Basic research

Key words

pericytes, gene therapy, vascular grafts, angiogenesis

Animal types	Life stages
Mice	juvenile, adult, aged

Retrospective assessment

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

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 find new cures for patients suffering from heart attacks and poor circulation in the legs.

A retrospective assessment of these aims will be due by 21 April 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 it's aims and if not, why not?

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

Why is it important to undertake this work?

Cardiovascular disease caused by the narrowing of arteries and capillaries that provide oxygen and nutrients to the heart, brain, and legs, is the number one killer in the UK. Current treatments reduce the risk of death after a heart or a brain attack and delay the need of foot amputation as the last resource to save the life of patients with very poor circulation to the legs; however, many patients continue to have a poor quality of life. This

is because current treatments are not enough to create new blood vessels around the occluded ones. In addition, grafts used by surgeons to create a new route around the blocked section of the artery tend to occlude after few years from implantation.

What is needed is a definitive solution for building up new blood vessels of different diameter, ideally large arteries to carry blood around the narrowed artery and capillaries to transport oxygen and nutrients to the suffering tissue. To reach this goal we will investigate new methods based on:

Testing drugs that can encourage the formation of new arteries and capillaries. For instance, using a protein which protects people who live a long life (centenarians) from suffering from blood vessel occlusion as they become old.
Producing artificial tubes containing human cells to create grafts like a real artery

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

In the medium term, 5 years from now, we expect that this research will demonstrate the proposed solutions are safe and capable of being done. We also expect to obtain new knowledge of how these treatments work. We will publish the results in medical journals where the acceptance for publication is warranted only after careful evaluation by expert reviewers. This will be a demonstration that the research is novel and accurate and has an important medical impact.

In the long term, the project aims to make these new cures available to the patients. This will require (1) preparation of documentation of main findings supporting efficacy and safety, (2) approval from agencies responsible for introduction of new medical treatments.

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

In the short/medium term, during the next 5 years, the work will generate new information about diseases that cause heart attack and poor circulation to vital organs in the body. We will pay particular attention to vascular cells that have not been investigated sufficiently in the past, such as pericytes, which are regenerative cells lining around the small and large vessels. One major goal is to incite the pericytes to act as building blocks to rebuild new and well functioning blood vessels. We will also work in the lab at producing new tube grafts containing pericytes and other vascular cells. We expect that, once implanted, these grafts containing human vascular cells will be superior to the ones used by surgeons to bypass blocked circulation.

In the long term, the work is expected to lead to clinical trials in patients. In due course, the work will be of direct benefit to patients and reduce the burden on the national health system and society caused by the disabling effects of vascular disease.

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

The full demonstration that the proposed methods are valid and applicable to patients require skills and expertise that cannot be found in a single laboratory. We will therefore collaborate with other investigators, including doctors, surgeons, and scientists, to achieve the best results.
Clinicians are the best partners for maximising the impact of our research toward patients' benefit, but also to inspire new ideas from the bedside back to the bench. Scientists, with whom we collaborate, can bring new technologies and methods to the research.

We have established contacts with several small and large pharmaceutical companies (where some of our previous research fellows have relocated) and consult them frequently to see if they can be interested in helping us to develop our research and make faster the application to the clinic.

Results will be disseminated through scientific publications and presentations at national and international meetings. This is the most effective method to speed up the progress of science among the experts in the field. We will make sure that methods and results in publications contain enough details for other scientists to repeat the experiments and confirm or extend the acquired knowledge. The data will be communicated, either they confirm (positive results) or reject (negative results) the initial hypothesis. The report of data will follow the current international guidelines.

We have extensive experience in communicating the results through press release, having had articles covered in national news media (radio, television, and newspapers). The team is also experienced in communicating with lay persons through seminars to patients' forums and public lectures. We will use all the above methods to communicate results to the public and patients' associations after consultation with the Research and Development office.

Species and numbers of animals expected to be used

- Mice: 1350

Predicted harms

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

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

We use mice, which are the mammals with the lowest neurophysiological sensitivity suitable for these studies. Wherever possible, we use the least severe model for our investigations. The disease we want to investigate occurs during adulthood and advanced age, which requires using adult and older models.

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

The animals will be subjected to surgical procedures to create the condition of blood flow blockade to the heart or limbs as occurs in patients. In other experiments, animals will not be operated but the natural evolution of vascular disease will be evaluated. Treatments will be given through local injections or systemic route. The duration of experiments will last from 2 weeks to 4-6 months, and we plan to use ~1350 animals during the 5 years of the project.

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

Transitory pain after surgical induction of ischaemia will occur like that experienced by patients with a heart attack. Occasionally, we expect loss of weight and difficulty in breathing caused by heart failure and poor ambulation due to reduced blood flow to the lower extremities occurring for the maximum duration of 2 weeks.

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

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

50% mild, 25% moderate, 25% severe.

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

- Killed

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

We will be using mice for these studies because the complexity of cardiovascular disease and the efficacy of proposed treatments cannot be effectively tested in non-animal systems. However, preliminary studies of cell functions will be carried out before engaging with animal studies. In addition, simulation experiments are planned.

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

Cellular models. Simulation of blood flow alterations using theoretical modelling.

Why were they not suitable?

Because the final demonstration of efficacy requires the complexity of a living organism.

A retrospective assessment of replacement will be due by 21 April 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?

Based on an average number of 30 animals per experiment for a total of ~45 experiments in 5 years.

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

Well designed and correctly analysed experiments can lead to a reduction in animal use whilst increasing the scientific validity of the results. To this aim, we constantly refer to the NC3R's Experimental Design Assistant as a guideline.

Here, we summarise the main steps considered in the experimental design to reduce the number of animals, control bias and ensure that the results are scientifically valid.
Pilot studies comprising a small number of animals to generate preliminary data and/or allow the procedures and techniques to be solidified and “perfected” before large-scale experimentation.

The minimum number of needed subjects will be calculated based on the expected average benefit and the expected variability of the benefit (assessed from the literature or pilot studies).

The allocation of animals to different groups of treatment will be at random: 1) to avoid biases, 2) to guarantee that groups have the same probability to receive a treatment, and 3) to control experimental variability. When planning attribution of animals to groups, the assignment of animals at random to different groups and sub-groups will be improved by dividing animals in blocks to achieve minimal variation.

We prefer to use genetically selected strains of mice because they show a more homogeneous response to the disease, meaning lower variability will allow a reduction in number of animals needed.

Important variables as sex, age and weight of the animal should be similar among the groups, again allowing reduction in variability.

Our facilities provide state of the art in environmental enrichment. We ensure that all the animals are exposed to the same enriched environment.

Collection of data will be done in a manner that the treated and control group have their measures collected at similar time and by the same investigator.

Biases will be avoided by ensuring that researchers analysing experimental outcomes are unaware of the treatment received (blinded) until the final statistical analysis.
Data will be treated according to the principle intention-to-treat, where all participants who are randomised are included in the statistical analysis and analysed according to the group they were originally assigned, regardless of what treatment (if any) they received

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

We based our optimal number of animals and experiments based on experience in previous successful projects, pilot data and computer modelling. We also referred to the NC3R's Experimental Design Assistant to ensure that only the minimize number of animals are used.

We will use the best available technology and make sure that all the equipment is regularly calibrated for precise measurements.

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

Mice are the less neurological developed species that can be used to test the therapeutic interventions we hope to progress into the clinical application. We will use the best measures and practices to attenuate pain, discomfort, infections, and stress. We will adopt refined microsurgical techniques to minimise the adverse effects of surgery. All animals will be carefully monitored after surgery and recorded individually. Wherever possible animals will be group housed and provided with enriched environment. Interventions to assess pain and suffering are refined to provide the maximum benefit for restoring wellbeing without interfering with the physiology of the animals. Therefore, observation frequency is calibrated to the risk of adverse events, which is highest during post-operative surgery. The definition of stress and pain severity is addressed using sensitive scales that have been elaborated for specific use in the specific protocol, for instance to identify low level of pain in mice with heart attack or occluded leg arteries. We also designed the protocol in a way the goal of the research is reached before animals reach the most severe outcome.

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

Less sentient animals, e.g. fish or reptiles, cannot be used because do not reproduce the type of response a human being put in place following injury of the adult cardiovascular system.

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

We have monitoring charts and post-operative charts in place which will be adjourned and improved as new evidence emerges from real experimentation or the available literature.

Where appropriate, the animals will be trained to drug administration regimen associated with the lowest possible stress.

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

Surgical procedures will be undertaken in line with the recommendation set out in the LASA and institutional guidelines for aseptic surgery. Injection and drug administration will be conducted in line with NC3Rs recommendations.

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

We will keep informed about advanced in 3R by reading the literature, attending courses and webinars. In addition, we will attend at least 1 meeting every year on animal welfare, such as the RSPCA/UFAW Rodent and Welfare Meeting or other 3Rs symposium.

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

17. Implications of therapeutic inhibition of the complement system on infectious and non-infectious inflammatory diseases

Project duration

5 years 0 months

Project purpose

- Basic research

Key words

Complement System, Kidney injury, Microbial infection, Lung injury, Monoclonal antibodies

Animal types	Life stages
Mice	adult, juvenile, neonate, pregnant, embryo
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 validate the utility and efficacy of a novel therapeutic technology to reduce lung and kidney injury in some diseases. We will also look for possible side effects that may result from using this technology like an increase in the risk of infection with different pathogens and how to reduce this risk.

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

The complement system (a group of circulating proteins in the blood) is an important component of the immune system that participates in recognition and killing of invading microbes organisms. Activation of the complement system also triggers the inflammatory response. Uncontrolled activation can cause tissue injury and the development of some inflammatory diseases. Development of inhibitors that block the complement system in the blood will open a new avenue for novel approaches and strategies for the treatment of inflammatory diseases disorders. Unfortunately, the use of complement inhibitors may increase the risk of microbial infection in patients. Hence, it is important to address the fundamental interactions between the host immune system and different pathogens during the course of active infection. We will also introduce a new therapeutic approach for treating microbial and fungal infections using specific antibodies produced in mice and rats. These antibodies will be evaluated for their therapeutic benefits in mouse models.

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

Our research in the next five years will provide novel findings that will help to identify novel therapeutic approaches that will help to alleviate complement-mediated tissue injury, especially in the case of haemolytic uremic syndrome (HUS), lupus nephritis and ARDS. ARDS is one of the major complications of SARS-COV-2 infection that was responsible for increasing the rate of mortality among COVID-19 patients. It is now very well known that activation of the complement system in lung tissues has a major role in developing ARDS. Our work will evaluate how complement inhibition will improve lung function in a mouse model of ARDS induced by LPS or acid.

In addition, our results will provide a better understanding of how the immune system behaves towards invading pathogens, especially in case of complement deficiency. We will also study how the complement system interacts with different pathogens including Klebsiella pneumoniae, an encapsulated Gram-negative bacterium that was associated with increasing the rate of morbidity and mortality among COVID-19 patients during the current COVID-19 pandemic. The knowledge that we will add on how the immune system interacts with different pathogens in this project especially with K.

pneumoniae will help to develop new strategies for the treatment of bacterial and fungal infections using vaccination protocols or by monoclonal antibodies that binds to the microbes and facilitate complement-mediated bacterial killing. We always publish our new findings in high-impact factor scientific journals to disseminate them to the scientific community. Unsuccessful approaches will also be highlighted in our publications and discussed with our collaborators.

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

In the short term, the outputs will be communicated to academics working in the field of immunology, where we will assess both the benefits and the harmful effects resulting from complement activation and address the delicate balance between complement activation and down-regulation in health and disease.

In the long term, we will introduce a new and well-defined study in the field of complement immunology during infection that will help to better understand how the immune system fights microbes, and how to avoid the harmful effects resulting from uncontrolled activation of the immune system. This work might also introduce new therapeutic approaches that will help to reduce kidney injury in lupus nephritis and HUS and improve the outcome in these patients.

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

Our group has a close collaboration with experienced Senior Scientists in our field, who have established track records in mouse models of experimentally induced infectious disease, stretching over several decades whom I meet on a regular basis to discuss results and future experiments. A complete survey on the published data has already been done and we know exactly what is published in our area of study. We will use the most refined and the most reliable models for our infection studies as well as for HUS and lupus nephritis models. We always publish our new findings in high-impact factor scientific journals to introduce it to the scientific community. Unsuccessful approaches will also be discussed with our collaborators and will be mentioned in our publications. Sharing animal tissue from our well-designed experiment with our collaborators will help to maximise the benefits.

Species and numbers of animals expected to be used

- Mice: 2200
- Rats: 50

Predicted harms

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

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

We will use adult wild-type and adult complement-deficient mice that lack one or more of the essential components of the immune system. Using these mice will give a clear overview of how the absence of these immune components will affect the infectivity and pathogenicity of different pathogens included in this project. We will also use adult MRL-lpr/lpr (Lupus) mouse, a strain that is known to spontaneously develop lupus nephritis and kidney injury after 14 weeks of birth. This model will help us to validate how inhibition of the complement system will improve kidney function in such mouse model.

We will use adult mice in our models of diseases because the immune response at that stage captures the essential traits of many bacterial infections and tissue injury in humans. For monoclonal antibody production, we will use adult mice and rats because the animals at that stage have a well-developed immune system that will initiate an immune response. This immune response stimulate B cells to differentiate and produce antibodies.

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

In our mouse models of microbial infections:

Mice will receive a specific bacterial/fungal dose, or it is toxin via internasal or oral administration as well as by injection.
Mice will be observed for disease progression.
Immunomdulatory and/or complement inhibitors therapeutics could be injected at any time before or after the infection.
Blood samples will be taken at different time points post-infection to assess the bacterial load in blood to avoid any complications that may increase the severity of disease progression beyond the expected severity limit.
Our experience shows that the infection study in HUS model will last for 3 weeks while other infection models will last for 7 to 14 days.

In the case of lupus nephritis model:

Mice at the age of 14 weeks will start to develop signs and symptoms of renal injury.
At that age we will treat mice with immunomodulatory and/or complement therapeutics that will reduce complement-mediated kidney injury.
In some experiments, mice will be treated with complement inhibitors therapeutics/immunomodulatory compounds at earlier ages (10 -12 weeks).
This study will continue for 8 to 10 weeks
All animal experiments will be randomised and people who are assessing the animal will not be aware of whether they are assessing control or treated mice.
In the case of ARDS model:
Mice will receive a specific dose of LPS or diluted HCL via internasal (of by injection in case of LPS only)
Mice will be observed for the symptoms of ARDS.
Immunomdulatory and/or complement inhibitors therapeutics could be injected at any time before or after the infection.

For monoclonal antibodies production:

Mice/rats will be immunised with a specific recombinant antigen.
Animals will be boosted 4 to 7 times until a high antibody titre in blood is detected.
Animals will be euthanised and spleens will be collected.

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

In models of Haemolytic uremic syndrome (HUS) and acute respiratory distress syndrome (ARDS), a robust inflammatory response occurs shortly after infection where mice show signs and symptoms that exceed the moderate severity limit. Animals will show symptoms such as stary coat, piloerection, lack of grooming, and hunched posture as well as a reduction in their activity within the cage, respiratory distress, and lethargy. In HUS model, mice injected with purified Shiga toxin (a toxin produced by specific strains of E. coli that cause kidney injury) may also display hind limb paresis, and shivering. A decrease in body weight by up to 20% will also be observed. Mice will be immediately euthanised if not respond to external stimuli.

In mouse models of infection, mice will show signs and symptoms of moderate severity limit including a reduction in their activity within the cage and a decrease in body weight. Animals will be closely observed as frequently as necessary and at least every 6 hours to ensure these symptoms will not last for more than 12 hours. If these signs persist for over 12 hours or if an animal begins to deteriorate at any time, it will be immediately killed.

When mice show signs and symptoms of severe severity limit, mice will be observed and scored every 2-3 hours once they develop signs and symptoms of disease progression to ensure that these symptoms will not last for more than 12 hours. If these signs persist for over 12 hours or if an animal begins to deteriorate at any time, it will be immediately killed. Lupus nephritis is inflammation of the kidney due to an auto-immune disease known as systemic lupus erythematous. With lupus, the body's immune system targets its own body tissues causing kidney inflammation and kidney injury. In a mouse model of lupus nephritis, mice will start to show some complications depending on the stage of the disease progression and the age of the mouse. From 1416 weeks old, mice will show symptoms of mild disease severity such as a decrease in body weight (510%) and a slight increase in some biological markers that reflect how kidneys are functioning such as blood urea nitrogen (BUN) and creatinine levels in the blood. Older mice will show a moderate decrease in body weight (10-15%) and significantly elevated BUN and creatinine levels. Because the strain of mice that we will use in this model spontaneously develop auto-immune disorders, mice will also show some other side effects such as enlarged lymph nodes and skin lesions that will be considered during the course of our experiment. Immunisation of mice or rats either subcutaneously or via intraperitoneal route has a limited side effects and we do not expect any change in the animal behaviour. These side effects include swelling, redness and inflammation at the site of the injection.

Animals will be humanely killed once they reach the predetermined endpoint for this protocol.

General signs and symptoms of different degrees of severity limits

	Mild	Moderate	Severe
Piloerection	Partial piloerection could be observed especially after dosing where mice would recover within 24-48 hr if no other clinical signs observed	Staring coat-marked piloerection	Staring coat-marked piloerection
Body posture	Hunched posture could be observed especially after dosing where mice will recover within 24-48 hr if no other clinical signs observed	Hunched posture with stiff movement	Hunched with no movement (frozen)
Behaviour and activity	No deviation for normal behaviour	Animal shows subdued behaviour pattern and activity when provoked	Unresponsive to external stimuli and provocation (lethargy)
Respiration	No deviation for normal behaviour	Shortness of breathing	Laboured respiration
Tremors	Not expected	Mild tremors could be observed	Persistent tremors
Paresis	Not expected	Not expected	Only in case of Shiga toxin toxicity
Prostration	Not expected	Intermittent prostration	Prolonged prostration
Maximum Body weight loss	Weight loss up to 10%	If clinical signs do occur weight loss up to 15% (end point) may be observed	In case of clinical signs and weight loss up to 20%, mice will be euthanised. In case of no clinical signs weight loss up to 20 where mice will be allowed to recover for 48 h as long as body weight loss not exceeded 25%. If no recovery is observed mice will be euthanised

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

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

500 adult mice will be used at a severe severity limit (All animals).
1500 adult mice will be used at a moderate severity limit (All animals).
1200 adult mice will be used at a mild severity limit (All animals).
50 adult rats will be used at a mild severity limit (all animals)

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

- Killed

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

We will study how the immune response mediates tissue injury in some auto-immune diseases such as Haemolytic Uremic Syndrome (HUS) and lupus nephritis. Our work will also evaluate the role of the complement system during acute respiratory distress syndrome (ARDS), a lung injury that is always associated with the activation of complement with subsequent production of cytokine storm that might cause respiratory failure and death.

In addition, we need to study the complex interaction between the innate immune response and different pathogens. These processes involve a complex interaction between immune cells, plasma proteins, the blood vessel walls (vascular endothelium) and organ-specific cells that cannot be modeled using ex vivo (using animal tissues in cell culture) or in vitro (in test tubes rather than in animals) systems.

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

Prior to animal experimentation extensive in vitro studies using different techniques to assess the ability of different proteins from the immune system to bind bacteria such as (ELISA and FACS analysis techniques). Opsonophagocytosis assays (engulfment of bacteria by white blood cells isolated from human blood) will be performed to identify which strains/serotypes of the pathogen will stimulate the targeted pathway of the complement system. Assessment of the functional activities of the complement inhibitors will be completed first in-vitro using ELISA and FACS analysis to evaluate the specificity and efficacy of these complement inhibitors. We considered the possibility of limiting the infection experiments to ex-vivo studies using blood from human volunteers as a source of complement and cells. Ex-vivo studies do not reproduce the course of the infection in multi-organ model (i.e in animals)

Why were they not suitable?

It is necessary to study microbial interaction with the immune system in a whole mammalian organism to appreciate the impact of medical treatments on:

The spread of the bacteria between different organs in the body
Tissue pathology in various organs
The effects of the progressive escalation of immune responses
In addition, we need to assess the degree of kidney injury as a result of uncontrolled complement activation in some autoimmune disorders such as lupus nephritis and HUS. This study requires the tissue to be exposed to different complement components and

recognition molecules for a prolonged period of time to achieve tissue injury and there is no available ex-vivo model for such complications.
In ARDS mouse model, severe lung injury must be induced to mimic the critical situation in humans which is normally associated with massive infiltration of inflammatory cells and activation of the complement system with the initiation of cytokine storm, a condition that cannot be replaced by ex-vivo models.
For the production of monoclonal antibodies, it is essential to use spleen cells from mice/rats. Other alternative techniques are not suitable for our project.

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

Our long-term experience in animal work, using good experimental design helped us to give the estimated numbers of animals that we will need to use in each experiment. We also consulted with experienced scientists in our field and within our group. In addition, we consulted a departmental statistician who gave us advice and help whenever needed. Our previously similar animal models published in high impact factor scientific journals were also a useful guide to estimate the number of animals that will be used in this project.

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

Whenever possible we perform preliminary studies using smaller numbers of animals. We also try to adjust our work to use only one control group that can be used for different experiments. We use the smallest possible experimental number of animals for each experiment being very careful that this does not affect the accuracy of the results. To calculate the smallest number of animals that we can use, calculations based on advanced statistics and mathematics in addition to other published results were taken into considerations. Online tools such as experimental design assistant (EDA) from NC3Rs website were used to perform sample size calculations. To increase the quality, reproducibility and translatability of our animal studies we will follow PREPARE guidelines (Adrian et al., 2018). Animal tissues from different experiments will be kept frozen and/or embedded in paraffin blocks that can be used later if more tissue analysis is 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 always run pilot experiments using a small number of animals to assess the feasibility of the study.

In some experiments we will use bacterial toxins instead of using the bacteria. This will help to minimise the side effects where mice will recieve only the calculated dose of the bacterial toxins that show the symptoms and the signs of disease without exposing animals to the bacteria. We will run pilot experiments to test the potency of the bacterial toxins and so large stock of bacterial toxins will be purchased to avoid repeating this step every time we buy a new batch.

We would not start any animal work in large groups until we have preliminary positive and encouraging data. Tissues from pilot experiments and major experiments will be kept frozen or fixed in paraffin blocks for future use. For genetically modified mice, we will follow a breeding protocol that gives us only our gene-targeted mice by breeding deficient mice whenever possible to avoid the production of unnecessary mice.

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

All animal models in this work are designed to induce the required severity limits necessary to mimic the disease progression in humans. All the necessary procedures will be taken to achieve high levels of welfare to the animal starting from good husbandry and animal care before starting the experimental procedures. We will use the most refined mouse model of infection for each pathogen. During the experimental procedures in the infection studies (Protocols 1,2 and 5) All requirements will be taken to cause less stress to the animals during the experimental procedures. e.g. In the case of i.n (intranasal) infection we will use light anaesthesia to reduce animal stress and maximise the volume being delivered. We will use small gauges and fine needles to minimise trauma in the case of intravenous and intra-peritoneal drug administration. Mice will be monitored for any unexpected signs and symptoms of disease progression to avoid exceeding the allowed severity limit. Mice will be euthanised at early endpoints to avoid exceeding the allowed severity limit.

In protocol 5, mice will be infected with LPS from E. coli or acid (diluted HCL) to induce ARDS. In this model, we will use the lowest possible concentration of LPS or acid to induce lung injury. Also, mice will be monitored closely to avoid the disease severity exceeding the allowed severity limit. Mice will be also monitored for signs of disease progression including stary coat, piloerection, lack of grooming, hunched posture, decrease in body weight as well as a reduction in their activity within the cage. Mice will be also monitored for respiratory dysfunction such as rapid breathing, shortness of breath, or

laboured respiration. We will also use a highly purified form of LPS with the lowest concentration of protein and RNA contaminants.

In the lupus nephritis (kidney injury) model (Protocol 3), we will use a specific strain of mice that spontaneously develop kidney disorders and validate our complement inhibitor therapeutics to prevent the onset of disease progression. Lupus-prone mice will survive normally without any complications until they reach the age of 14 weeks when they start to develop signs and symptoms of lupus nephritis and kidney injury. These symptoms include elevated levels of creatinine and blood urea nitrogen and increased albumin and protein levels in urine. We will not inject any compounds that could accelerate kidney injury but ameliorate or prevent inflammation and tissue injury. We will use therapeutic compounds that are expected to minimise kidney injury and improve renal function.

We will immunise mice and rats with antigens emulsified with any of the commercially available adjuvants. The irritation of the skin is mainly due to the high concentration of the adjuvant (50% alum). We will decrease the concentration of the adjuvant (less than 50%) to avoid ulcer formation at the dorsal back of the animals. An optimum concentration of 25% Alum will be used especially with highly antigenic antigens. S.C injection of alum will be under light anaesthesia to reduce the risk of leakage of alum into the dermal layer of the skin that may lead to the formation of granuloma and skin irritation. The site of injection will be changed every time to avoid skin irritation. We will also use the smallest possible size of needles, minimal numbers, and frequency of repeated injections.

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

We need to study the immune response towards bacterial infection and how the immune response might cause tissue injury as a result of uncontrolled activation of the complement system. This will require animals to be exposed to the insults for a relatively long time (days or weeks) and this cannot be done in anaesthetised mice. In addition, using immature life stages of animals is not a good choice to study the immune response, especially since immature developmental stages of animals are likely to have an immature immune system and therefore will provide results that cannot be extrapolated to reflect the responses expected in mature animals.

Production of monoclonal antibodies requires animals with a mature immune system to elicit a proper immune response when immunised with different antigens. Animals at immature life stages will not produce the immune response required for the production of antibodies.

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

Pilot experiments will be always performed before doing the major experiment to avoid unnecessary use of a large number of animals. Tissues from pilot experiments will be also kept to be used as controls for future work. Mice will be closely monitored for any sign of disease progression. Mash (soaked mouse pellets which are easier to access and eat) will be used during our studies if needed to minimise weight loss. Any pain or stress will be diagnosed as early as possible, and animals will be culled by humane methods (schedule 1 methods) if necessary. S.C injection of alum will be under light anaesthesia to reduce the risk of leakage of alum into the dermal layer of the skin that may lead to the formation of granuloma and skin irritation. We will also use the lowest possible concentration of alum required to immunise the animals in this project.

A proper environment including shelter and a comfortable resting area will be provided to the animals. All clinical signs and manifestations will be scored in scoring sheets. These scoring sheets will be kept and discussed after each experiment to refine our procedures.

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

Our group has an excellent reputation in the field of complement immunology and we keep ourselves updated with all new and novel experimental designs and models in our field. This includes non-animal models. All newly published data in our field are discussed internally within our group to enrich our knowledge and that could help to find novel experimental designs or other non-animal models that could help in our study. In addition, all our experiments will be conducted in a way that allows us to publish our results according to the ARRIVE guidelines. we will follow all guidelines provided by LASA, PREPARE and NC3Rs.

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

By following the updates on NC3Rs and NORECOPA (websites as well as additional guidance literature from Laboratory Animal Science Association (LASA). Related events such as conference and symposium will be attended.

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

18. Infectivity and strain behaviour of prions

Project duration

5 years 0 months

Project purpose

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

Key words

BSE, Scrapie, Zoonoses, Infectivity, Strain Typing

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?

To determine potential prion infectivity in various tissues that they may be risk assessed for consumption by animals or man or to validate prion disinfection methods.
The strain typing of prions to determine the strain properties of prion that are responsible for known and emerging animal Transmissible Spongiform Encephalopathies (TSEs) with the intention to identify animal TSEs that can behave like BSE with particular emphasis on the zoonotic potential and the ability of Bovine Spongiform Encephalopathy (BSE) to overcome species barriers.

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

TSEs are chronic, incurable and fatal diseases of the brain caused by pathogens which mainly consist of a single protein termed prion. TSEs cause diseases in both humans and animals, including diseases that are zoonotic (passing from infected animal to man such) as BSE. All these diseases are currently untreatable and ultimately fatal.
This research is linked to statutory TSE surveillance schemes and it aims to minimise the impact of TSEs in animal and public health. The main focus of the research activities is to refine and enhance the existing bioassay methodology in order to improve the delivery of reliable data from sensitive surveillance cases as soon as possible.

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

The outputs and data that will be generated from this project will inform on the pathogenesis of TSEs, strain diversity of prions, qualitative and quantitative data regarding tissue infectivity, evaluation of decontamination methods and new in vitro diagnostic tests.

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

A large amount of this information is required by and has direct impact on government policy so it will be firstly communicated to the relevant authorities/laboratories as soon as data become available. Data will be submitted to and peer-reviewed by funding bodies, peer review via preparation of manuscripts for publication and presentation at national and international meetings.

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

As well as scientific publication, the research group is a member of various European and International collaborations, and advises policy makers in the UK and the OIE (World Organisation for Animal Health).

Species and numbers of animals expected to be used

- Mice: 9,000

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.

Transgenic mice are the biological system of choice for these studies. With the different prion transgenes inserted from cattle, sheep or bank voles the various transgenic lines

reflect the susceptibility of theses host species. Compared to the host species they offer considerable advantages on speed (months rather than years) and physical size, social structure and husbandry requirements are conducive to humane care in containment laboratory settings.

The established model uses mice at a few weeks of age as they are fully immunologically developed and dependent on the type of experiment being undertaken may have to last up to 1000 days.

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

The mice used in the project are transgenic. The transgenes are not harmful and all breeding is done under this project, Protocol 1.

The mice used for experimentation will be given a general anaesthetic under which an identification microchip is inserted and a small amount of material that is likely to contain a TSE is injected intracerebrally. The mice will be recovered from the anaesthetic and then if the material contain TSE's some months later they will start to develop clinical signs of TSE. They are clinically assessed regularly by trained staff, once the clinical signs are confirmed the mouse is euthanised and the clinical and pathology information is combined for analysis.

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

There will be impacts around the intracerebral injection of TSE for which anaesthesia and pain relief will be given.

The other impact will be as they develop clinical signs of TSE. They are regularly clinically assessed by trained staff, once the clinical signs are confirmed the mouse is euthanised. One of the transgenic lines currently used (TgSHPXI) has an adverse effect linked to its metabolism of fat in the mature animal (Abdominal Fat Necrosis). It generally occurs around 8mths of age in between 4 to 8% of mice of this line and once identified requires the affected mouse to be euthanased as it is progressive without a cure.

Due to the longevity of mice kept post inoculation, signs associated with ageing may be observed, e.g. intercurrent disease, incontinence, tumours, reduced mobility. Such incidences are treated as welfare cases and acted upon via NVS and/or NACWO instruction.

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)?

35% mild (breeding only), 55% moderate 15% severe .

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

- Killed

A retrospective assessment of these predicted harms will be due by 14 March 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?

Non-animal alternatives such as immunohistochemistry, Western blot, PMCA and RT-QuIC are used for statutory diagnosis and in research projects. However, none of these methods can assess infectivity or define fully strain characteristics. Only when information about infectivity or the finer detail of prion strain is required will the mouse bioassay be used.

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

TSEs agents lack nucleic acids and do not induce immune responses. Therefore none of the immunological and genomic approaches available can be applied to detect them.
In pursuing our efforts to minimise animal use we are committed in trying new approaches in diagnosing TSE agents and characterizing their strains. Therefore we have undertaken research into emerging technologies in the TSE field including cell culture , organ culture and PMCA were tested to identify prions.

For the last 30 years there have been several attempts to use cell cultures for detection of infectivity or for strain typing. However, there has been incremental progress in this field confined mainly to mouse adapted experimentally produced prion strains. The organ culture methodology is more recent but it has proved unreliable and similarly with the cell cultures it is not used any more in TSE diagnosis or research.

From these techniques only Protein Misfolding Cyclic Amplication (PMCA) promising in amplifying prions in vitro. However, this amplification may not necessarily be associated with infectivity. The organisation has contributed to this work under the previous licence and will further support the validation of this method as it represents the only realistic possibility in replacing partially mouse bioassays. Real Time Quaking Induced Conversion (RT-QuIC) is a more straightforward and reliable method for amplifying prions in vitro based on the same principle as PMCA. This technique is currently being established at the organisation included in the same project as bioassay and experiments have already been designed to compare it with mouse bioassays for TSE diagnosis.

Also in collaboration with other organisations we assess the ability of transgenic fruit fly (Drosophila Melanogaster) bioassays as an alternative to mammalian bioassays for detection of infectivity.

Under this Project Licence any emerging in vitro or non-mammalian bioassay method will always be validated against animal bioassays to identify if they can be adopted as suitable alternatives.

Why were they not suitable?

Currently only mouse bioassay can give the information about infectivity or the finer detail of prion strain as a clinical and pathological output is required to determine disease phenotype.

It is possible that in future PMCA, RT-QuIC or transgenic fruit flies may partially replace mammalian bioassays in TSE detection testing. However, currently no alternatives exist for the role of mammalian bioassay in strain typing.

A retrospective assessment of replacement will be due by 14 March 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 mice requested for this research represent a maximum estimate of the numbers required given current policy demands on established and emerging TSEs.
Based on data from the previous licences the number of animals under genetically altered mouse breeding protocol has been calculated as a realistic estimate to support production of mice for the experimental protocols.

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

Statistical input for studies has been and will continue to be sought to ensure validity of data from the organisations biostatisticians.

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

Research work during previous projects demonstrated that transgenic mice have superior sensitivity and specificity compared to wild type mice. As a result only transgenic mice are included in this licence. In infectivity experiments this results in a reduction from 20 down to 8 mice per group. When wild type mice were used for strain typing experiments serial passages were mandatory requiring at least 52 mice to characterise a strain. In contrast use of transgenic mice for similar experiments requires only 8 mice as strain typing can be achieved during first passage and serial passages are seldom required.
Three of the transgenic lines are homozygous. However, the fourth line cannot be kept homozygous, and there is no alternative homozygous line that expresses the same transgene. The breeding of all lines is linked to experimental requirements thus avoiding any excess breeding.

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

This project uses small panels of transgenic (genetically modified) mice for TSE strain typing. These mice have no animal welfare issues associated with the transgene. Anaesthesia and analgesia are used for the initial and only injection of potentially infectious material intracranially. Then there is an incubation period of generally several months during which time the mice are clinically normal but regularly neurologically monitored by trained staff. When consistent signs of TSE infection are seen the animal is humanely euthanised.

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

The evaluation of the pathology to define strains requires a fully developed central nervous system and clinical signs can only be observed in conscious fully developed animals.

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

We currently use post-operative analgesia, and monitoring regimes. The majority of inocula used are sterile for conventional pathogens, however in a small number of unavoidable cases there may be a concern over the inocula containing endotoxins. These inocula are heat treated and will initially only be given to a single mouse. If this mouse does not show any adverse reactions for 7 days the rest of the group will inoculated. If the mouse shows adverse effects it will euthanised. The inoculum then is diluted in 9 volumes of saline (1:10 final dilution) and the diluted inoculum is used to challenge a single mouse initially and if the mouse does not show any adverse reactions for 7 days the rest of the inoculations resume. If the mouse shows adverse effects it will euthanised and a further dilution (1:100) is tested as described above. If the single inoculated mouse shows any adverse effects in the 7 day post inoculation period then no further attempts to challenge mice with this inoculum are made.

When there is a concern over the presence of endotoxins increased post-operative monitoring regime is used to identify animals suffering from the effects and euthanase them if they are not going to recover from the effects.

For the diagnosis and identification of different types of TSEs at end of the experiment the mice have to have consistent clinical signs before humane euthanasia. Experience has shown taken to early, there are not sufficient pathological changes in the brain for analysis.

A fat metabolism issue has been identified in a small percentage of one of the transgenic lines. It occurs when they are mature (approximately 8 months of age) and the animals are euthanased when the problem is diagnosed.

Regular monthly meetings involving the NVS, NACWO, animal care and laboratory staff , personal and project licence holders to ensure current knowledge is brought to bear. All aspects of the performance of the bioassay are discussed, what is going well and if there was anything that could be done better. If there are any suggestions for refining the procedure they will be considered and if appropriate, incorporated into the protocol.

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

Home Office The Harm–Benefit Analysis Process
Home Office Guidance to ASPA
Home Office Code of practice
RSPCA Guidance on Welfare of mice

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

I have regular contact with the NIO, NACWO and NVS through various forums and use of the library function which can scan for relevant publications. In developing this work I have been in contact with other researchers outside of the organisation who also specialise in the TSE field and any viable alternatives to mouse bioassay will be continually pursued.

A retrospective assessment of refinement will be due by 14 March 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. Intestinal failure in neonates and adults: mechanisms, protection and repair

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

Ischemia, Reperfusion, Nectrotizing, Colitis, Stem Cell

Animal types	Life stages
Mice	adult, neonate
Rats	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.

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 project is to further our understanding of the pathophysiology of intestinal failure and test the efficacy of novel stem cell derived therapeutic strategies that may have implications for the treatment of these conditions in humans.

A retrospective assessment of these aims will be due by 16 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 it's aims and if not, why not?

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

Why is it important to undertake this work?

Lack of blood flow induces damage to the gut during neonatal as well as adult period of life, which results in severe consequences to human health. Presently, there are no treatments to cure patients who experiences such episodes. Previously, studies have shown that molecules produced by stem cells promote tissue regeneration when these molecules are given to an individual from whom the stem cells were initially isolated. Here we will test for the first time whether stem cells or molecules that they produce derived from one animal or human can alter the disease progression of a gut of another animal that has experienced periods of ischemia.

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

Knowledge gaps need be filled regarding the mechanisms of intestinal failure in two settings: Ischemia reperfusion injury (IRI) and Necrotizing enterocolitis (NEC) .
Improved scientific understanding of the mechanisms of injury by NEC and its sequelae (short bowel syndrome and neurodevelopmental complications) is essential for the development of effective therapies against the disease. This will be achieved through analysing the tissue at the histological level, profiling the expression of key proteins that control the major mechanisms underpinning tissue homeostasis, as well as key gene markers reporting on tissue health.

A short-term benefit of this proposal will be to define the mechanisms of pathology and how they can be modulated by stem cell factors in rodent models
The key outputs will be the generation of new information, which will be communicated via publications in peer reviewed international journals.

Provision of this information will allow usage by third parties.

The outcomes of these studies will be made available to interested parties through a number of routes. Firstly, we will publish our work in international peer reviewed journals. Our work will also be communicated at national and internal conferences and workshops. All raw data will be made available upon request following clearance for the provision of this information from the University Data protection unit.

The long-term benefits of this project will be the translation of knowledge gained in establishing the stem cell secretome based therapy to these pathologies. This long-term goal will require a tiered process that involves safety testing, testing in a non-rodent model for efficacy before entering a phase one clinical trial (first in human). Our collaborators already have an established pig model for both conditions which will accelerate the translation of positive results from this licence should they arise. Furthermore, the translational route will be accelerated by partnering with clinical teams who are closely involved in the training aspects of this project.

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

The short-term benefits of this proposal will be gaining mechanistic insight into the two indications at multiple levels, ranging from whole organ function down to the identification of molecules that are perturbed as a conscience of the diseases. The identification of aberrant molecules is likely to be of particular benefit to third parties as they themselves could become therapeutic targets. Therefore, the short-term benefits are like to be important to not only the general scientific community but also to groups around the world working on these diseases.

Long-term benefits include the development of novel efficacious therapeutic strategies for patients with NEC and IRI for their improvement of their quality of life. This is an important target as NEC and IRI is associated with significant morbidity (short- and long-term) and mortality. Caring for such patients is difficult for relatives and healthcare professionals and has significant impact on limited resources. In order to achieve this, it is essential to appreciate that NEC and IRI are multi-factorial diseases. In the present project, we account for this by using two distinct models of “NEC-like” injury. The therapies we will investigate include stem cells or factors derived from stem cells in both a rat and mouse model of IRI and in rat for NEC by various routes of infusion relevant to clinical translation in animals at appropriate stages of development relative to the indication.

The clinical use of differentiated or undifferentiated stem cell conditioned media provides an attractive, less invasive, safer alternative (minimising cancer, reducing immunological risks) to stem cell transplantation. The benefits could be in future be reaped by those who suffer with ischemic-induced damage of the gut.

However, this is only the first step towards use in the community. Future studies will require safety testing a process that is likely to take two to three years after the successful completion of this project.

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

We will communicate the results arising from this study by presenting our progress at both national and international conferences and meetings as well as communicating completed studies in the form of publications.

Species and numbers of animals expected to be used

- Mice: 1000
- Rats: 2300

Predicted harms

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

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

Rodents are being used in this project as they can be used to develop the symptoms of the diseases that are the focus of this project: Ischemic Reperfusion injury (IRI) and Necrotising Enterocolitis (NEC).

The IRI work and work on NEC will be conducted on adult rodents and new-born rodents respectively, as these developmental ages mimic the times of respective disease onset in humans. Both animal models were first developed in rat due to the size of organs that need to be manipulated. We will establish the protocols in the rat and then move to the mouse for IRI. The mouse is a more attractive model due to logistical reasons in the first instance as they are more readily maintained (smaller cages, and cheaper to maintain) Secondly after demonstrating that we can perform IRI in mice we hope (through amendments to the license) to work on transgenics.. If unsuccessful, then we will conduct all our studies in the rat.

All work on NEC will be carried out in rat due to the size of newborn pups and the type of intervention that is required to induce NEC. This work in the first instance would be technically challenging, although not impossible in mice.

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

Typically, rodents will be maintained until the desired age and then be induced to develop a disease state, which will be achieved either through surgical intervention (Ischemic Reperfusion injury (IRI)) or treatment comprising of exposure to reagents and stressful conditions (Necrotising Enterocolitis (NEC)).
Thereafter they will be treated with a stem cell secretome, which has potential therapeutic properties.
The treatment will be allowed to have time to work, during which time the rodents will be monitored to assess the impact of the treatment.
The experiment will be terminated at a set period. The animals will then undergo physiological examination which will allow us to gauge how well the tissues are working.
Thereafter the animals will be killed, and tissues collected for further investigation aimed at revealing how the treatment has altered the course of the disease.

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

The interventions described in this license will compromise the working of the gut, which will have impact on both body weight and potentially other physiological processes.
Examples of adverse effects could include hunched posture, piloerection, anorexia, septicaemia, abdominal pain, inactivity, foam coming out of the mouth.
Additionally, rodents may show signs of general malaise and decreased activity.
We will implement rigorous monitoring regimes which will allow us to identify any signs of adverse effects and depending on the nature of the adverse effect we will either stop the intervention followed by a recovery protocol or humane kill the animal to prevent further pain, suffering and distress.

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

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

The most severe category is severe which will be experience by no more than 15% of the animals in this proposal. A small proportion of animals will be used in studies to evaluate whether the treatments improve survival.
However through the use of a rigorous monitoring regime informing of when an animal shows signs of no longer being recoverable, we will perform humane killing to minimise pain, suffering and distress.

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

- Killed

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

The objective of the project is to explore the clinical benefit of stem cell secretory factors on ischemic gut disease in adult and neonates. Mice and rats are the lowest sentient species that can be used to reach our objectives. Alternative experimental models for research into gut diseases have been developed including those carried out in test tubes or with whole animals including amphibians and fish. However, none of the latter faithfully replicate the condition in humans.

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

With some aspects of the project, experimental animals can be replaced by in-vitro testing, and as outlined in the programme of work such in-vitro testing will replace intact animal experimentation wherever possible in the project. Such in-vitro testing will include appropriate dissociated cell culture, cell line testing and testing on organoid culture preparations appropriate for each disease model. Organoids technology is relatively new and allows scientists to grow a 3D collection of cells that have all the major cell types found in the human gut. Furthermore these have been used to develop models of necrotizing enterocolitis. We have extensive expertise in the development of gut organoids starting with tissues harvested from rodents.

Why were they not suitable?

While in-vitro studies are very informative on the impact of a disease or condition on a particular tissue, they have very little value when the nature of the disease or condition acts in the whole body. While organoids are incredibly informative when addressing key developmental issues related to the gut, they are presently still not a good model for studying the types of diseases investigated in this study. Organoid 3D architecture is not as regular as seen in vivo. More importantly organoids lack several essential components of the living digestive tract, such as the enteric nervous system, the vascular system, lymphatic systems and functional adaptive and innate immune systems. However co-culture protocols are being developed that allow numerous cells types to be grown at the same time. But, as more components are introduced, these compromise the development of the primary gut tissue.

Nevertheless, we will assess progress for outcomes that overcome these limitations. Thereafter, we can use these as testing ground prior to work in animals.

A retrospective assessment of replacement will be due by 16 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 number of animals estimated will allow us to carry out comprehensive studies to evaluate the efficacy of stem cell-based treatment for ischemic gut injuries. The investigations will involve examining the animals as well as tissues using a battery of tools which necessitate the provision of starting material for each study. The experiments have been designed to generate statistically significant data, which allows us to reach quantitative conclusions regarding the efficacy of the intervention.

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

Generally, advice will be sought from our Consultant Statistician, where appropriate, to ensure a statistical design that is used is efficient and minimises the number of animals required yet maintains sufficient precision and power. For example, advice will be sought when a specific pre-clinical model introduces large subject-to-subject variation. Furthermore, a number of studies including our own have shown that the impact of IRI and NEC effect more than just the primary tissue under investigation, that being the gut. Therefore, in order for an intervention to be examined further for possible use in humans, it is now apparent that an intervention should be examine at the tissue functional level as well as in non-gut tissue, in particular the nervous system. We have developed a pipeline that extracts the maximal amount of data from each animal by not only determining gut function whilst the animal is alive, but then to isolate as many tissues as possible after the animal has been killed. This approach means that a single animal will generate data related to a wide variety of bodily functions.

We hope that the advent of robust and complex organoid based in-vitro culture systems will allow us to generate guiding and pilot data that allows us to conduct more precise experiments in live animals. For example when robust gut complex gut organoid protocols become available, we will carry out experiments to establish optimal doses of condition media before transitioning into animal based studies.

We will also use experimental protocols for both conditions that have been optimised over a number of decades for our studies. These protocols are the most robust yet and will have an impact on the number of animals being used. Again we will survey literature for developments that further refine them in-order to reduce animal numbers for a particular outcome.

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

The proposal aims to minimize animal usage by drawing on our extensive experience in this area of research. These insights will be deployed not only to keep the number of mice

and rats to their lowest possible number but also to conduct the experiments in the shortest time span thus minimizing animal suffering.

A retrospective assessment of reduction will be due by 16 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.

For both ischemic-reperfusion injury (IR) and necrotising enterocolitis (NEC) it is necessary to induce damage to the gut. Pain and suffering will be minimised through use of appropriate anaesthetic and analgesic regimes. The models selected provide the most data on paracrine mediated stem cell induced repair of tissue damage and will generate data for our company and others to develop definitive therapies while answering basic questions on stem cell biology.

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

Small rodents (mouse: rat) are the simplest appropriate pre-clinical models to study these diseases and their potential amelioration. While there are species differences in physiology between humans and rodents, these are minimal and the use of species phylogenetically closer to humans is not required by the appropriate regulatory bodies and thus is not proposed as part of this project. Rodents are the animals of choice since they are amenable to surgical interventions whose outcome leads to tissue destruction very similar to IR and NEC.

Non-vertebrate models including nematode worms and fruit-flies do not share the same physiological parameters as humans and are thus not informative for this type of study. Fish and amphibians have gastro-intestinal systems more similar to mammals than non-vertebrates. However, they are quite distinct to mammalian guts including major changes to the 4 distinctive layers found in rodents and humans. These changes could reflect their great reliance on an aquatic environment for their survival and differences in the diets. They are not good models for the diseases being investigated in this project.

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

The procedures to induce IR and NEC have been developed over decades and refined to delivery intended scientific outcomes with the least suffering to the animals. We will use the most optimised versions of these protocols available.

Importantly these protocols have been developed and refined, in order to best mimic the human condition and at the same time deliver this desirable outcome with the use of the smallest number of animals, by our partners who will be intimately involved in this project. These include some of the most eminent neonatal surgeons in the world. They will provide the expertise and oversight to this project. They will not only roll out the project but also ensure that all experimenters are fully competent in performing all aspects of the project. As outline in our lifespan study, the use of paired animals (1 untreated: 1 treated) allows us to reach our endpoint (evaluating the impact of the intervention on survival) with the fewest possible animals.

For all surgical interventions, monitoring of the animals during recovery may be extended or increase in frequency if required. Furthermore, appropriate anaesthetic and analgesic regimes may be deployed to help manage pain.

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

We will seek to carry out the experiments using best practice advised by differing parties including:
Laboratory Animal Science Association (LASA) for best practice guidelines for administration of substances and genetically modified mouse welfare guidelines.

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

We will seek advice from the Home Office inspector as well the named veterinary surgeon for alternative approaches using either less sentient animals or non-animal systems that allow us to achieve our goals.

A retrospective assessment of refinement will be due by 16 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?

20. Investigation of influenza virus infections in animals as models for human 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

Influenza, Virus, Infections, Pathogenesis, Vaccines

Animal types	Life stages
Ferrets	juvenile, adult
Pigs	juvenile, adult
chicken, duck, turkey	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.

Objectives and benefits

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

What's the aim of this project?

The aim of this project is to improve animal and public health and welfare in relation to influenza virus infection. This will be achieved by improving our understanding of viral pathogenesis and dissemination as well as by investigating intervention strategies applicable to viral transmission in animals and humans, including across the human-animal interface. Animal models of human disease will be employed to investigate these factors.

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

Animal Influenza viruses have the capacity to infect humans and so can have a Public Heath impact. Indeed, pandemic influenza is included in the UK National Risk register of Civil Emergencies. This licence therefore provides the essential capability to safeguard the UK by enabling research into influenza viruses that have the potential to cause pandemics in humans. Scientific results are communicated to stakeholders who include academia, biopharmaceutical companies and government organisations in the UK, EU, USA as well as internationally.

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

The Objectives for this licence will support scientific capability to investigate influenza viruses that are constantly evolving, necessitating continued monitoring and research. Objective 1 will specifically provide key reagents and tools to characterize influenza virus antigens, assess the antigenic relatedness of different influenza virus strains and data will be supplied to inform the selection of candidate vaccine viruses (CVVs) by international organisations.

Objective 2 will underpin characterization of influenza infection and outcome (pathogenesis) parameters and transmission of virus infections in a susceptible animal species (pigs and ferrets) as an animal model for humans. Disease intervention strategies will be investigated to inform epidemiological assessments and disease mitigation strategies. The studies will also include pre-clinical investigation of intervention strategies such as candidate vaccines and antiviral drugs.

Objective 3 will allow questions concerning species barriers and risks at the human-animal interface to be addressed.

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

Beneficiaries will include funders commissioning the research carried out e.g. funding agencies in the UK (MRC and BBSRC) and EU, governments (Defra, UK Department of Health, US-CDC) and biopharmaceutical companies conducting fundamental research. International organisations (Offlu, WHO) will also benefit from knowledge-sharing. Ultimately the general public and global influenza community will benefit from the enhanced capability to inform influenza risk and disease mitigation strategies worldwide.

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

The outputs of this work can be maximised through participation in collaborative studies with external organisations. As detailed, the establishment already has representation on several organisations and also has joint-funded projects that are active or in negotiation. This allows co-ordination of research efforts and good use of animals. Research findings are presented at external meetings and published.

The establishment ensures dissemination of knowledge to stakeholders, including Defra and PHE, in accordance with the UK Government data sharing policy and also within the remit for engagement with international organisations eg OIE, FAO, OFFLU, US-CDC, USDA and WHO. In the case of notifiable disease or increased risk, information is disseminated through expert reports and disease risk assessments.

The establishment ensures active engagement with stakeholders, for example through the organisation's evidence and policy teams.

Species and numbers of animals expected to be used

- Pigs: 400
- Ferrets: 425
- Domestic fowl: 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 pig and ferret species can be naturally infected with influenza viruses of relevance for human infection and are therefore biologically relevant hosts. In addition, influenza virus infection results in similar responses in the respiratory and immune systems in humans, pigs and ferrets. For these reasons, both pigs and ferrets are considered valid animal model species for human influenza virus infection. Where possible, an alternative to live animals is sought, for example cell or organ culture. However, for certain scientific questions where complex virus-host interactions need to be studied, it is not possible to replace a live animal. For example, vaccine efficacy investigations require study of the multifaceted interactions between virus and host immune system.

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

Procedures will involve inoculation of influenza virus with or without prior treatments. Treatments may include immune stimulation (eg inoculation of immunological reagents such as vaccines or antigens) or administration of antiviral therapies. Substances will be delivered by injection or into the nasal cavity. The study outcomes will generally be followed by analyzing longitudinal blood and nasal samples. Some studies to assess virus transmission (either directly or indirectly) will require co-housing of contact healthy animals with infected animals or introducing animals into a virus-contaminated environment. In some cases, for co-housing transmission studies, an avian host species will be infected as the natural host for an avian-origin strain of influenza virus. Study duration will depend on the study design and scientific question to be addressed. Generation of an immune response normally requires 4-10 weeks. Influenza virus infection is normally monitored for a duration of 5-14 days.

Single housing of ferrets is required for block design studies investigating virus transmission. This designs allows scientifically robust data to be obtained using the lowest number of ferrets whilst maximizing the number of replicate groups.

This design is deemed best practise by influenza research groups worldwide to maximise the scientific benefit while using the lowest possible number of animals and minimising adverse effects. (Belser J. et al ., 2022. DOI link: https://doi.org/10.1128/mbio.01174-22)

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

The main potential adverse effects are the clinical signs resulting from influenza virus infection which are predicted to be similar or milder to infection in humans. The severity of avian-origin influenza virus infections may vary from mild to severe in avian hosts and the severity in mammalian hosts is frequently unknown, although from limited experience, the maximum severity is moderate. Clinical score sheets are used to ensure humane endpoints are applied, to thereby minimize suffering. Transient mild adverse effects may also be experienced as a result of restraint during sampling or inoculation. Anaesthesia will be applied if anaesthesia is not more severe than the procedure itself.

If required to import ferrets from Europe, there will be an increased journey time. This transport will be done by a professional animal transporter company authorised under EU Regulation (EC) No 1/2005 for the protection of animals during transport and related operations, to mitigate any effects of the longer journey time.

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

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

Protocol 1:
Ferrets and pigs: Mild 100%

Protocol 2:
Pigs: Mild 99%, Moderate 1%

Protocol 3:
Ferrets: Mild 95% Moderate 5%

Protocol 4
Ferrets: Mild 95% Moderate 5%
Pigs: Mild 99%, Moderate 1%
Chickens, Ducks, Turkeys: Mild 75%, Moderate 25%

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

- Killed

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

Only viruses of scientific importance will be selected for full assessment in animals. A complete biological system is frequently required to fully study the course of clinical disease and virus-host interactions during infection and particularly the immune response of the host. For example, investigations into the mechanisms of virus transmission between animals or the effectiveness of disease interventions such as vaccination cannot be studied in non-animal alternatives.

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

In vitro and *ex vivo* methods will be developed and used where appropriate, for example: continuous cell lines and organ or tissue explant cultures.

Why were they not suitable?

These alternatives are suitable to address some, but not all influenza research questions. Alternatives to animals cannot be used, for example, to address questions such as virus-host interactions, transmission, mechanisms of disease induction by a virus (pathogenesis) or vaccine efficacy.
Modelling of risk pathways in agricultural settings e.g. in pig herds and assessment of risk or mitigating approaches at the animal-human interface also requires live animals of the relevant livestock species and animals representing human models.

A retrospective assessment of replacement will be due by 10 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?

Numbers have been estimated based on previous research programmes and project licences.

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

The use of a statistically valid minimum number of animals per study will be determined based on expert advice from a professional Biostatistician. Animal studies will be

designed in a consistent manner so that inter-study comparisons and data analysis can be performed.

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

Animal studies will be designed to maximise collection of biological materials and, where feasible, run in parallel. This will potentially reduce the number of control groups required and therefore increase the data output and research questions that can be addressed.

A retrospective assessment of reduction will be due by 10 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.

Ferrets represent a valid human model for influenza virus infection and pigs can serve either as a human model or as a natural livestock host species. Poultry (chickens, ducks and turkeys) represent a natural livestock host species for investigating transmission of avian influenza viruses from animals to humans or vice versa.

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

In studies involving study of virus-host interactions, it is not possible to use less sentient animals as study requires use of the biologically and epidemiologically relevant host species with a complete immune repertoire.

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

The species chosen are valid animal models or are a biologically relevant host e.g. pigs are the agriculturally relevant host for swine influenza and also represent a valid model for human influenza viruses. Animals are acclimatized in the study accommodation before procedures are carried out. Pilot experiments are used to refine protocols for the study of viruses with unknown properties e.g. to establish dose, route and timeline of infection required for infection and transmission.

The research team strive to continually improve clinical score monitoring systems and refinement of humane endpoint(s). The Clinical Score sheets used in this licence reflect refinements made over the course of the preceding licence. Animal species have their own

specific and disease-relevant clinical observation criteria and score sheets. No animal will be allowed to progress beyond the described humane end point using a 2-3 times daily monitoring system. On site veterinary teams and animal welfare officers (NVS and NACWO) are integral to each study. Clinical signs serve as study endpoints when the scientific objective does not require progression of disease.

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

Best practice guidance and information is obtained from NC3Rs, IAT and the RSPCA. Publications and articles are also reviewed during the approval process prior to each individual study.Other researchers in the same field of expertise may also be consulted. Where specialist training is required, interinstitutional exchanges and training visits are organised. Our team follows the ARRIVE2 in reporting data from animal studies.

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

The Establishment applies the Culture of Care in animal studies. The Establishment frequently attends or organizes external meetings on laboratory animal welfare e.g. NC3R, RSPCA and IAT meetings. Staff attending these meetings communicate relevant information by providing meeting feedback reports locally. In addition, the Establishment has a Species Group Care and Use Committee and all PILs and NACWOs are invited to attend meetings. Specialist topics are presented and refinements, such as environmental enrichment, are communicated and opportunities are used for implementation. In addition, specialist knowledge exchange is organised by field and lab exchanges with other organisations conducting influenza research.

A retrospective assessment of refinement will be due by 10 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?

21. Investigating the role of the extracellular matrix in 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

No answer provided

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

Objectives and benefits

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

What's the aim of this project?

Our previous work has identified that a genetic defect (a mutation) in a protein called collagen affects many tissues in the body and causes stroke, blindness and kidney disease for which there is no treatment. This project will investigate how these mutations in collagen cause disease, and we will determine if we can interfere with the mechanisms of these mutations to inform on possible new treatments.

A retrospective assessment of these aims will be due by 27 January 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 it's aims and if not, why not?

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these

could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Disease such as stroke, heart, eye and kidney disease are a major burden on Society and for many of there is an urgent need for better treatments. We have previously identified that mutations in collagen can lead to genetic forms of stroke, eye and kidney disease. By determining the mechanisms by which collagen causes these diseases, we will significantly increase our understanding of how these diseases develop. As these mechanisms may represent therapeutic targets, this will also guide the development of new and more effective treatments.

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

Outputs from this project will include the generation and publication of scientific manuscripts. In addition, oral and poster presentations will be generated and presented at local, national and international meetings.

A major output will be the increase in knowledge of how collagen plays a role in disease. Collagen has been implicated a large variety of diseases, many for which there are no treatments. Increasing knowledge gained in this project will help other researchers and industry in their research investigating these disease and/or other types of collagen. Finally, this project will also help the workforce in the UK by providing high quality training to PostDoctoral Researchers and PhD students in multiple disciplines.

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

Impacts from this research will benefit directly other researchers, as well more indirectly and in the longer term will inform the general public and clinicians.
Researchers will benefit through publication of manuscripts and presentation during the course of this project. This also applies to clinical researchers. In the longer term the information on potential treatment targets for vascular disease may impact on patient management and ultimately treatment. In so doing this would be a benefit for the general public and the NHS.

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

Outputs will be maximised through publications, oral presentations and public engagement activities including press releases. In addition attending and presenting at scientific conferences will be used to disseminate information gained.

I am part of several multi-centre programmes of work with international collaborators. This will maximise the global reach of this work.

Species and numbers of animals expected to be used

- Mice: 3400

Predicted harms

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

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

This project focuses on the mechanisms of disease due to Col4a1 mutations with the aim of identifying therapeutic approaches. In addition identified mechanisms and treatment will also be investigated other vascular disease models for example Ehlers Danlos Syndrome due to Col3a1 mutations.

The animal models used are well established models for these diseases. For example, the identification of the human disease caused by collagen IV mutation was based on the analysis of mouse models with mutations in the same gene. This illustrates that mice are a very accurate model of the human disease and a powerful model to investigate how the disease develops and to analyse potential treatment strategies. In addition there is a wealth of genetic tools available to researchers and protocols are well established and standardised.

The mice models have been previously generated and recreation of other animal models such as zebrafish can be more difficult due to species differences: the cardiovascular system of mice has a much higher homology than the zebrafish cardiovascular system to the human cardiovascular system.

The use of well-developed protocols and pilot studies minimises the welfare costs to the animals. All possible measures will be undertaken to minimise animal stress as it is well established that stress has a strong influence on cardiovascular parameters such as blood pressure.

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

The vast majority of animals will be used for breeding and maintenance and analysis will be performed on tissues collected post-mortem. A minority of animals will undergo in vivo phenotyping protocols such as measurement of blood pressure and magnetic resonance imaging (MRI) to analyse brain haemorrhaging. The phenotypes of animals may be altered through administration of substances or altered diets with the aim of ameliorating the disease and identifying its underlying pathways.

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

The adverse effects to the animals include the development of eye, kidney and vascular disease due to the mutation. Other very rare adverse effects are associated with the use of anaesthesia and protocols have been optimised to minimise adverse effects such as correct dosing and close monitoring of animals. As we will investigate older animals of more than 12 months old, we expect these to develop signs associated with normal ageing. In addition some of the adverse effects of the mutant animals can also increase with age. Close health monitoring of these animals will be performed to control the adverse effects.

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

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

We expect that the animal models (>95%) will have a mild severity limit, and <5% will have a moderate severity.

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

- Killed

A retrospective assessment of these predicted harms will be due by 27 January 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?

Currently no tissue culture and/or in vitro systems are available that accurately model the development of diseases that affect multiple tissues phenotypes, or their progression with increasing age.

Consequently, animal-based approaches are required to investigate the effect of mutations in genes and proteins on whole body biology or increase our knowledge of how diseases develop. In a similar manner to determine how a disease develops and whether a novel therapeutic strategy is able to modulate the disease, animal work is required.

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

Whenever possible, we will employ in vitro approaches including cell culture to perform the molecular analysis of the mutations and how they affect how cells function. These cell culture experiments will also be used to investigate the efficacy of a treatment strategy. Data obtained from these cell culture experiments will then guide the animal-based work.

Why were they not suitable?

No tissue culture and/or in vitro systems are available that accurately model the development of diseases that affect multiple tissues phenotypes, or their progression with increasing age, necessitating research using animal models.

A retrospective assessment of replacement will be due by 27 January 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?

Our extensive experience of working with animals combined with published research has generated a large amount of data that was used to estimate the number of animals required. Much of this has been based on our previous published research using these model and the techniques employed within this project. This has enabled us to establish the size of appropriate animal cohorts. In addition our experience with, and detailed knowledge of the used animal models has enabled us to develop appropriate breeding schemes and identify the number if animals that need to be generated. This experience has resulted in a significant decrease in the numbers of animals used.
Statistical analysis combined with pilot experiments has also been used.

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

The molecular analysis of mutations will be performed where possible in cell culture experiments. In addition, we have performed large mutagenesis screen on invertebrates, rather than in mice, which has signficantly reduced the animals used in this project. Data from these approaches will then guide our animal based work.
Where possible experiments on animal cohorts will be coordinated so that experiments will begin simultaneously for the entire cohort to allow optimal use of animals and reduce the number of animals. Statistical advice is sought to optimise approaches to ensure that maximum information is gained from the lowest number of animals. In addition, pilot studies are performed to determine how many animals are needed for an experiment, reducing overall the number of animals. Animals will be randomised, both sexes will be used and researchers will be blinded to the genotype and treatment status. This ensures effective blinding to prevent unconscious bias.
All procedures will be undertaken in accordance with current guidelines as listed on NC3Rs (http://www.nc3rs.org.uk/our-resources) comply with current good practice including 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?

To reduce the number of animals required we will collect multiple tissues of each animal and coordination of animal studies within the laboratory will ensure optimal use of animals. Performing multiple phenotyping assays will reduce animal numbers and provide an accurate characterisation of co-occurring phenotypes within one animal.

A retrospective assessment of reduction will be due by 27 January 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.

This project uses mice as animal models due to the available genetic tools and standardised phenotyping protocols. The mice models have been previously generated, model the human disease, and recreation of other animal models is difficult due to species differences.

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

Although zebrafish is extensively used in analysing the development of the cardiovascular system, rodents are still the standard species for adult phenotypes. Due to size difference it is much easier to analyse adult mice compared to zebrafish and the rodent cardiovascular system has a much higher homology than the zebrafish cardiovascular system to the human cardiovascular system.
Human disease due to mutations in collagen progress with age and age is a major risk factor for stroke in the general population with the risk of stroke most increasing significantly above the age of 55 . It is therefore necessary to recapitulate these age points in our experiments through the analysis of mature adult mice (3-12 month of age) and in some cases older mice to investigate the impact of increasing age on disease.

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

Several approaches are adopted to implement refinement.
This includes pilot studies to develop expertise, optimise protocols and determine endpoints before the actual analysis. Measures will be undertaken to minimise animal stress. This includes training; for example animals will be trained before measurement of blood pressure. In addition whenever possible grouped housing will be utilised and animals are monitored daily. Further intensive monitoring will be performed where appropriate including on older animals.

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

All procedures will be undertaken in accordance with current guidelines as listed on NC3Rs (http://www.nc3rs.org.uk/our-resources), this includes the ARRIVA guidelines.

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

Our animal work is performed with guidance from the NVS and Home Office Inspectorate, and we will keep abreast about advances in the 3R via the NC3R's website.

A retrospective assessment of refinement will be due by 27 January 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?

22. Lipid regulation of cardiovascular disease

Project duration

5 years 0 months

Project purpose

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

Key words

Cardiac disease, clotting, high fat diet, specialised fat molecules,

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.

Objectives and benefits

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

What's the aim of this project?

The primary aim of this work is to determine the role that newly discovered lipids (fats) called "eoxPL" play in cardiovascular disease and abdominal aortic aneurysm, specifically through their ability to modulate blood clotting. It has been recently found that these lipids are made in the blood vessels of mice with both disorders, and that mice that are not able to make these lipids are protected from disease. Studies have shown these lipids can alter different aspect of vascular disease by modulating both clotting and underlying vessel wall inflammation. We now need to better understand the molecular mechanisms involved in order to identify new ways to prevent or treat these diseases.

A retrospective assessment of these aims will be due by 02 March 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 it's aims and if not, why not?

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

What are the potential benefits that will derive from this project?

Cardiovascular Disease CVD causes 26% of all deaths in the UK; which equates to over 150,000 deaths each year or one person every three minutes. Along with this, abdominal aortic aneurysm is a complex silent disease, often co-existing with CVD (such as aneurisms and atherosclerosis), that has no effective treatments current, and is of high mortality. These diseases represent a massive public health concern, as well as a considerable economic impact.

The newly discovered lipids generated by enzymes (called lipoxygenases) appear to play a major role in the progression of cardiovascular disease and aneurysm formation, but the specific ways in which these lipids act still needs further investigation. The potential benefits of this work could include new treatments to prevent cardiovascular disease or aneurysms, or identify blood proteins that could be used to identify “at risk individuals”. We may also discover new drugs that could be developed for treatment of bleeding disorders.

Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

Mice (wildtype and genetically altered strains) will be used, and the total number will not be greater than 12,500 in total. Only a proportion (3,500) of these will undergo complex biological testing, the rest will be for breeding purposes

Predicted harms

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

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?

Of the 3,500 mice that undergo biological testing only a very small proportion, less than 35, may die as a result of complications that result from procedures.

Sudden death caused related to Ang II aneurisms.
For one of the knockout mice (or double knockouts) treated with an aneurism agent (Ang II), there is a risk of sudden cardiac death. Daily checks are conducted on the mice that are receiving Ang II, and mice showing adverse effects will be humanely killed. In addition, any mice found dead whilst under protocol 3 will be necropsied to evaluate whether an aneurism rupture has occurred

Breeding defects observed in knockout mice.
The knockout (ApoE) breeders show a reduced breeding pattern, compared to other knockouts and higher pre-weaning loss rate, for this reason animal care staff perform regular monitoring on mouse pups and license holders conduct weekly visual inspection on these mice.

Skin lesions induced by high fat diet in ApoE-/- mice
To assess this, daily health screening will be conducted on the mice. Should mice not respond to treatment over a course of 3 days then mice will be humanely euthanised.

Surgical procedure to implant minipump
Where surgical procedures are applied, additional appropriate control measures are in place for all adverse effects to ensure that all animals will be monitored closely, and appropriate action taken. All animals will be humanely killed if adverse effects cannot be treated.

A retrospective assessment of these predicted harms will be due by 02 March 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 why you need to use animals and why you cannot use non-animal alternatives.

To study cardiovascular disease and aneurysm, the use of mice is unavoidable because this can only be studied properly in vivo.
Disease development involves the coordinated interaction of multiple cell types with the specific micro environment and physical stress/strain parameters which cannot be modelled faithfully with cell or computational models. We also extensively use non-sentient alternatives including cultured cells, human tissue obtained post-operatively from patients and healthy volunteer human blood.

A retrospective assessment of replacement will be due by 02 March 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 you will assure the use of minimum numbers of animals.

Based on previous work, appropriate group sizes that give a defined statistical power will be used. Results will be monitored as studies are undertaken to determine whether subsequent experiments could use fewer mice
Where possible, we will breed lines as homozygotes.
We will cryopreserve lines we are not actively using and provide tissues to both internal and external collaborators.

A retrospective assessment of reduction will be due by 02 March 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

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

We will minimise suffering by applying procedures which where possible are non-invasive, and only lead to short periods of minor discomfort and stress and ensure less than 1% of all mice experience more adverse effects such as aneurism related deaths. Further to minimise harm to the animals, animals will be monitored weekly and then daily (whilst undergoing procedures) and where there is any concern, advice will be sought from the Named Veterinary Surgeon and Named Animal Care and Welfare Officer, and prompt appropriate action taken. Pain relief and appropriate anaesthesia will be used as standard for surgical techniques or when needed to reduce pain. Habituation will be used to acclimatize mice to restraint stress. A Treatment plan for skin lesions is in place so that as soon as mice present with symptoms treatment can be started immediately.
Mice represent an excellent system in which to study cardiovascular disease and aneurysm, since they possess many of the same genes as humans and also display similar disease progression. Mice are also an ideal species due to our ability to genetically and environmentally modify them.
Mice with a gene mutation (*ApoE* gene, a protein that is important in handling plasma lipids/fats) are susceptible to elevated blood lipids and cardiovascular disease. As such these mice represent a robust mouse model of cardiovascular disease that have been extensively characterised over the last 20 years. This again makes them an ideal model to study the pathophysiology of this disease.

A retrospective assessment of refinement will be due by 02 March 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?

23. Measuring brain activity of fish during humane slaughter

Project duration

4 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
  - Improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes

Key words

Fish, humane slaughter, electrical stunning, electroencephalography

Animal types	Life stages
Tilapia (Oreochromis niloticus)	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 the project is to develop and apply a method to measure brain activity of fish around the time of slaughter, to allow the development of stunners that render them unconscious immediately and until they are killed.

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

In 2010 it was estimated that between 37 and 120 billion farmed fish were slaughtered. Ensuring humane slaughter of farmed animals, including fish, is an essential part of ethical food production. Humane slaughter requires that the animal is rendered immediately unconscious and does not regain consciousness until it is dead, this mostly requires some form of stunning. At present there is only effective pre-slaughter stunning for a small proportion of farmed fish. To ensure that the stunners are effective we have to measure the brain activity of the fish. This project will develop and apply methods to measure the brain activity of fish around the time of slaughter.

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

The project should result in new products leading to commercial opportunities and publications affecting worldwide improvements in animal welfare. It will produce a non-invasive (externally applied) system to monitor brain activity in a variety of fish species. It will facilitate the development of humane stunning equipment and allow evaluation of existing stunning systems.

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

The ability to monitor brain activity in animals around the time of slaughter is essential to ensure that the stunning is effective, and that they do not regain consciousness. The findings of the project can be rapidly implemented due to a rising demand in the sector. This project will benefit the welfare of farmed fish and farmers using the equipment and all those interested in the ethical production of farmed fish.

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

This research proposal is a collaboration between industry, academia and the wider supply chain. The project will expand and refine our ability to humanely stun fish by widening the range of environments and fish species for which effective practical and objectively validated humane stunning systems can be supplied. Following scientific validation of stun parameters, full scale prototype stunning equipment will be installed in fish processing plants for additional commercially important species.

Species and numbers of animals expected to be used

- Other fish: No answer provided

Predicted harms

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

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

We will initially use trout, salmon or African catfish, since these are the species on which the majority of work has been conducted and published. Subsequently we will focus on the commercial target species: initially we will work with tilapia and subsequently if time and resources permit pangasius catfish and Atlantic salmon.

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

Most of the fish will be anaesthetised and have their brain activity recorded. Some will then be stunned (rendered immediately unconscious) using an electrical field in water.

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

We anticipate that the vast majority of the fish will experience no more than routine handling before being anaesthetised or stunned. Anaesthesia is a common husbandry practice based on addition of anaesthetic to the water. While this does cause a mild stress response it does not appear to cause any pain, suffering, distress or lasting harm. The fish that are stunned will be rendered immediately unconscious (<1 second) and will not regain consciousness before they are killed. However, in order to identify the effective lower limit of stunning it is possible two fish may suffer a brief electric shock before being humanely euthanised.

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

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

For the test trout and African catfish 100% will be sub-threshold or mild.
For each of the three target species (Tilapia (n=150), Pangasius (n=150) and Atlantic salmon (n=150));
Non-recovery 33% (Tilapia), 33% (Pangasius), 33% (Atlantic salmon)
Mild to moderate not greater than 63% (Tilapia), 61% (Pangasius), 67% (Atlantic salmon)
Severe not greater than 4% (Tilapia), 6% (Pangasius)
All the fish will be humanely killed by a schedule 1 method at the end of the procedures.

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

- Killed

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

This project aims to develop methods to measure brain activity around the time of slaughter to make the slaughter of fish more humane. This can only be achieved through the use of live fish.

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

Where possible we will use dead fish for testing our equipment but once we reach the stage of measuring brain activity, we need to revert to live fish.

Why were they not suitable?

Brain activity can only be measured in live animals.

A retrospective assessment of replacement will be due by 24 February 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?

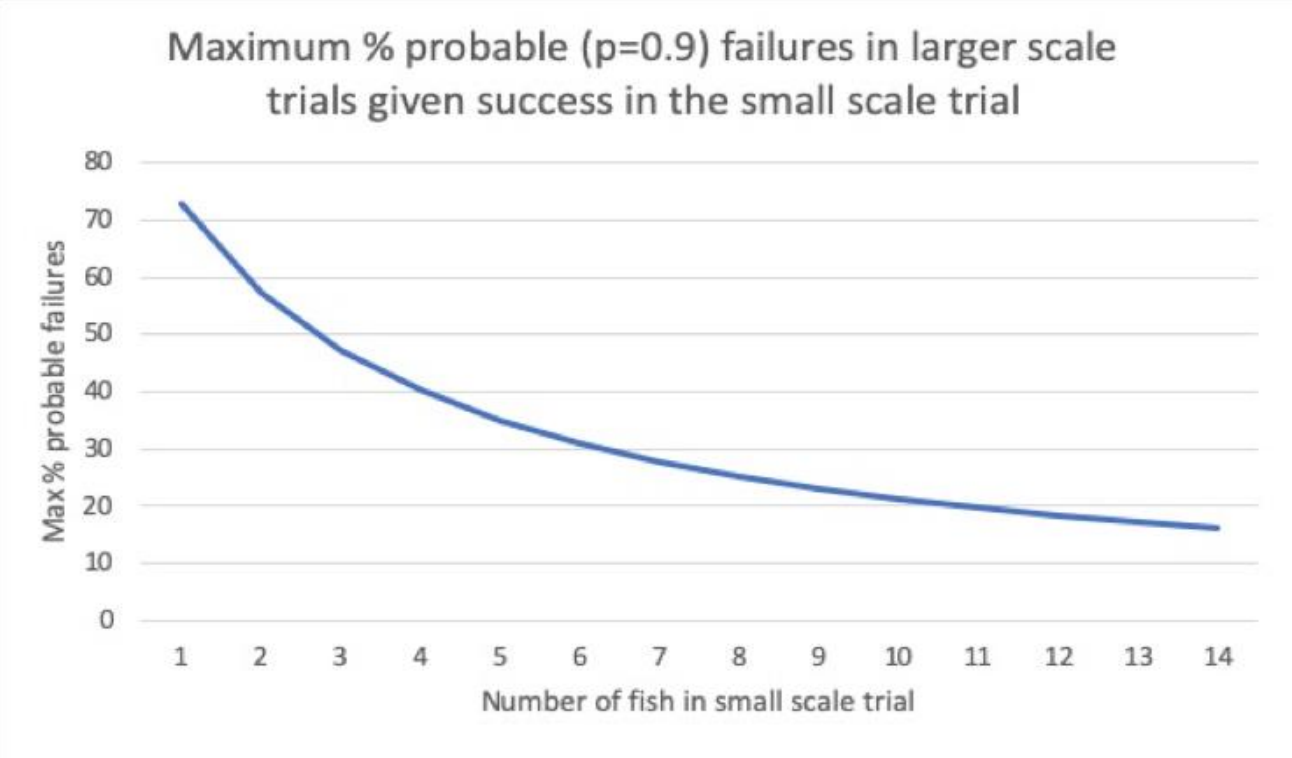
Reduction

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 UK Animals Scientific Procedures Legislation (ASPA), Development of systems and procedures will be conducted with individual fish. Once we have systems or which appear robust at the individual fish level, we will use 6 fish per trial.

Success in 6 fish gives a reasonable compromise between power and numbers of animals used. Success in 6 fish suggests that failure rate in larger scale studies will be less than 31% (p=0.9), see graph.



Proportion in a single sample using Wilson's method (Altman, D (2000) Statistics with Confidence: Confidence Intervals and Statistical Guidelines, 2nd Ed. BMJ Books, 254pp.) Eventually experimental trials will result in stunning parameters that will be used to inform commercial processes. At this stage although the activities will not be controlled under the UK Animals Scientific Procedures Legislation (ASPA), we will continue to monitor the efficacy of the stunning system to detect low prevalence problems that are unlikely to be detected in small scale experimental studies.

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

There is an inevitable compromise between reducing the number of animals and improving confidence in the results. We worked with a professional statistician to reach such a compromise. When using stunning equipment prior to slaughter there are some problems that only occur very rarely. We will not try to detect such problems under experimental conditions, since this would require the use of a very large number of fish most of which would not produce any useful information. Rather we have another phase of the project which will monitor large numbers of fish in commercial systems (outwith the UK). This will not be experimental and therefore will not covered by ASPA but will allow us to detect issues that only occur very occasionally e.g. 1 in 1,000 fish or even less frequently.

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

The research group has a great deal of expertise in all the key areas, minimising the need for developing new methods or equipment. In addition, we have an agreement with international groups which will allow us to exchange information and work in each other's laboratory. As a result, we will avoid duplication of effort in the two sites and both groups will benefit from advances made by either group.

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

We will use the external application of electrodes to the head of fish to measure brain activity. This is far less harmful or distressing than the alternative surgical implantation of electrodes. However, the external application of electrodes is not a proven technology for our target species and we may need to revert to the use of needle electrodes (inserted into peripheral muscle and connective tissue surrounding the skull) during development or in the event that the external application of electrodes is not viable.

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

We are developing systems for monitoring the slaughter of commercially important species and therefore need to work with those species.

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

Humane slaughter is an essential part of ethical food production and Electroencephalography (EEG) or measurement of brain activity is essential for determining if methods of stunning are humane.

Currently accurate EEG measurement in fish requires surgical implantation of electrodes. The insertion of electrodes directly into the brain of fish, may result in pain and discomfort, requires surgical anaesthesia, delicate surgery, and that the animals are restrained post-surgery. The method being developed and applied here will simply require the application of a suction cap to the head of the fish. While the fish's activity will then have to be restricted, they will not have to be tightly restrained. This not only drastically reduces the severity of the procedure but will also allow data to be collected under commercial conditions.

In the event that EEG signals cannot be obtained externally (e.g. where fish anatomy includes a layer of tissue between the skull and skin) needle electrodes will be inserted through the tissue layers (skin, connective tissue and muscle) towards the skull but not into the skull, of a sedated fish. Prior to recovery from sedation a local anaesthetic (e.g. lidocaine) will be applied to the site of the needle insertions.

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

We will apply the NC3Rs guidelines - Institutional framework for the 3Rs, and Responsibility in the use of animals in bioscience research.

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

The hosting institution runs a compulsory program of training and refresher courses to ensure all staff using regulated procedures are aware of current developments. The Animal Welfare and Ethical Review Board and Named Information Officer also circulate any relevant information on a regular basis. The licence holder will also maintain a watch on the relevant literature.

A retrospective assessment of refinement will be due by 24 February 2025

The PPL holder will be required to disclose:

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

24. Modelling organ fibrosis and cancer 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

fibrosis, cancer, therapy, inflammation, chronic disease

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

Retrospective assessment

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

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 organ fibrosis (scar formation in damaged tissue) and liver cancer develops as well as identify new drug targets and test new medicines for the treatment of these diseases.

A retrospective assessment of these aims will be due by 01 June 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 it's aims and if not, why not?

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

Why is it important to undertake this work?

It is estimated that tissue fibrosis, which can affect any organ in the body, contributes to up to 45% of deaths in the developed world. The number of people diagnosed with organ fibrosis, particularly liver fibrosis, are increasing every year. Currently, only two drugs are licensed to treat fibrosis in a rare lung disease, however, there are no medicines approved to treat other lung disease or fibrosis in other organs.

Liver fibrosis is also a risk factor for developing liver cancer, and with Sorafenib, the only drug used to treat advanced liver cancer, only extends life expectancy by 3 months. Therefore, there is an urgent need to better understand the disease and develop improved liver cancer models for drug testing.

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

Work conducted under this project licence will generate data and new knowledge that will help advance our understanding of how organ fibrosis develops and what changes at the level of molecules and cells make the disease get worse. Our work will help advance knowledge the disease process and identify new drugs or drugable targets, which limit scar formation in damaged organs or prevent liver cancer.

We aim to identify biomarkers of disease (e.g. proteins or DNA in the blood that are released from the damaged liver) or imaging tools to help assess how advanced the scarring or cancer is 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 or identification of new pathways which when targeted yields therapeutic benefit.

Ultimately, the long term aims is to benefit patients either through development of new diagnostics (biomarkers or imaging tools) or new treatment strategies.

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: Up to 50,000 breeding and 39,100 experimental animals

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.

Tissue fibrosis and liver cancer develops over many weeks/years therefore adult mice are used.

To study disease biology and ask how a protein effects the disease process 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. Genetically modified mice are readily available therefore mice provide an optimal system to study the disease. Additionally the research tools/reagents needed to investigate disease mechanisms and test therapies are available and are able to be translated to higher mammals.

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

Chemical models of liver or lung fibrosis 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 liver disease model mice receive bi-weekly injections of the chemical into their belly, whist in the lung model a chemical is inhaled into the lung. For some liver fibrosis models chemicals may be placed in the drinking water. We also use chemicals to induce liver cancer, where a mouse receives a single injection in the belly and cancer develops over many weeks.

Surgical models will be used to study liver disease, kidney disease, liver cancer or assess liver regrowth after partial removal of the organ. Pain relief is always given as needed. The liver disease models accurately mimic's a different type of liver fibrosis, where the bile duct is tied preventing flow or bile in to the intestine, bile spills into the liver and causes damage, this mimics a disease called cholestatic liver disease. To study liver growth, up to 70% of the liver is removed and then allowed to regrow over a period of 5 days (when normal mass is restored). Liver cancer cells are injected into the liver to study liver cancer and then test drugs. Cancers may develop over a few weeks. 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 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. Other dietary models include the methionine and choline deficient (MCD) diet model we expect mice to lose weight but develop fat induced liver disease.

Skin inflammation (redness and swelling caused by white blood cells) and scarring will be induced in the mice by either giving agents, which irritate the skin or chemicals that cause scarring. Punching two small holes in the skin and then watching them heal will be used to study how skin wounds heal. Pain relief is given as required.

Liver cell death will be promoted by giving mice chemicals or drugs at toxic doses (e.g. paracetamol) or inflammatory molecules, doses may be lethal or sub-lethal and mice will recover after a short period of feeling unwell.

Bone marrow chimera (a bone marrow transplant): normal or genetically modified (GM) mice will be irradiated to remove the white blood cells and then given new immune cells from a donor mouse of a different background e.g. normal in to GM.

All of our disease models are used to test therapies for that type of chronic disease or cancer.

In these models we may wish to perform non-invasive imaging whilst the animals are asleep, assess well-being using behavioural test or measure blood pressure. We may take blood samples to assess disease stage or drug metabolism. In disease models we might perform glucose tolerance tests.

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

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.

Surgical models: adverse effects due to surgical complications may include bleeding. Mice were the bile duct has been tied, occasionally become jaundice (skin becomes a yellow colour) or get swelling in the belly. Supportive care such as fluids, soaked diet and a warm environment will be provided. Pain relief is always given in surgical models and animals are carefully monitored by the surgeon and an assistant prior to and during the surgery to ensure that animals are sufficiently anaesthetised throughout the procedure.
In the dietary models most mice will gain weight, but not to a point where mobility is impaired, but in the methionine and choline deficient (MCD) diet model we expect the mice to lose weight.

Liver cancer model, mice will develop liver cancer over a period of up to 60 weeks but this will not result in liver failure.

Liver cell death caused by toxic chemicals or drugs will be either lethal where mice are humanely killed when very sick and the liver starts to fails or sub-lethal where mice will fully recover.

Lung fibrosis; mice will develop lung disease over a period of up 28 days and can lose weight during the first week of the models but regain weight after this time.
Skin fibrosis; the skin may become red (inflammation) and the skin will become thicker.
Skin wound healing; the wounds created by “skin punching” are superficial and therefore do not bleed. These models last up to 28 days.

Kidney fibrosis; mice will develop kidney disease over a period of up 18 days and can lose weight during the first week of the models but regain weight after this time.
Heart fibrosis will be induced by increasing blood pressure, which stresses the heart by chemical infusion or by surgically restricting the aorta. Mice can lose weight during the first week of the models but regain weight after this time.

Bone marrow chimera: Mice will always receive replacement bone marrow after irradiation, however, they may be sick and lose weight due to the effects of the irradiation but will recover once the immune system has been replaced. Rarely the transplantation of the bone marrow may fail, if this occurs (animals fail to regain weight) then the animal 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 therapies will cause significant adverse effects.

In all models Pain relief is given 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)?

Only mice will be used in this program of work. We estimate that ~80% animals used will in procedures will be mild including breeding mice to maintain a colony, for experiments and to take tissue or perform mild models of disease such as acute liver injury or dietary models of disease. Cell isolation from liver tissue in non-recovery procedures comprise ~1% of experiments. Approximately 16% of animals will be of moderate severity, whilst up to 3% 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 01 June 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?

We are studying complex diseases which develop and heal over many years.
Tissue fibrosis, a term which describes scar formation in an organ as a result of tissue damage is caused by many different cells in the organ changing their behaviour to try and heal the wound. If the damage is only small or limited to one insult, then the scar will dissolve. However, when the organ is repeatedly damaged over and over again, the scars persist and then reduce the ability of the organ to perform normal daily tasks. When disease is very advanced an organ can fail. Fibrosis occurs not only as a consequence of damage to the tissue but also through recruitment of white blood cells to the injured tissue, therefore signals from outside the affected organ are an important of the disease process.

Cancer, is also a complex disease, which involves many different cell types but is more likely to develop in a diseased or fibrotic organ e.g. liver disease.

This research will identify proteins which either cause or limit fibrosis or cancer. To prove that they do disease models may be performed in mice which are "genetically engineered" and lack the protein of interest. Alternatively, we may wish to test drugs which we believe will limit tissue fibrosis or cancer growth.

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 human cells in culture (including cell lines) to understand and model the biological processes involved in causing tissue fibrosis and cancer or to perform drug testing.

We have started testing/developing 3D cell culture models as an alternative method for drug screening and understanding the biology of scar cells or cancer cells.

We have recently optimised a new technology to culture very thin slices of tissue from human liver, lung and kidney to model fibrotic disease and test scar-limiting drugs. We are now developing methods to model other fibrotic diseases. These technical advances will help minimise use.

Why were they not suitable?

Whilst these are useful tools, there are limitations of cell cultures systems, these include; 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.

Organ fibrosis 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. Recreating all of these internal and external organ damage signals is difficult to model in culture. Using genetic engineered mice will help advance our understanding of the disease to help identify new targets for drug discovery.

Such systems are extremely useful for aiding our basic knowledge and for initial drug screening, and we use these systems to reduce animal use.

A retrospective assessment of replacement will be due by 01 June 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?

Yes, we have used knowledge from previous studies to mathematically calculate the minimum number of animals needed in each group to generate 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.
We have performed audits of our previous research studies 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 animal models of organ fibrosis and cancer. 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.

A retrospective assessment of reduction will be due by 01 June 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.

All of the disease models chosen are the lowest severity model that can be used to answer our research questions.

Accumulation of scar tissue in response to organ damage, namely fibrosis, can affect any organ in the body and is caused by many different types of injury. Scarred organs are also at a higher risk of developing 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.

For example, chemical models of liver fibrosis or lung has been refined for many years and is a very predictable model, therefore we can predict the disease stage 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. In the liver disease model mice receive bi-weekly injections of the chemical into their belly, whist in the lung model a chemical is inhaled into the lung. We also use chemicals to induce liver cancer, where a mouse receives a single injection in the belly and cancer develops over many weeks.

Surgical models will be used to study liver disease, liver cancer, kidney disease or assess liver regrowth after partial removal of the organ. Pain relief is always given as needed. The liver disease models accurately mimics a different type of liver fibrosis and is used to test therapies for that type of liver disease. To study liver growth, up to 70% of the liver is removed and then allowed to re-grow over a period of 5 days (when normal mass is restored). Liver cancer cells are injected into the liver to model liver cancer. To induce kidney disease the ureter is surgically tied to prevent urine flowing from the kidney to bladder, which causes kidney injury and fibrosis.

To model diet induced liver 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 disease.
The most refined model is always used.

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

Organs contain lots of different types of cells and these cells all talk to each other to ensure that the organ does its job properly. When an organ is damaged, all of the different cell types are needed to help heal the injury. White blood cells also enter the organ at the site of injury to help clean up the damage and heal the wound. However, when organs are repeatedly damaged this healing process becomes abnormal and fibrosis occurs. Fibrotic diseases in the liver, lung, skin, kidney, heart and liver are complex processes that develop many weeks/years. Fibrotic organs are also at a higher risk of developing cancer. 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.
Genetically modified mice, which lack a protein which is thought to be important in the development of tissue fibrosis are used to help understand the disease process better but also to help identify new drug targets to treat organ fibrosis and cancer.

Fibrosis is an inflammatory driven disease. 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.

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.
For surgical models we use good surgical techniques and operating theatres/equipment to minimise the risk of infection. The bile duct ligation model is a surgical model of liver fibrosis. 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. By working with the vet team and animal behaviour/welfare scientists we have developed a clinical scoring system to help assess the animal's well-being and level of disease.

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

For all models and optional procedures good practice guides will be used to help refine the model as described in the ARRIVE guidelines and Laboratory animals special article 2015, 49 (s1).
LASA Working Party guidelines on assessment and control of severity will be used throughout the project to determine if any animal is suffering distress.
Workman et al in 2010. British Journal of Cancer (2010) 102, 1555–1577 NCRI guidelines will be used to perform and monitor liver cancer studies.
Should distress occur immediate actions as described in the individual protocols would be taken to reduce an animals suffering.

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.

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 01 June 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?

25. Mouse models of prostate cancer initiation & progression

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

prostate progenitors, castration-resistance, immune system, genetic drivers, cancer

Animal types	Life stages
Mice	adult, aged

Retrospective assessment

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

Objectives and benefits

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

What's the aim of this project?

Our aim is to improve the understanding of prostate cancer initiation, progression and disease spread (metastasis), in particular the contribution of genetic alterations occurring in diverse cells of origin. Ultimately, by the use of our mouse models, we aim to develop new treatment strategies which can be translated into the clinic.

Retrospective assessment

Published: 22 December 2023

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

No

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

Our aim was to improve the understanding of prostate cancer initiation, progression and disease spread (metastasis), in particular the contribution of genetic alterations occurring

in diverse cells of origin. Ultimately, by the use of our mouse models, we aimed to develop new treatment strategies which could be converted into human use. In particular we had four aims:

1. To improve our understanding of initiating prostate stem and precursor (progenitor) cells and their dependencies on the location and environment around the cancer cells (microenvironment), to drive prostate tumour initiation and progression to aggressive, lethal tumours.

2. To define the role of specific genetic pathways, including those highly mutated in prostate cancer in promoting tumour cell content differences (heterogeneity), therapy / castration resistance, to develop novel therapeutics for prostate cancer.

3. To provide insight into the spreading (metastatic) process of prostate tumours.

4. To assess the role of the immune system in the growth of prostate tumours.

When we started this work, we focused on improving our knowledge of the early stages of prostate cancer, particularly the molecular events (e.g. elevated quantity of tumour-promoting genes) and interactions between the constituent cells that drive development, as it could improve our ability to identify aggressively growing tumours at the early stage of diagnosis. Thus, we exploited our previous discoveries on the mechanisms responsible for the resistance to male hormone (androgen) deprivation therapies, which are the standard of care for the treatment of prostate cancer patients in the clinic. To minimise the appearance of resistance to therapy, we were especially interested to investigate the key biological mechanisms that mediate changes in gene regulation within the cancer cells (signalling pathways) that can synergise with androgen deprivation, opening a window of opportunity to target tumours at very early stages of the tumoral growth process, with the potential to intercept tumours' progression towards incurable stages. This was of potential clinical importance since data from our work could allow novel therapeutic interventions before the occurrence of clinical castration-resistant prostate cancer. Additionally, our studies have taught us that initiation of tumour growth in older mice is more reflective of the scenario found in patients, which has been key for the transfer of our newly identified tumour-dependent pathways into patients' care.

The work was impacted by the global COVID pandemic which meant that a significant period of time was lost during lockdown where studies could not be run, and we therefore focused on the first three objectives. None-the-less the work conducted under this license did lead to several important observations that have been published or are currently in the process of being published. These include development of a novel, genetically-driven prostate cancer model that better resembles human disease (informed and used to address Aim 1 and Aim 2); identification of a new mechanism that can make cancer stem-like cells more sensitive to killing by androgen deprivation abrogating metastasis initiation (Aim 2 and Aim 3); establishing new combinations of chemotherapeutic agents, targeting multiple molecules at once, to reduce the tumour growth and the number of resistant cancer stem-like cells (Aim 1, 2 and 3); development of new animal models to further explore novel therapeutic agents and their role in tumour initiation and resistance to current standard of care therapies (Aim 1 and 2).

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?

Prostate cancer (PCa) has become the first leading cause of cancer-related mortality in men in the UK, accounting for 15% of all male cancer deaths. PCa patients exhibit highly variable clinical behaviour; some have indolent disease whilst in others it is lethal. Localized treatments, which include radical prostatectomy, and radiotherapy, are highly effective for prostate cancer patients. However, 20-30% cases relapse. A main challenge for treating prostate cancer patients is a lack of sufficient patient stratification, which result in standardise treatments to a highly heterogeneous, often multifocal disease. Moreover, currently available immunotherapies have limited effects in PCa patients, highlighting the need to study in depth the prostate local environment for development of adequate therapies. The use of mouse model systems, with a complete immune system will allow us to address those questions and pursue novel therapeutic interventions.

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

The expected outputs of this project would include:

New knowledge regarding the prostate tumour initiating cells, with particular focus on cell types sustaining more aggressive tumours, leading to the lethal stage. The research will contribute to the prediction of PCa subtypes by cell origin, their associated disease outcome and defined their specific molecular network to be targeted to abrogate tumour progression.

New knowledge regarding the requirement for several genes or cellular pathways for the function of normal or malignant prostate cells (e.g. inflammatory, stress response pathways), with a particular focus on those selectively required for tumour maintenance, metastasis and therapy resistance. The research findings will be published in academic journals and will be of interest to scientists in the field.

Identification of specific candidate therapeutic targets in PCa, which would be of particular interest to identify new biomarkers and to develop novel therapies to translate into personalized medicine.

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

We anticipate that a large part of benefits stated above would be seen within the 5-year duration of the project. The main beneficiaries of these will be other scientists, health professionals as well as pharmaceutical companies working on prostate cancer. We anticipate that patients won’t be able to benefit from this work within the time frame of this project licence, but might benefit in the next 10 years. Patient benefit might be via better classifying prostate cancer and understanding better which patients are likely to respond to current treatments, as well as novel immunodulatory combinatorial strategies.

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

Our findings will be made available to other scientists through collaborations, publication in high quality journals and presentations at scientific conferences and meetings. Our Institution has a policy of ensuring that all publications generated are available on free access to all.

Species and numbers of animals expected to be used

- Mice: 3000

Predicted harms

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

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

Cancer development is dependent not only on the changes occurring within the transformed cells, but also on the interactions of the cells with their microenvironment. Mice are more comparable to humans than less sentient model systems (fish, invertebrates) in pathophysiology and show higher levels of conservation in nucleotide and amino acid sequences. This is important as we intend to use reagents such as small molecule inhibitors and antibodies that have been developed to target human proteins. While this has elucidated many changes in cancer cells, it provides little information about the local environmental factors influencing early-stage cancer development in life. Also certain hallmarks of cancer, such as spread of cancer and blood vessel formation, are impossible to study in vitro (cell culture). Therefore, mouse models are important for studying the in life aspects of human prostate cancer development. Mouse models have been engineered to develop tissue-specific cancers, which accurately mimic their human counterparts, and have potential applications to test the effectiveness of novel cancer therapeutics. Moreover, non-protected species and less sentient species do not have prostate, so we would be unable to use them for animal models of prostate cancer involving the injection of prostate cancer cells in the organ where they are found in man. Embryonic stages would not provide us with a sufficient window to follow tumour development and besides it is not feasible to perform the desired interventions in embryos (such as tamoxifen injection in pregnant females). Therefore, adult mice are to be used.

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

Normal and genetically altered mice will be used to investigate the role of certain specific molecular pathways in the induction, progression and metastatic potential of prostate cancer cells.

The animals will have tumours grown in them initiated through either use of cancer prone genetically modified mice; by the use of inducing agents or by the implantation / injection of existing tumour cells / tumour pieces. For one of the protocols to investigate the spread of cancer we will inject cancer cells into the heart chamber with the aim of delivery to bone sites. However, we do not know the eventual locations until we complete the first study. It is possible that there could be multiple site tumours, but we will monitor the mice very closely to measure the total tumour burden and limit to that which is necessary to achieve the scientific target. To mimic some of the testosterone driven prostate tumour processes, some of the mice will, on occasions be castrated, under general anaesthesia and given post operative pain killers (analgesia). Other surgical procedures requiring general anaesthesia and analgesia are tumour implantation (into specific organs of interest), tumour biopsy (a gold standard method used clinically) and the implantation of small drug delivery systems under the skin.

To monitor the tumour growth, a combination of methods will be used: the use of callipers (for subcutaneous implanted tumours) and imaging under light general anaesthesia will be performed on a number of occasions to monitor tumour growth. On occasions it will also

be necessary to inject, by one of several possible different routes, chemical agents that will allow better imaging of the internal tumours. The majority of animals are not expected to show signs of adverse effects that impact on their general well-being. Very rarely the severity of these signs may be such that the humane end points may be reached (i.e. 20% loss in bodyweight) and the mice culled humanely. The majority of the procedures will result in no more than transient discomfort and no lasting harm.

In order to evaluate the contribution of immune cells in the progression of prostate cancer and associated metastasis, some mice will also have endogenous immune cells destroyed by radiation therapy. These same mice will also be injected with replacement immune cells. Some mice will also have current and potential new therapeutic agents administered to help understand both mechanisms involved in the cancer development but also as a means of assessing the usefulness of new potential treatments for prostate cancer and the subsequent spread. Some mice will have a second challenge of tumour administration to determine the duration of therapy efficacy.

As prostate cancer is a disease of old age, and we will also want to investigate how the cancer spreads and develops in other organs, it will be necessary to continue to follow these mice for up to 28 months.

All the mice will be humanely culled at the end of the experiment.

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

The impact of tumourigenic mutations are not expected to cause any adverse effects per se as these in most cases only manifest following administration of inducing agents. Following inhalation of inducing agents mice carrying tumourigenic mutations are expected to have prostate tumours. It is possible that the tumour growth might affect normal physiological functions (such as eating, locomotion or breathing). However, mice will be observed daily and any side effect that cannot be managed satisfactorily will result in humane culling of the animal.

Injections would only cause very transient pain.

After surgical procedures we will monitor mice for signs of pain and administer effective pain relief for as long as it is required.

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

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

The vast majority of mice are only expected to experience the mildest clinical symptoms due to tumour growth before they are humanely sacrificed. It is possible that there could be, for a small proportion of the total mice used, multiple site tumours following injection of tumour cells into the heart chamber. Additionally, some mice will experience the discomfort of repeated (daily) injections of therapeutic agents or oral delivery with a specialist tube. We will aim to utilise the least stressful route of administration wherever possible.

A minority of mice will undergo surgery and these will be anaesthetised for the operation and receive pain killer post-operatively until pain subsides. Some mice will also have

repeated anaesthesia for the purposes of imaging the internal tumours. Whilst loss of consciousness may be distressing this is not painful.

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

- Killed

Retrospective assessment

Published: 22 December 2023

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

As expected and in accordance with our license, the impact of tumour-promoting mutations did not cause adverse effects per se before treatment as these in most cases only manifest following administration of tumour promoting (inducing) treatments. Following injection of inducing treatments into the abdomen (intraperitoneal), which only caused a very transient pain, mice carrying tumour-promoting mutations developed abnormal prostate cell growth which evolved to localised prostate tumours.

As it was possible that tumour growth might affect normal body functions / activities (such as eating, locomotion or breathing), experimental mice were observed daily. In close collaboration with the animal care team, where side effects couldn’t be managed satisfactorily to prevent any pain (very rare) the mice were killed humanely.

We used 291 mice and the vast majority (>88%) experienced mild clinical symptoms (only inducing treatment injections under the skin) due to tumour growth before they were humanely killed at the scientific endpoint. Some mice experienced the discomfort of repeated (daily) injections of therapeutic agents or oral delivery of a drug with a specialist feeding tube. We utilised the least stressful route of administration in each treatment.

A minority of mice underwent castration surgery (removal of testes), and these were anaesthetised for the operation and all of the mice undergoing surgery received pain-killer post-operatively. Some mice experienced repeated, but brief, anaesthesia for the purposes of keeping them still whilst imaging the internal tumours. Whilst loss of consciousness may be distressing this was not painful.

A small proportion of the total mice used (8, <3%) were found dead in their cages overnight for unknown reasons (post-mortem analysis were conducted, but did not identify any cause). These mice were reported to the Home Office in the Annual Returns for the year.

Animal use

Year	Sub threshold	Mild	Moderate	Severe
2023	0	6	3	0
2022	0	76	10	0
2021	0	71	73	6
2020	4	25	15	2

Year Sub threshold` Mild Moderate Severe

Replacement

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

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

While valuable studies of human cancer are performed using tumour material and cell lines derived from both mice and human samples, the mechanistic understanding of cancer pathogenesis and its dynamics from tumour initiation to metastasis requires use of living animals (in vivo). In particular, cancer development and spread involves a plethora of interactions between cancer cells and their surrounding host and their behaviour is governed by multiple signals originating from both their immediate neighbours and from distant tissues.

Genetically engineered mouse models (GEMM) have been designed to develop cancers, which accurately mimic their human counterparts, and have potential applications to test the effectiveness of novel cancer therapeutics. This cannot be replaced by in vitro studies or indeed even in different in-vivo models such as zebrafish or insects which remain far less complex than their murine counterparts. . Additionally, inducible mice and tissue-specific Cre recombinases are used to restrict mutations to the biologically appropriate tissue.

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

Use of animals will be minimised by making use of computer modelling (e.g. prostate cancer patients' data) and in vitro model systems. In particular, we will use a variety of in vitro approaches to investigate how manipulation of nucleic acid based targets alter cell behaviour in cultured cancer cells (prostate) prior to undertaking in vivo studies. Methods to be utilized include cell biology techniques to measure organoid-growing capacity, cell proliferation, survival, migration, invasion, etc., biochemical and molecular biology techniques such as western blotting, enzymatic assays, proteomics, RT-PCR, etc. to study protein function. In addition, we will use molecular pathology (e.g. immunohistochemistry) to substantiate findings from our in vitro models in human tumour samples.

Why were they not suitable?

The study of cells in culture (in vitro) provides us with clues on the mechanisms of cellular processes in a simple and valuable context, which allows the establishment of hypotheses regarding the function of cells in a living animal. However, these systems do not recapitulate the complex cellular interactions described above.

Retrospective assessment

Published: 22 December 2023

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

Wherever possible work was conducted using cell lines grown in culture in the laboratory (in vitro). For example, the direct effect of therapeutic agents on prostate cancer cells was studied extensively using cultured cells and previously-frozen primary cancer cells before

taking the cells into mice. These studies identified mechanisms by which different treatments may kill tumour cells or lead to the expression of molecules that can influence treatment responses and potential mechanism of resistance, and therefore identified potential synergy between different treatments to minimise or abrogate tumour progression/metastasis.

We also used computer modelling (in silico) to measure signalling pathways associated with tumour progression and current standard of care therapies. Important observations arising from these studies have been published in scientific journals. However, as described above, resistance mechanisms involve complex interactions between multiple signalling pathways within the cancer cells. Moreover, cancer cells have considerable crosstalk to the surrounding cells both within a tumour and from circulating cells. This means that is incredibly difficult to replicate the multitude of interactions that occur between different cells, in different locations, and at different times within the body, using a culture flask in vitro or with a computer modelling. In addition, any predictions from the computer models need to be confirmed in living organisms.

This meant that whilst in vitro and in silico studies were important and useful, animal models remained essential to the development of novel therapeutic combinations.

Reduction

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

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

The overall aim will be to generate models whereby a measurable effect e.g. reduction in tumour volume or tumour incidence following manipulation of a gene of interest or treatment with a drug can be determined using the minimal number of animals.
Data available from the literature or from pilot studies are used to perform power analysis to determine an appropriate sample size for the definitive experiment. In general, we will use a sample size capable of detecting a 40% practical difference with 80% power and 95% confidence.

Based on past experience, group sizes of between 10 and 30 animals (dependent on the readout, fewer for transplanted tumours compared to spontaneous tumours in GEMM mice) per experimental group suffice. However, for an experiment to be well controlled and meaningful, we may include more than one experimental group. For instance, in implantation experiments where we deplete a gene in a cell line or prostate subpopulation using shRNA, we will use two independent shRNAs targeting the gene as well as a control shRNA. Moreover, we would typically examine more than one model cell subpopulation/line. Likewise, we may use several doses of a drug, or several different drugs or treatment combinations to test a hypothesis. Considering power, the number of experimental groups, and the number of genes and drug targets we are interested in, we have then estimated the total number of mice to be used over the licence lifetime.

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

Use of animals will be minimised by (i) making use of in vitro model systems wherever scientifically justified, (ii) use of in vivo bioimaging to follow disease development and response in real time (rather than culling cohorts of mice at defined time points), (iii) inducing tumour initiation by injecting virus in vivo (iv) the use of pilot experiments to test for the extent of an expected phenotype prior to a full scale confirmatory experiment (thus avoiding full scale experiments that may lack sufficient statistical power), and (v) the cryopreservation in multiple aliquots of prostate cancer samples (which eliminates a requirement for continuous production of cohorts of mice with experimentally initiated prostate cancer).

For our engineered models an efficient breeding strategy will minimise the number of mice used to obtain the desired genotype.

Experiments will be appropriately controlled and mice of the same age, genetic background and source used to reduce the variability of results and to produce highly consistent data. Wherever possible and appropriate, a single group of animals will serve as a control for duplicate experimental group. The proposed experimental designs and methods of analysis of the results will follow statistical guidelines and involve discussion with our bioinformatician scientist to provide sufficiently powered studies, minimizing the number of animals used in each experiment. The design of individual experiments will generally involve factorial designs, which maximise the information obtained from the minimum resource.

We will be conducting and recording our experiments to be able to publish our results following the ARRIVE guidelines [https://www.nc3rs.org.uk/arrive-guidelines] and will use randomisation, blinding etc. where appropriate so as to minimise biases. Furthermore, additional resources may be used to aid experimental design such as the NC3Rs experimental design assistant tool (https://www.nc3rs.org.uk/experimental-design-assistant-eda).

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

Pilot studies will be performed if applicable and, after analysis of the results, group sizes for subsequent experiments will be determined based upon these data. As far as possible, multiple parameters will be evaluated in a single mouse. Live imaging of the same animal at multiple time points also greatly reduces the numbers required.

Retrospective assessment

Published: 22 December 2023

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

Use of animals was minimised by: (i) making use of in vitro model systems wherever scientifically justified, (ii) developing in vitro 3D model systems for functional assays as described above (iii) use of in vivo bioimaging to follow disease development and response in real time (rather than killing different groups of mice at defined time points), (iv) inducing tumour initiation at a specific timepoint, so all tumours were able to be monitored from the beginning and also reduced the number of mice required for each experiment, (iv) the use of pilot experiments to test for the extent of an expected characteristics prior to conducting larger experiments (thus avoiding larger experiments

that may lack sufficient statistical power), and (v) the freeze storage in multiple aliquots of prostate cancer samples (which eliminated a requirement for continuous production of groups of mice with experimentally initiated prostate cancer).

For our genetically engineered mouse models an efficient breeding strategy minimised the number of mice used to obtain the desired genotype.

Experiments were appropriately controlled, and mice of the same age, genetic background and source used to reduce the variability of results and to minimise the risk of inconsistent data. Wherever appropriate, a single group of animals served as a control for duplicate experimental group. The proposed experimental designs and methods of analysis of the results followed statistical guidelines and involved discussion with our bioinformatician scientist to provide sufficiently powered studies, minimizing the number of animals used in each experiment.

We conducted and recorded our experiments to enable publishing our results in accordance with the ARRIVE guidelines [https://www.nc3rs.org.uk/arrive-guidelines].

Refinement

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

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

Mice have been chosen for the study because they represent the least sentient species from which meaningful experimental data can be generated, while exhibiting considerable genetic and biological similarities to humans. Other less sentient non-mammalian species, such as Danio rerio or Xenopus, which lack an urogenital system that is comparable in complexity and anatomy to that of Homo sapiens have been considered and rejected as models.

The conditions under which the experimental animals are kept and used for the proposed procedures are designed for the least possible disruption of natural behaviour and quality of life. We constantly work to improve husbandry and procedures to minimize actual or potential pain, suffering, distress or lasting harm and/or improve animal welfare in situations where the use of animals is unavoidable. As detailed in each protocol, there is provision for the appropriate anaesthetic and analgesic regimes as well as appropriate culling methods following guidelines on administration of substances to mice by Morton et al (2000) and working with cancer models (Workman et al 2010).

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

Only a mammalian prostate cancer model system has the potential to accurately mimic both the anatomy and complex cell biology, including microenvironmental interactions, of human normal and tumoural prostate. Furthermore, there is considerable experience in the wider scientific community regarding the use of mice as a model system for human malignancies and many reagents exist for the phenotypic characterisation of mouse cells.

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

The conditions under which the experimental animals are kept and used for the proposed procedures are designed for the least possible disruption of natural behaviour and quality of life. We constantly work to improve husbandry and procedures to minimize actual or potential pain, suffering, distress or lasting harm and/or improve animal welfare in situations where the use of animals is unavoidable. As detailed in each protocol, there is provision for the appropriate anaesthetic and analgesic regimes as well as appropriate culling methods.

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

Relevant published literature will be used as template for experimental design and decision making (Workman et al., 2010. Guidelines for the welfare and use of animals in cancer research. BJC, 102, 1555-1577).
We will follow guidelines of good practice [Morton et al., Lab Animals, 35(1): 1-41 (2001); Workman P, el al. British Journal of Cancer, 102:1555-77 (2010)] administration of substances will be undertaken using a combination of volumes, routes and frequencies that themselves will result in no more than transient discomfort and no lasting harm. Guidelines for Body condition score. [Ullman-Cullere, Lab Anim Sci. 1999 Jun;49(3):319-23]

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

By reading 3Rs literature and participating in 3Rs workshops locally and nationally. Through discussing refinements with our NACWO, NVS and HO inspector. I am also a member of the AWERB and I am a regular attendee of our Retrospective Review meeting and the 3Rs Poster session, both of which take place annually.

Retrospective assessment

Published: 22 December 2023

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

Due to our experience and work on our previous license, we developed the models described in this project licence. To our knowledge they were the most appropriate models to address the role of prostate progenitors in tumour initiation. Furthermore, these models allowed us to track tumour cells from the beginning of the transformation process due to fluorescent markers, which facilitated the reduction of animals required per each experiment as detailed above. The conditions under which the experimental animals were kept and used for the procedures were designed for the least possible disruption of natural behaviour and quality of life in our state-of-the-art animal facilities. We applied the appropriate anaesthetic and pain control (analgesic) regimes as well as appropriate methods for humanely killing the mice when scientific endpoints were reached.

We also used the following published best practices:

- Relevant published literature was used as template for experimental design and decision making.

We followed the guidelines of good practice [Morton et al., Lab Animals. 2001. 35(1): 1-41; Workman, el al. British Journal of Cancer. 2010. 102:1555-77] and administration of substances were undertaken using a combination of volumes, routes and frequencies that themselves result in no more than transient discomfort and no lasting harm, as well as the guidelines for body condition score [Ullman-Cullere, Lab Anim Sci. 1999 Jun;49(3):319-23].

Additionally, we used either cupping or tube-handling of mice; only used needles once and used environmental enrichment (nesting material, gnaw blocks and houses).

26. Mouse Models to Investigate Neurological Dysfunction

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

No answer provided

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.

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 characterise highly refined preclinical mouse models to help us understand specific intellectual disability and neurodegenerative disorders in humans.

A retrospective assessment of these aims will be due by 10 January 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 it's aims and if not, why not?

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

Why is it important to undertake this work?

The most common cause of death in England and Wales is neurodegeneration (roughly equal first with ischaemic heart disease in Scotland and slightly less impactful in Northern Ireland but still a major killer). Neurodegenerative diseases include Alzheimer's disease, Parkinson's disease, motor neuron disease including amyotrophic lateral sclerosis, spinal muscular atrophies.

Neurodegenerative disease also makes up aspects of syndromes and a prime example is the occurrence of Alzheimer's disease in Down syndrome: having the neurodevelopmental disorder Down syndrome (the most common known genetic form of intellectual disability) is the single biggest risk factor known for early-onset Alzheimer's disease.

We have almost no treatments for neurodevelopmental cognitive deficits (such as Down syndrome) or for neurodegenerative disorders in humans, let alone cures, although they massively impact our lives. Nor do we understand how a neurodevelopmental disorder like Down syndrome results in a neurodegenerative disorder later in life. We require many different types of models, including cells, human tissues, ex-vivo preparations and animals, to be able to understand these human conditions. We will use mice to show us why and how disorders develop from the early embryo to old age, investigating the interactions between different cell types, in order to ultimately understand disease (for example, Down syndrome, intellectual dysfunction and neurodegeneration) and find treatments.

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

At the end of our project we aim to have new information about the causes of dysfunction in the central and peripheral nervous system that arise from specific genetic mutations affecting cognition and locomotion, and leading to neurodegeneration. We aim to shed light on the earliest processes, some of which may be suitable targets for therapeutics, or for developing much needed biomarkers of specific diseases.

We will also have new, highly refined, animal models, for therapeutics testing including by genetic manipulation such as the use of new gene-therapy based medicines, or gene editing, for example.

Examples of benefits that we expect from current studies from include:

Being able to continue to map specific deficits in memory to a small region of synteny to human chromosome 21 in mouse Down syndrome models. In previous studies we have identified five candidate genes for a specific memory deficit in a mouse Down syndrome model; we expect to be able to start assessing the role of such genes during the course of this licence and thereby deliver potential future therapeutic targets, thus in the long-term, outside of the scope of this licence, helping to ameliorate memory deficits in this disorder. In addition, we are aiming ultimately to identify other genes (possibly up to 3-5) that modulate Alzheimer's disease in Down syndrome. We have yet to identify these genes, but current work is giving important new information which confirms there are genetic targets that can be modulated in Down syndrome-Alzheimer disease, and potentially in Alzheimer disease in the euploid population.

Using a combination of mouse models and human immortalised pluripotent stem cells we have been able to determine novel gene transcripts in motor neurone disease. During this

project we aim to determine the functional significance of these variants on motor neurone disease pathology.

All studies are aimed at eventually taking us to therapeutics for the disorders modelled, and in all cases we work closely with clinicians and other functional/clinical experts to ensure that knowledge is disseminated and opportunities for therapeutic developments are exploited.

All of this research takes place with regular and frequent interaction with a range of specialists including clinicians who are experts in the disorders we study, and basic scientists including behaviouralists, neuroimagers, cell biologists, molecular biologists and other experts, to help ensure we fully capitalise on the information from our mouse models and relate this back to the human condition.

All of this research will be published and disseminated to non-scientists through the charities that fund our work, and the patient/carer groups, and to scientists/clinicians through publications and presenting work at specialised scientific meetings including both academic and industrial audiences, and lay meetings.

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

Our research has impact over the short-term and in the long-term, after completion of the project.

In the short-term, the biomedical research community will be the main beneficiaries of our work, in that we will create new resources (mouse models) and disseminate new data on our specific disorders of interest and their underlying mechanisms. The mouse models will help in refining models to address specific questions on the pathology of our diseases of interest. The information on affected cell types, aberrant molecular pathways, aberrant changes detected by neuroimaging, aberrant behaviours, interactions between neurons and other cell types will inform biomedical researchers about early stage disease changes. In the long-term, the resources and data we produce (both of which will be freely available, long-term, either in biorepositories (mice) or in the literature (data)) will be used by us and by others to help provide the basis for producing therapies for the diseases of interest, and possibly also biomarkers for diagnosis and understanding disease progression.

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

Our ethos is that all individual mouse models should be studied by the widest group of experts possible, so that we maximise our understanding of phenotype. We work on neurological disorders, but to fully understand these disorders, we need experts in heart, skin, etc., to assess our mice holistically. Therefore, we have a wide-ranging set of long-term collaborators from different fields to whom we send samples for analysis, and if we find novel phenotypes we will always go to the experts in that area to ask them to follow up our findings.

All new knowledge is disseminated via publication, meetings, talks, academic and lay. Unsuccessful approaches are equally important and we also publish these in our papers and where possible as individual papers.

As well as conventional scientific press we also have an active social media presence and regular communication with other media (newspapers, radio).

Species and numbers of animals expected to be used

- Mice: 42400

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 work with mice because we can relatively easily manipulate the mouse genome to create animals that exactly mimic the genetics of people who have the disorders we work on. Also, until recently it was very difficult to manipulate the genome of many species, other than mouse, and so there is a wealth of mouse strains that are helpful for biology and which are compatible with the novel mice we make.

We study all life stages, from embryos through to old age, because we are interested in the functioning of the nervous system in different disorders, relevant to neurodevelopment and to later neurodegeneration -- noting that generally we do not know when neurodegeneration starts.

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

Typically, mice will be used for breeding, to keep our mouse colonies going.
There is no 'typical' regime for all our mouse models, the experiments depend on where we are with understanding the features of the mice we are working with.
For example, we have different protocols to assess the whole, awake animals for locomotion, memory, behaviour:

To assess locomotion in our mouse models, typically we may look at how mice walk, and how far they walk, by giving them free access to a running wheel and then timing how long they are on the wheel, and how far this adds up to running over a particular time period. We might also assess truncal strength by putting a mouse onto a wire hanging above a cage, and timing how long it takes for the mouse to haul itself onto the wire and run along it to the cage edges.

To assess memory, typically we may place a mouse into a box shaped like a capital T - the mouse is placed at the bottom of the T and will explore along the stem up to the T-junction and then turn right or left. We will then take the mouse out of the box, and put it back at the bottom of the T. Because mice are foraging animals, a normal mouse will again run up the stem of the T but then turn in the opposite direction, because it wants to explore. Mice with memory deficits may have forgotten which direction they first went in, and go in the same direction. Thus, we can detect if mutations cause memory deficits.

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

Depending on the model of neurodysfunction, we expect to see different features in the mice, most of which will be progressive, most of which are mild or moderate.
For our Down syndrome models, these animals have relatively mild learning and memory deficits, but live to old age - effects are mild but throughout life.

For the motor neuron disease models, these have loss of locomotion which in most cases is mild and throughout life, with our physiological models. Some transgenic models may develop severe symptoms by age four months, but we rarely work with such mice, and then only for a few specific experiments such as to determine the effect of introducing a gene knockout (by crossing in such a locus), as a test for possible therapeutics.
Similarly, for the Alzhiemer's disease models, some are known to have seizures in up to 20% of mice by 6 months of age, but again, these are transgenic models and so overexpress the gene of interest which may lead to the seizures. We have no plans for working with such mice.

For the movement disorders, the phenotypes are also related to locomotion.
We do not have reason to believe that pain is part of any of the phenotypes we study. There may be some weight loss depending on the mutation, but generally this is insufficient to be a welfare issue.

In a small number of strains, locomotion and weight loss will be severely affected and mice may reach humane endpoints within a few months.

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

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

25000 mice on the mild breeding protocol are not expected to suffer any adverse effects and the majority will not have any obvious features that are different from other mice.
We primarily work with models that express the normal or a mutant gene at the same level as occurs naturally – this is unlike transgenic mice for example, in which the gene level is usually many times above normal. These mice are ‘physiological models’. Therefore, most the features of our mice are mild and slowly progressing.

The majority of our mice with disease causing GAs are physiologically expressing models with mild features in the heterozygous state (i.e. with one copy of the mutant gene, not two) and therefore most will be bred under the mild protocol. When breeding new GA mice, or generating new crosses (e.g. generating mice with two copies of the gene of interest), mice will be bred on the moderate protocol in the first instance. Up to 25% of the 10000 mice may be show 'moderate' features.

In a minority of cases, GAs may lead to severe phenotypes during breeding ages, due to for example, neurodevelopmental abnormalities; up to 50% of the 1000 mice may exhibit these features under this protocol.

Up to 400 mice undergoing a protocol involving surgery will be non-recovery after terminal anaesthesia.

Protocols for characterising the animals, without surgery, involve up to 6000 mice, where up to 90% of animals may experience 'moderate' features.

Severe characterisation protocols will only involve up to 1000 mice, with less than 50% expected to experience severe features.

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 10 January 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?

We are studying diseases of the nervous system. For one set of disorders that involve cognition, many cell types are involved, and we can neither model behaviour other than with the whole animal, nor do we yet know about all the cell interactions and the effect of development so we have to work with animals.

For another set of mainly neurodegenerative disorders, scientist cannot yet grow the mature neurons that we work on, in dishes, nor can they grow the multiple different cell types together that represent the older human brain, in the body with environmental, hormonal, metabolic and other important influences, including ageing. Therefore, although the mouse lifespan is much shorter than that of humans, nevertheless mice go through an ageing process that mirrors our own, and we can study neurodegenerative disease in mice.

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

Lower vertebrates and invertebrates are used in the field to understand the interplay between genetics and conserved molecular pathways. However, our goal is to understand disease from a systemic viewpoint within the complexities of the mammalian nervous system. Critically, our most refined humanised models involve large genomic replacements – to understand human mutations in the most physiological way possible – that are not currently possible to engineer in other model organisms with available technology.

Why were they not suitable?

Scientists do not know how to grow the adult neurons we need to investigate, cells in dishes cannot recapitulate the complexity of developing and ageing in a body, and the effects such as hormones and metabolism, and we cannot study behaviour on cells.

A retrospective assessment of replacement will be due by 10 January 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?

This is based on a combination of previous throughput and power calculations for individual upcoming experiments.

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

We use the minimum number of animals to give us a statistically meaningful outcome, based on a prior statistical tests such as power calculations. We are careful to use control groups that are age-, sex-, genetic background matched, in order to reduce cohort sizes.

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

We design breeding plans to work with the minimum number of mice that will give us all the experimental and control genotypes that we need. We undertake small scale pilot studies to determine effect sizes for power calculations. We aim that as much tissue as possible is frozen/fixed and banked within our laboratory for use to use for future studies, and to share with collaborators, and this reduces the need to breed and age mice.

A retrospective assessment of reduction will be due by 10 January 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.

The mouse is currently the only mammalian species that we can use to examine the full complement of parameters that are measurable in behavioural and physiological changes, cellular and molecular changes arising from neurodysfunctional disorders, in concert with

our ability to tailor the genome of these animals to maximise the information gained from each mouse. We also try hard to develop protocols that detect early changes, such as behavioural changes, prior to major deterioration; such detection can only improve with time and as we learn more about mechanisms including from the very earliest stages. Note that we tend to work with physiological mouse models that have mild phenotypes. Refinement within our experiments includes processing primary cell lines (e.g. fibroblasts from ear clippings) (and these are immortilised and no further biopsies need to be taken), for multiple future use to ask questions at the cellular level. We also work with human cell lines, such as fibroblasts and induced pluripotent stem cells, to complement, our mouse studies, to validate our findings with the human condition.

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

We are studying neurodysfunction in the mammalian nervous system, and while mice are different from humans at all levels, we are sufficiently close in evolution that basic mechanisms are almost always conserved, as are genes -- which is not the case in non-mammals. Therefore mice are the model of choice to study mammalian neurodysfunction, also given the number of different mouse models available to the research community that informs our research.

Other vertebrates may be used to understand some of the biology of the disorders we study, but are not the best at recapitulating pathology such as, for example, behaviour. Similarly, while many aspects of the central nervous system are conserved from humans to invertebrates and may help us understand disruption to these tissues, lower organisms such as fish or flies do not harbour the complexity and intricacies present in mammals, and mammalian models better recapitulate complex pathological changes in disease. For example, invertebrate models often carry far fewer genes than mammals and do not have the paralogues or even orthologues found in the mammalian genome, making modelling of some disorders impossible. Genetic systems in fish may be relevant for some disorders but the manipulation of the fish genome is nowhere near as sophisticated as that for mouse, and cannot currently give us the refined models we work with for human disease, and again, fish may not have the specific genes we wish to investigate. For complex genetic disorders such as Down syndrome (a chromosomal disorder) the technology simply does not exist to model such features in any system other than mouse.

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

For all tests it is important that mice have no additional stress, therefore mice are handled by experienced operatives only, calmly, and are habituated to testing rooms as well as arenas if possible.

For all handling at all three sites of availability, tunnel handling or cupping are used for all mice, for the benefit of all animals.

For all tests mice are only housed in modified cages or arenas for the minimum time needed to gather meaningful data.

Mice undergoing phenotyping tests have increased monitoring and are removed from tests if they appear to be suffering from an adverse stress reaction, or other unexpected adverse effects of the phenotyping tests.

Through experience, we have refined methods for handling and caring for a minority of GA mice strains that demonstrate aversion to handling and disturbances. In such strains, we limit phenotyping procedures (do not perform phenotyping tests that elicit an adverse reaction), take extra care when changing cages and performing welfare checks (moving more slowly and quietly), and only change cages when absolutely necessary to reduce disturbances.

Mice that have been given anaesthetics are continuously monitored -- in the case of sedation until they are fully recovered. These mice then have regular extra checks when they are taken back to their home rooms. When general anaesthetics are necessary, the combinations with least adverse effects will be used.

Pipelines are designed with thought given to the overall experience of the mouse and the number of type of tests any one animal will go through.

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

Routes and volumes for administration of substances are taken from LASA guidelines. All sites of availability conform to the highest level of quality control on all fronts including husbandry, phenotyping and administrative processes.

Standard operation procedures for most tests have been generated using data and expertise from multiple animal houses and can be found at REDACTED guidelines will be followed at all times.

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

We receive regular information and updates on the 3Rs from all three sites of availability where we work. We also attend 3Rs events. When we develop 3Rs advances ourselves, we present these at 3Rs events.

A retrospective assessment of refinement will be due by 10 January 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?

27. Neurophysiology of Reward

Project duration

3 years 2 months

Project purpose

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

Key words

brain, reward, learning, decisions

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

Required at inspector’s discretion

Objectives and benefits

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

What's the aim of this project?

The main reason for our work is to advance knowledge about how humans process reward information in the brain. Our work aims to improve our understanding of how rewards are valued based on key characteristics and how this information is used by the brain when making decisions about which reward is best. To do this, we need to know how the brain processes economic (reward) choices that are the most fundamental processes for survival.

We define the word ‘rewards’ scientifically as stimuli, objects, events, situations or activities that are crucial to our individual survival and that of our species. We are interested in how reward mediates learning, approach behaviour (getting closer to an object or stimulus because an animal or human likes it and/or wants it), economic decision making (the process underlying the choice between rewards) and emotions.
The basic way reward and economic decisions are processed in the brain appears to be very similar between humans and non-human primates. This means the monkey work we

do can be used to understand the fundamental brain processes in the healthy and the diseased human brain.

As a result, our work will contribute to our understanding of the processes that drive drug addiction in humans, which is a disorder in reward processing and economic choice. Similar abnormal reward processing occurs in obesity, which is a prevalent and growing human disorder in the early 21st century.

Retrospective assessment

Published: 28 July 2023

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

No

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

1) Stating reward value: we discovered that rhesus monkeys can learn an auction task in which they freely state how they evaluate rewarding juices. Dopamine neurons reflect in their reward responses how the mionkeys evaluate internally the value they state in the auction. Two papers have arisen from this part of the work undertaken on the PPL.

2) Preferences revealed: we discovered that the single-dimensional subjective reward value of multi-component choice options in monkeys decreases with satiety and is coded in neurons in the orbitofrontal cortex in a similar manner as in human reward structures revealed by functional fMRI neuroimaging. Two peer reviewed papers have arisen from this part of the work undertaken on the PPL.

3) Getting the best reward: we discovered that the choices of rhesus monkeys comply with the continuity axiom (which states that probability and magnitude should be traded off against each other) of Economic Choice Theory. Further, monkeys' choices comply with the independence axiom (which states that adding common rewards to all choice options should not change preferences), which corresponds closely to choices of humans. Neurons in monkey orbitofrontal cortex show diverse relationships to amount and probability that converge to precise coding in the neuronal population. Additional data analyses indicate that choices in monkeys adjust to different types of risk. Reward is also optimised during effort in monkeys. Six peer reviewed papers have arisen from this part of the work undertaken on the PPL.

4) Choosing nutrient rewards in social context: we discovered in a non-social precursor experiment that monkeys show specific nutrient and sensory preferences for different rewards. The study is currently being extended to neuronal recordings in the amygdala and, separately, forms a part of a future project on choice complexity in monkeys. The social component of the study will also follow. In a separate direction, a behavioural study on monkey cooperation completed under a previous PPL is being prepared for publication. Two papers have arisen from this part of the work undertaken on the PPL.

According to the achievements stated above, the following benefits have arisen:

1) Basic knowledge: the published and to-be-published results listed above testify to this point.

2) Necessary data for research using other methods: the most likely uses of these results relate to human functional fMRI imaging (as mentioned in point 4.3.4 above) and to work on rodents that is becoming more popular and will benefit from the higher precision of work on monkeys. These biological data provide also a basis for computer models.

3) Building foundations for future research: our use of specific economic concepts makes this work foundational for the new field of neuroeconomics. Our focus on dopamine and frontal mechanisms makes this work important for understanding the normal neuronal mechanisms that have become defective in drug addiction.

4) Health: our work relates to vulnerability of reward systems in drug addiction, obesity, attention deficits and many other human disorders involving the brain.
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.

What are the potential benefits that will derive from this project?

By understanding the brain signals underlying the reward and economic decision processes will enable us to understand the basic building blocks of reward directed behaviour and explain why such decisions may go right or wrong.

It is now thought that cells called neurones (nerve cells) in the brain detect rewards but not how such ‘reward signals’ detected by these neurones lead to the choices we make. There are good learning and decision theories available, and we will use them to formulate and answer our questions. For example, how does the brain detect fatty and sugary food and liquid rewards that lead to obesity that is so harmful to so many people? Other examples of our research will look at how uncertain rewards are processed and how this may lead to irrational choices that are harmful to humans.

Knowledge of normal processes is fundamental if we are to understand what goes wrong in reward disorders such as obesity, risk taking and drug addiction. Any, even minor, advances are likely to help the search for treatments that could save thousands of lives. In addition, because in our daily life we receive many different rewards, we also need to understand how the brain interprets the rewards other humans are getting and how this affects the way we behave. To understand why we misjudge rewards in friendly and unfriendly situations may help us understand how to reduce social conflicts. The knowledge gained from these experiments should help us to understand the abnormal processes in reward addiction, obesity, gambling, attention deficit disorder and other disorders, and provide further steps to improve human health and welfare.

Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

Macaca mulatta (rhesus monkey, old world monkey), 7-9 animals (transferred from current licence).

Predicted harms

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

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?

Our monkeys live in rooms that house 2 to 4 animals. This is considered an appropriate number to ensure their welfare. Unfortunately, some animals will fight as they get older and try to establish hierarchies. This can result in injuries and the need to separately house animals for their safety. A separately housed animal will always be able to see, hear and communicate with the other animals living in the same room. If socially compatible, separately housed monkeys will have periods of time during which they are allowed to have direct contact with other monkeys; these contacts will enable them to groom each other and to conduct other social activities; however, these contacts this will be closely supervised.

It takes us several months to train our animals to enter the laboratory from their living area and to perform specific tasks. These tasks include making choices by moving a joystick in front of a computer monitor to obtain a fruit juice reward. It is not unusual for monkeys to remain focused and busy on these tasks for several hours on a given day. During these periods, the animals sit in a custom designed ‘primate chair’ (a Perspex box) in a natural position.

When performing brain recordings, we restrain the animal’s head movements by connecting an implanted head holder to the primate chair. For experiments where this is required we slowly and carefully train each animal. This can take several months.
Once trained the monkeys are visibly relaxed and will fully engage in their tasks. Indeed, the animal’s comfort, cooperation and engagement are essential if we are to obtain useful scientific results. An animal that is not comfortable, or ill, will not perform their task properly; thus, it is in our interest as researchers to make sure our animals are comfortable. If a researcher detects lack of comfort in an animal sitting in the primate chair in the experimental room, we stop and give it a break. We then search for and eliminate the reason for their discomfort. If the situation cannot be remedied during that day's session, we stop the procedure for the day, give the animal any remaining liquid, and return the animal to its holding cage. We do not test the animal in the experimental room before the reason for the discomfort has been eliminated.

During our experiments we control each animal’s access to food and fluid. However, when we test the behaviour or record from and/or stimulate the brains of our animals, we ensure that each animal always receives its daily fluid and food requirements, and we make sure particularly attractive foods, such as fruits are provided. Basic, nutrient, dry food (monkey chow, dried fruits, nuts) is available at all times in the home cage. We carefully monitor each animal’s weight so we can adjust the feeding regime if necessary. We provide free food and water access at least one day every week, and we give our animals week(s)-long breaks every few months.

In order for us to be able to record the activity of neurones in the brain we have to undertake several surgical procedures on our monkeys. These surgeries normally take about 5 to 9 hours during which our animals are fully anaesthetised (asleep and unable to feel pain). Just like humans undergoing surgery in a hospital, our surgery is performed under fully sterile conditions, and with subsequent pain relief provided as advised by the veterinarian.

Brain recordings require each animal to undergo surgery during which metal implants are attached onto the skull. The first surgery is often a metal restrained post. Months later a small chamber may be implanted onto the skull. In a small number of cases, an additional surgery or two may be required to repair an implant. Following surgery, we often locate brain areas we intend to study deep in the brain by using one or more x-ray sessions during which the animals are under sedation anaesthesia. During these sessions we record the activity of neurones, which enables us to specifically identify the brain centres that respond when we touch different parts of the body (which otherwise would disturb an awake animal). We also sedate our animals every few months in order to thoroughly clean their implants. This means each animal will undergo full general anaesthesia for a surgical procedure up to a maximum of four times in its lifetime, plus several periods of sedation anaesthesia for the shorter procedures such as implant cleaning.

We record the activity of individual nerve cells in fully conscious (awake) animals. This is done by inserting microelectrodes (extremely fine needles, less than one fifth of a millimetre) through a small skull opening within the implant chamber into a specific brain area. The stability of these recordings requires head restraint. These sessions normally last up to 4 hours, exceptionally up to 5 hours, after which the head restraint is removed and the animal returned to its home cage. The head restraint schedule will continue for up to 30 months, and in rare exceptions 48 months. These recording with head restraint are performed up to 5 days each week for several months, after which the animal is given a one- or two-week break before returning to testing.

Electrode insertion does not cause the animal pain, because the dura, which is the tissue covering the brain, has lost its ability to detect pain, and because there are no pain receptors inside the brain. Each animal is likely to experience occasional mild discomfort when sitting for extended periods and after having experienced fluid restriction in the hours before testing starts. However, the occasional discomfort is mild and not sufficient to stop the animal from making sophisticated decisions when offered different choices in order to obtain a reward. The way the rewards are presented is carefully designed to enable the researcher to detect the lack of comfort during the experiment and in the recorded data (for example the monkey will make poor choices or no choices at all). With more intense discomfort that agitates the animal or affects its voluntary choices, we search for the reason and remedy it immediately, or return the animal to its holding cage as described above.

We also inject very small volumes (microlitres) of solutions containing molecules that will label the nerve cells we are interested in the specific parts of the brain. The use of these labels helps us identify the exact neurones for recordings or stimulation using small optical fibres (less than one fifth of a millimetre). Such injections may be done under sedation, in which case this will add to the total number of sedations an animal receives.

Surgery or inserting electrodes is not without risk. Animals can develop brain infection or oedema (swelling), surgical stitches may open, paralysis or mild muscle weakness on one side of the body may occur, or mild and almost undetectable seizures have been seen in rare cases. We treat these conditions with antibiotics, anticonvulsants, pain medication, and other medication as advised by a veterinary surgeon. If affected animals do not show signs of improvement within 12 hours, they are humanely killed. In addition, if the animal has not recovered within 12 hours in cases of pain or complete limb paralysis, 24 hours in cases of brain oedema, or 48 hours in cases of systemic infection and wound healing, the animal is humanely killed. Any animal that develops more than mild, localised and transient seizures that require medication using antiepileptic drugs for more than 24 hours

will be humanely killed. Complete paralysis has never been observed and is unlikely to occur and would result in immediately humanely killing the animal.

In summary, each animal will undergo behavioural training for about 1 year after which it will undergo neuronal recordings for up to 30 months, and in rare exceptions 48 months. At the end of this time, each animal is killed humanely.

Retrospective assessment

Published: 28 July 2023

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

2.2 Does the information provided in the table reflect the projected incidence of harm provided in the adverse effects for each protocol?

• The number of rhesus monkeys subjected to regulated procedures under the current PPL was 9. They were:

1) Tigger: Actual Severity: Moderate

2) Ulysses: Actual Severity: Moderate

3) Vicer: Actual Severity: Severe

4) Wicket: Actual Severity: Moderate

5) Wurzle: Actual Severity: Moderate

6) Yaa: Actual Severity: Moderate

7) Yum-yum: Actual Severity: Moderate

8) Athos: Actual Severity: Moderate

9) Aragorn: Actual Severity: Severe

• The monkeys experienced the following harms, as listed in the PPL application:

1) Injuries from group housing: monkeys Athos and Aragorn had a fight that resulted in two puncture wounds in Aragorn that did not require sutures (according to the NVS). This harm concerned 1 of 9 monkeys used in total during our PPL period (11%). Further, two monkeys were separated in their own cages from other monkeys in the same large enclosure for 7 months (Yum-yum) and 15 months (Wurzle) to prevent fights after having observed their behaviour. During this whole period, the animals had visual contact with other monkeys. Given the social incompatibility of these monkeys at their age range, we could not introduce new partners due to the upcoming closure of the laboratory. This harm concerned 2 of 9 monkeys used in total during our PPL period (22%).

2) General infections unrelated to intracerebral electrodes, stimulation and injections (1 monkey affected): monkey Aragorn had an infection: On the 15 of July 2022 the implant presented granulation tissue with superficial infection and was cleaned daily. A first course

of antibiotic (ceftiofur) was prescribed and bacteriology swab for culture taken. This treatment had little effect on the pus production, and Aragorn's weight loss reached 10% of what it was the month prior. On 2 August 2022 the animal was reviewed by the NVS under sedation, the implant was thoroughly investigated, and channels between the craniotomy chamber and the margins were found. A large collection of pus between the bone and soft tissue was found at the back of the implant. Blood was taken and revealed a small ratio albumin/globuline. On reception of the bacteriology results, the antibiotic was changed to a more appropriate antibiotic for the infection. On Monday the 08 August 2022 the weight loss reached 1.9 kg, equivalent to 15.58% (from 11.98kg to 10.08kg) and therefore was above our licence threshold. A Condition 18 report was send to HOI, who allowed exceeding the 15% and requested daily updates. The animal briefly reached its lowest weight of 9.64kg compared to 11.87 at 4 weeks before, which amounted to a loss of 18.79% but recovered quickly afterwards and reached 2 month later its initial weight of 11.98kg. By the end of the experiment on 17.3.23 his weight was 15.5kg. We helped weight recovery (and stopped the >15% weight loss) by keeping the animal on ad-lib free water during the whole antibiotic treatment period. We suspect the weight loss was most likely due to the infection, and the antibiotic treatment helped to regain the weight. In addition, we supplemented his usual monkey diet by giving him rice and yogurt daily for two weeks. This harm concerned 1 of 9 monkeys used in total during our PPL period (11%).

3) Implant breakage or complete breakoff: monkey Vicer lost his implant (Oct 5, 2020), which is classed as Severe in our Actual severity classification. The HOI was informed but did not require an Condition 18 report. The animal was euthanised because the loss of its implant created a wound on the skull that could not be properly closed and also made its use for the experiment impossible (the animal was not found dead). This harm concerned 1 of 9 monkeys used in total during our PPL period (11%).

2.3 Describe any unexpected adverse effects and include the percentage and the number of animals affected. How were these episodes managed and recorded?

• Monkey Aragorn drank huge amounts of water (October 2022). This phenomenon was classed by the HOI as not being Condition 18 material.s Aragorn underwent an assessment of the free water intake without any restrictions that was required for the controlled fluid intake under the PPL. It was noticed that the animal repeatedly drank 2–4 litres of water / 24 hours every day since 10.10.22. This volume far exceeded what is typically expected for monkeys of his size (13 kg during that period) in our experience (~600-1000 ml/day) and what Aragorn previously drank when given free access to water in August. The NVS was consulted, and urine and blood samples were taken. Results from both samples were normal, and neither liver nor kidney damage were apparent. The animal was eating and behaving normally and was not exhibiting any other symptoms. We initiated specific analyses that produced the following results: biochemistry was unremarkable for liver and kidney's function; glucose and electrolytes were in normal range. These results ruled out any kidney or liver issue problems and metabolic diabetes. We managed Aragorn until this animal reached the scientific end point. The main clinical hypothesis, confirmed by post-mortem histology, suggested a diabetes insipidus (adrenocortical hyperplasia, Cushing's disease) as likely origin the polydipsia. This harm concerned 1 of 9 monkeys used in total during our PPL period (11%).

2.4 What percentage and number of animals had to be killed or died unexpectedly before the end of the experiment?

• Monkey Vicer lost his implant and needed to be killed, as detailed above. This harm concerned 1 of 9 monkeys used in total during our PPL period (11%).

Replacement

State why you need to use animals and why you cannot use non-animal alternatives.

To understand higher brain function and provide crucial information for improving the treatment of human disorders, we need to investigate biological signals in the intact working brain. Understanding individual cells is the key for understanding higher brain function because individual cells are the basic units of the brain that process information. Only by doing this in monkeys can we understand how individual nerves function in relation to behaviour at a level found in humans.

Computer simulations, tissue studies and human brain imaging do not permit researchers to study the activity of individual brain cells.

Other animals such as insects or rodents do not have the complex human like behaviour, the highly developed brain structures of interest (e.g. frontal cortex and connected structures), do not permit sufficiently precise distinctions between reward, decision making and movements, and thus do not allow identification of reward and decision brain cells. Despite some progress in investigating the activity of brain cells in humans, systematically controlled experiments still require the use of animals.

Retrospective assessment

Published: 28 July 2023

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

It is not possible to do behavioural experiments with non-animal alternatives while recording single-neuron action potentials wiht their high temporal and spatial resolution crucial for understanding neuronal information processing. However, we have used, throughout major parts the >20 years of this HO project ('Neurophysiology of Reward'), human fMRI neuroimaging. This fMRI method does not allow single-neuron resolution, but it can complement the relationship between behavioural tests and brain activity. We have also used machine learning algorithms (decoders and classifiers) to obtain more detailed knowledge about how neurons might control the behaviour of monkeys, but we have not used outright neuronal modelling, as that would not have allowed us to advance the major steps in neuroeconomics that we desired.

Reduction

Explain how you will assure the use of minimum numbers of animals.

Our study design is based on the need to produce reproducible, relevant and scientifically robust data. For example, we compare the activity of a neuron after a reward against the

activity of the same neuron before the reward. We can also compare a neuron's activity between several rewards.

We also use modern machine learning tools ('decoders', 'classifiers') that predict the animal's choice by looking at the responses of brain cells to previous rewards, which demonstrates that the brain cell is involved in the decision process. We use computer models to translate behavioural theory into efficient experimental design, which limits the number of experimental tests we need to perform. We have developed advanced computer simulations that help us to understand and predict how behaviour and brain activity are related. We use these tools to make our experimental designs and research questions more specific and informative, which limits the necessary amount of experimentation.

Each neuronal study requires data from 60 or more neurones from a single animal, and a minimum of 2 animals to assure reproducibility across individuals. Sometimes 3 animals are necessary to obtain more robust results. On occasion we have found that the behavioural tests also require 3 animals.

To ensure that we make the best use of the data we gather in these experiments, we analyse the data using statistical methods including regressions, general linear models and machine learning tools. We also use these data to help us to plan and carry out the experiments in a more appropriate and efficient way, using the least number of animals and reducing the duration of behavioural training.

Our experiments are based on knowledge of formal, well established theories of how humans and animals learn and make choices. This theory based approach helps us address more effectively the scientific questions being investigated.

Our related human brain imaging studies help us select the brain structures for investigation at the single cell level in the current animal project.

Together, these approaches help us to avoid unnecessary experiments and ensure we focus only on those parts of the brain that are relevant to the behaviour under investigation.

Retrospective assessment

Published: 28 July 2023

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

The minimum number of monkeys required for one behavioural neurophysiological experiment were used (N = 2, sometimes 3 if not enough data were obtained from one or two animals). The project 'Neurophysiology of Reward') required several individual behavioural neurophysiological experiments, thus requiring more than 2-3 monkeys.

Post mortem samples from the hypothalamus of 2 monkeys for anatomical studies and post mortem brain samples from 1 monkey for measuring brain viscosity were provided to other researchers.

Refinement

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

We work hard to use methods that are the most refined, and we spend considerable time, effort and manpower changing and enriching our animal accommodation and our primate chairs. For example, at least once per month, an aquarium with animated artificial fish is placed in each room for the animals to watch. We locate the aquarium in the room where the animals live but outside their reach. We also show videos that we believe our monkeys find interesting, as judged by their behaviour.

Incidents of fighting can occur as part of the normal hierarchical/dominance structure of monkeys. We supervise special interaction time for animals that need to be separated from others, either temporarily or longer term. The supervision sessions last for several hours each day and involve our experienced laboratory technician being present inside the holding room throughout that time. An animal that needs to be separated from others over longer periods will be able to see, hear and communicate with the other animals living in the same room and, if socially compatible, will have direct interactions for grooming and other social activities under supervision.

We provide layers of wood shavings on the floor of the animals' cages in order to mimic aspects of monkeys' natural foraging environment. The animals search for their daily diet (foraging), which also alleviates potential boredom.

We change the fruits and other foods frequently, which appears to keep the animals interested and stimulated. We add to this a variety of more interesting combinations of vegetables.

We use variable sized home cages which allows us to house groups of different numbers of animals. Inside the cages we provide hiding boxes, use video systems to monitor the animals' behaviour and interactions; supervised grooming periods serve to provide social encounters in socially unstable animals. We believe that the combination of all of these measures makes the life of our animals more interesting and reduces the potential for aggression and fights.

We improve the surgical techniques and materials we use and now use an even more tissue friendly material for head implants that binds well with the animals' natural tissue. We make a point of learning and evaluating different methods from new laboratory members joining us from other primate laboratories. We have improved our surgery techniques over the years through experience gained from working and talking to orthopaedic and plastic surgeons.

In addition to these specific points, we ensure that our choice of animal species, care and welfare methods, and scientific methods are the most refined by current standards as follows:

We follow the published guidelines of the NC3Rs and, together with our animal carers and technicians, regularly attend workshops and training courses on animal welfare.
We continue to refine our experimental and scientific approach. We interact and collaborate with other neuroscientists, biologists, medical doctors, statisticians, and data modelling experts nationally and internationally, to exchange knowledge and information gained from work on humans, different animal species and computer simulations.

We continue to remain up to date with the increasingly delicate details of psychological learning theories (so-called Rescorla-Wagner Theory of animal conditioning, and efficient 'Temporal-difference Reinforcement Learning' solving the Bellman value function for optimising reward) and economic decision theories (so-called Von Neumann - Morgenstern Expected Utility Theory, Kahneman & Tversky's Prospect Theory, and Revealed Preference Theory). These theories contribute conceptual frameworks for the experiments and provide more stringent approaches for addressing the scientific questions of reward processing and economic decision-making.

We look for answers to our scientific questions using a number of methods. These include advanced behavioural tasks, single-neurone recordings and computer modelling. In addition, we supplement our animal studies with human brain imaging. We do all this to ensure that our animal experiments address questions that can be answered by single-neuron recordings in monkeys; in other words, we make sure that our animal data will be relevant for humans and their pathological conditions. We refine all of these approaches so that our research can be rigorously peer reviewed by research grant awarding bodies and by the editors of the journals in which we publish our scientific data.

Retrospective assessment

Published: 28 July 2023

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

We refined neurophysiological recordings by using several NAN multi-electrode recording systems on several procedures. Each NAN system consists of several microelectrodes, rather than a single one, and thus allows to obtain data from several neurons from one penetration into the brain (and thus reducing the number of daily recording sessions required for a full data set and preventing potential harm to the animal caused by the daily recording sessions).

We introduced greater use of food-enriched toys and destructible toys into the cages, which kept the animals more engaged. See attachment titled ‘Laboratory Primate Enrichment Ideas’.

We used a behavioural scoring sheet for the daily monitoring of the animals’ behaviour, as well an actual severity monitoring sheet.

We also used a welfare monitoring sheet for pre- and post-op surgery.

We refined the scoring in 2020 to include activities that had recently taken place in the room. This helped us to see if animals leaving the room for the test laboratory was affecting other animals’ behaviour.

All animals received regular health checks and implant survey by the NVS about once every month unless potential health issues required more frequent checks. Regular reports were documented and sent to the HOI every six months unless potential health issues required more frequent documentation and HOI information. Frequent and spontaneous

health checks were done by one or two team members that had medical accreditation (including the team leader) and/or by the NVS. Daily health checks were done by one or several ream members under close supervision by the NVS and team members with medical accreditation.

With the knowledge we have now, we feel that the macaque monkey with its superb and experimentally well-controllable behavioural repertoire is the ideal animal for obtaining fundamental new knowledge about brain-behaviour relationships (rather than extending existing knowledge). This argument becomes particularly visible with the present failure to obtain similarly sophisticated and informative new data from marmoset monkeys and rodents, as far as precise behavioural choices and well-identifiable reward processes are concerned that can be differentiated from sensory and movement processes. The macque monkey, in careful and experienced hands, is a precious scientific resource that should be well preserved.

28. Neutrophils in infection and disease

Project duration

5 years 0 months

Project purpose

- Basic research

Key words

infection, neutrophil, macrophage, inflammation, cancer

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

Retrospective assessment

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

Objectives and benefits

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

What's the aim of this project?

This project will study the mechanisms that regulate inflammation focusing on neutrophil and macrophage responses and how they are affected by changes in hematopoiesis during infection, chronic inflammatory disease and cancer.

A retrospective assessment of these aims will be due by 30 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 it's aims and if not, why not?

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

Why is it important to undertake this work?

Neutrophils are crucial for immune defence but are also driving immune pathology when they become deregulated. Severe infections, cancer and atherosclerosis are the major drivers of premature death worldwide. Neutrophils play a critical role in all of these

diseases. Understanding how neutrophils protect against infection and more importantly how they are implicated in sepsis, cancer and atherosclerosis and other inflammatory conditions will provide new avenues of treatment for diseases that remain largely untreatable.

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

This project aims at uncovering novel mechanisms that drive sepsis, cancer and chronic inflammatory disease that could be targeted therapeutically in order to improve the lives of patients suffering from these diseases. In addition, it aims at addressing fundamental questions about the functions of neutrophils in immunity and disease. This work will be published in a number of scientific research publications and collectively present an inter-connected body of work that will advance our understanding of the mechanisms that fine-tune the production and function of neutrophils and regulate their interactions with the other parts of the immune system.

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

In the short-term (2-5 years): academics, researchers in academic institutions and the pharmaceutical industry. In the long run (5+ years) inflammatory disease, infection and cancer patients, clinicians and the general public.

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

Presentation in national and international scientific meetings and seminars at other research institutions.
Publication of high impact research papers, reviews and book chapters.
Collaboration with other basic and clinical researchers.
Disseminations via public engagement activities.

Species and numbers of animals expected to be used

- Mice: 25000

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 work with mice as they are well-characterized model organisms to study the immune system and model human infection and disease. In addition, countless critical genetic resources have been developed in mice that are invaluable for our work.
Nearly all of our immunological challenges are tested on young adult mice which have robust and mature immune systems.

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

The protocols in our project employ a number of procedures such as injections or administrations of microbes, immune or tumour cells of small molecule substances, intravenously, orally or intratracheally. Moreover, some mice receive an altered diet, such

as a western style high fat diet. In one protocol mice will undergo a minor surgical procedure to inject tumour cells into the pancreas. Depending on the experimental design experiments with microbial challenge last approximately 1 week. some mice may be infected on multiple occasions with small doses of microbes to evaluate the effect of chronic low grade exposure on tissues and immune cells. Some mice may be infected with higher doses that will cause pneumonia or a skin abscess. Other mice may receive microbes systemically that cause septic shock in order to understand the mechanisms that promote the condition as well as the mechanisms that protect against its onset. Tumour challenges last approximately 2 weeks, arthritis models 10 days and atherosclerosis dieting typically 6-12 weeks. Animals may also be induced to develop arthritis that spontaneously resolves after 2 weeks.

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

Infections typically cause weight loss and some transient discomfort characterised by reduced activity. Skin infections may cause abscesses that in some cases may ulcerate resulting in the animals having to be sacrificed. In more severe cases such as sepsis models, mice may experience systemic inflammation with some pain, lethargy, drop in body temperature and respiratory distress, which will be endpoints in the experiment. However, monitoring the temperature allows us to cull the animals before they reach these severe symptoms if meaningful results can be obtained in earlier stages of the condition. Tumours do not usually cause visible symptoms but some weight loss may occur. Large metastatic tumours may cause more significant weight loss and respiratory problems which will be endpoints in our experiments The atherosclerosis model does not cause any visible harm aside from weight gain and vascular plaques that are asymptomatic. The arthritis model will cause some limb swelling and may impact on mouse mobility to some degree.

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

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

The majority of our protocols are of mild or moderate severity. Only one protocol employing a model of microbial sepsis is classified as a severe protocol as mice are expected to develop symptoms associated with systemic hyperinflammation. However, the mice will be closely monitored and will be sacrificed as soon as these symptoms begin to appear.

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

- Killed

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

Our projects investigate the functional and mechanistic basis for inflammatory diseases. While certain aspects can be addressed using in vitro cultured experiments, to establish the relevance in vivo and to dissect the events that drive these diseases functionally in in vivo requires the use of animal models of disease, particularly since none of the diseases we are studying can be fully recapitulated and modelled in vitro using organoids. Sepsis is a complex disease implicating several different organs and cell types. Similarly, atherosclerosis and the interactions of immune cells with tumours must also be examined in their native in vivo environment.

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

We are employing human primary neutrophils and other myeloid cells to conduct many of our mechanistic experiments before proceeding to in vivo validation. We are also conducting descriptive studies with human clinical samples of sepsis and atherosclerosis. This approach reduces the number of mice we use in our projects. However, for certain projects as in sepsis we have relied on mouse experiments guiding subsequent mechanistic in vitro studies. The only real alternative to in vivo studies are organoids but they are not applicable to the diseases we are studying.

Why were they not suitable?

All of the aforementioned approaches are complementary to in vivo experiments, but unfortunately cannot replace animal work given that the questions that we are investigating have to be examined in the native disease context.

A retrospective assessment of replacement will be due by 30 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?

Pilot experiments and biological replicates

When setting up a new experiment, rather than using large groups of animals and having to repeat these large-scale experiments to refine the experimental parameters, we start

with very small groups (23 animals /group) and test several parameters we predict to be important for optimization.

Subsequently, we follow up the selected conditions that provide good experimental data with additional experiments consisting of small groups that we add to the original study until we reach statistical significance. This phased sequential experimental design reduces the overall number of experimental animals used in our studies since it limits the number of animals that participate in studies under suboptimal conditions.

In addition, we keep experimental groups small and divide mice over a larger number of independent experiments (biological replicates) in order to obtain better statistical significance from a smaller total number of experimental mice.

Power analysis

Prior to designing experiments we use published data and our own past experience to set the appropriate sample sizes. For most of the quantitative experiments, sample sizes may be set using power analysis, generally using a significance level of 5%, a power of 80%, and a least practicable difference between groups of 20%.

Imaging

We are also implementing imaging techniques that in many cases are non-invasive and allow us to monitor the progression of infection, inflammation and tumour growth, without sacrificing animals. In this manner we can constantly monitor experimental animals and select optimal timepoints to terminate experiments rather than using multiple animal cohorts sacrificed at different timepoints in order to capture the best timepoint for measuring parameters. This approach minimizes the premature termination of experiments which would require unnecessary repetition and prevents unnecessary suffering by not allowing experiments to proceed beyond the optimum time point.

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

As specified above, we conduct small scale pilot experiments to guide power calculations prior to expanding our studies. In many cases long term experiments are performed with in smaller groups and in a phased manner, allowing us to increase the total sample size progressively until the required statistical power is achieved. The early results of the magnitude of changes between experimental groups are filtered through online power calculation tools to estimate accurately the sample sizes required for publication. Through small sample sizes and pilot experiments we can also refine variables like microbial and treatment doses for subsequent experiments

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

Breeding: We monitor the breeding of our mice closely to ensure that only breed the number of animals we need for experiments without breeding excess animals that will not be used in any experimental protocols.

Multiple uses of tissues, future proofing: From each experiment we collect the maximum number of tissues, even though we may not need them all for the purposes of the present experiment. Given that we employ standard experiments in many of our projects, we keep

frozen tissues and samples from all these mice and organise them in a database. This allows us to easily access the tissues in the future without needing to replicate the experiment with new mice. This practice has reduced the number of experiments we have been conducting in the past.

Small pilot studies always inform our group sizes, as well as avoid unnecessary large scale experiments that do not show promising results in the small-scale pilot studies. If pilot studies fail to demonstrate signs that experiments may yield interesting data, then no follow up large scale experiments will be conducted and projects will take other more promising directions.

A retrospective assessment of reduction will be due by 30 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 employ several murine models of pulmonary and systemic infection using fungal and bacterial pathogens. We will also employ two models of sterile chronic inflammatory disease: murine atherosclerosis using the administration of high-fat diet and a transient model of rheumatoid arthritis using injection of auto-reactive antibodies against collagen. Finally, we will employ several models of primary and metastatic tumours.

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

These murine models are optimised to cause the least amount of suffering. They last anywhere from 24 hrs to several weeks and therefore the animals cannot be anaesthetised for this period of time. However, they are temporarily anaesthetised when undergoing certain procedures. Murine models of disease are very similar to human disease and recapitulate many of the attributes and mechanisms of pathology observed in human patients. They also have a very similar immune system with that of humans that is extensively characterised. In addition, over several decade a multitude of genetic tools and reagents that are specific for mice have been developed that are essential for our research.

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

Over the years we have refined several of our protocols, particularly by fine-tunning the rate of monitoring and infection doses in our severe sepsis protocol which allow us to

predict relatively accurately when WT and GA mice will develop sepsis. We have also refined the breeding pairs needed for maximal use of mice in experimental protocols. These are constantly adjusted according to experimental needs. The time-courses for atherosclerosis experiments have also been well characterised from our prior work, allowing us to estimate with accuracy the length of time allowed to obtain the degree of plaque formation needed for specific experiments.

We have also refine our anaesthetisation protocols which reduced the recovery time for mice and reduced the appearance of unexpected symptoms in response to infection.

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

We are aware of NC3Rs. We also discuss with colleagues in other research groups new improvements that lead to refinement. In addition we follow the literature and improvements in commercial reagents in a constant search for more efficient and better refined alternatives. This has led us to improve our arthritis model which is now 100% penetrant, requires fewer injections and much less time and is much more predictable and consistent than the traditional immunisation protocol.

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

Our institute regularly distributes newsletters and holds seminars to inform us on 3Rs and ways of improving our methods reducing the number of mice we use and refining our techniques both en mass and at a personal level. We also regularly seek advice from our veterinarian on how to improve our procedures.

A retrospective assessment of refinement will be due by 30 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?

29. Pharmacokinetics of Pharmaceuticals

Project duration

5 years 0 months

Project purpose

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

Pharmacokinetics, Medicines, Preclinical

Animal types	Life stages
Mice	juvenile, adult, pregnant
Rats	juvenile, adult, pregnant
Rabbits	adult
Guinea pigs	juvenile, adult
Cynomolgus macaques	juvenile, adult
Beagles	juvenile, adult
Minipigs	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.

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 work is to conduct relevant studies during the development of new medicines, to assess how much and how quickly the body absorbs the test medicine, and then how much and in what ways the medicine is changed within the body and then excreted.

A retrospective assessment of these aims will be due by 09 April 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 it's aims and if not, why not?

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

Why is it important to undertake this work?

The work in this project will allow drug companies to make decisions on which possible new medicines are most likely to be to worth spending more time and animal use in developing, and also then providing enough information for the government regulators to allow the safe administration of the materials to people. Without these studies, progression of new medicines to early human studies and to patients could not occur in the current regulatory framework.

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

Data collected will be information on the absorption of various potential new medicines, as well as how animals change and excrete these materials. Outputs will include simple measures like the amount of the medicine which is in the blood, urine or faeces, at various times after being given the medicine. These results can then be compared to the known or expected situation in people.

The data will be collected to the quality standards expected by government regulators in the UK, Europe and elsewhere, who will make decisions on whether these materials can be safely marketed and used in society.

Improved methods of conduct of specific data collection processes may be developed during the course of the project.

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

Our clients, typically commercial drug companies, will benefit from the provision of high quality data. This will to help them in their work to develop new and better medicines, to discontinue development of inappropriate medicines or to understand and manage the risks of new medicines given to people.

Work on this project may also provide data to inform ongoing human clinical trials. Enabling development of successful medicines will benefit society through diagnosis, treatment or prevention of disease.

The wider scientific community may benefit from publication of refined approaches to animal use.

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

Our organisation has colleagues who also have experience of such work in different parts of the world. Collaborations and information exchange with others within the organisation, helps to identify and spread information on successful and unsuccessful approaches. The licensee and colleagues have had on-going collaborations with NC3Rs on various aspects of the conduct of studies, and associated housing and husbandry methods for the animals, over many years.

The licensee and colleagues seek to disseminate information through presenting outputs at scientific conferences and contributing to publications in the scientific literature where relevant.

Species and numbers of animals expected to be used

- Beagles: 250
- Minipigs: 150
- Pigs: No answer provided
- Cynomolgus macaques: 250
- Mice: 5000
- Rats: 10000
- Rabbits: 100
- Guinea pigs: 50

Predicted harms

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

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

Many scientific studies have been conducted to demonstrate that the types of animals to be used in the project will provide results which reflect the likely outcomes for people. Where this is uncertain, comparison between results in different species may be conducted, to identify the most relevant for predictions of outcomes in people. The way in which each new medicine is absorbed, changed and removed by people may be known, or predicted from other information, or studies may be conducted to find this out. The animal type(s) to be used will be chosen based on this understanding, or to find this out. The stage of life of the animals to be tested reflects the age/stage of life of people who would receive the medicines.

Another big advantage of using the listed animal types is that these animal types may be recommended by specific guidelines on how to do this work, and the results of tests are known to be acceptable to the government agencies responsible for authorising use of the medicines in human volunteers and patients. Development of new medicines cannot currently be achieved without this approval by government agencies in the UK, elsewhere in Europe and in other parts of the world.

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

Animals will be given a potential new medicine by the same method that people would be exposed to them - most commonly by mouth, but may be by other routes, including by injection and application to the skin. The medicines are most commonly given once only, and samples such as blood samples are taken to assess how much of the medicine has been absorbed by the body. Similarly, samples of urine and faeces and expired air may be collected to see how much and in what way the body excretes the medicine. Collecting urine and faeces and expired air requires keeping the animals, normally singly, in a small cage which allows the urine and faeces to fall through a grid, typically for about a week. Some animals will have surgery conducted under anaesthesia, and with use of pain relief, to allow collection of bile and/or blood from animals after they have been dosed and housed as described above.

Animals may be humanely killed after the collection period, and tissues may be taken from the animals post mortem (after death), and analysed. Often, animals are kept, and after checks by a vet to confirm that no lasting harms have been caused, they may be used on repeated occasions.

Some animal use involves only taking blood samples, to conduct scientific studies using the blood only.

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

The process of dosing animals, taking samples and confining for collection of urine/faeces/expired air can cause a degree of discomfort during conduct, but not expected to be long-lasting. The dose of test medicines used is not normally expected to cause any significant harm for the animals. Surgery has the potential to cause pain or discomfort, but this is generally prevented or minimised by use of appropriate anaesthetics drugs and pain relief, under veterinary control.

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 harms described above are expected to fall very largely within the mild category, as most studies involve giving a non-harmful material and taking a series of blood samples. Where surgery is performed, or animals are single housed for a period of days for collection of urine and faeces, this would be noted as moderate severity, and may involve about a tenth to a quarter of the total number of animals. Severe outcomes are not anticipated; if seen in individual animals, these would be reported to Home Office.

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 09 April 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?

Non-animal methods are routinely used in some aspects of the development programme for new human medicines, but they are currently not able to sufficiently predict effects on whole body systems or to provide information on how much of a medicine is absorbed, changed and excreted. This information is essential, to confirm that possible new medicines should be developed, and to protect human volunteers and patients who may then take the medicines.

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

The organisation does conduct various non-animal tests as part of the development programme for new medicines, but as noted above, it is still considered essential by scientists and government regulators, to also do work using animals, which this project describes. Some studies are conducted using only blood samples taken from animals; taking the blood samples for this work is also included in the project.

Why were they not suitable?

There currently remains general scientific agreement, and agreement of government regulators, that to protect human volunteers and patients, non-animal alternatives do not, as yet, provide enough information to replace all animal studies.

A retrospective assessment of replacement will be due by 09 April 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 estimates are based on analysis of use of animals in an existing licence authorising work for the same purpose, combined with anticipated need for use to a similar extent.

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

There is no definitive guidance from government regulators on the numbers of animals to be used in the studies described in the project; the applicant and colleagues will use their extensive experience of related programmes, taking account of statistical significance and scientific advice as necessary, to use sufficient animals for studies to provide robust results. Generally group sizes are minimal in nature, using up to 4 animals per group or end-point.

In some studies it is possible to give more than one test item to animals at the same time, and be able to analyse the results, typically the blood levels of the different test items, thereby reducing the total 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?

Pilot studies may be used to investigate the potential of new designs or processes to improve outcomes, before being used in larger numbers of animals. Screening studies, using small numbers of animals, are designed to identify and eliminate materials with undesirable results, and so reduce the numbers of animal which are then used in the studies required by government regulators.

The extensive re-use of some animals enables an enormous reduction in the total number of animals used in the project.

A retrospective assessment of reduction will be due by 09 April 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.

Dosing of potential new medicines is by the same way as they would be given to people; most commonly by mouth, but including various injection methods, occasionally dosing by inhalation and by application to the skin. The methods used are generally very well established and commonly used by experienced staff at the establishment. Volumes of drugs to be given are in line with published guidance on minimising discomfort, and/or are known to cause minimal discomfort based on extensive experience at the site.
Blood sampling is a common need. We follow published guidance on methods and suitable volumes which can be taken while minimising harms to animals.
Restraint or confinement of animals is a common need, to allow collection of samples, generally urine and faeces. Methods used are those with which staff have extensive experience, and the duration of time is minimised wherever possible while allowing completion of the process so that tasks do not generally have to be repeated.
Surgery is required for a small percentage of the animals to be used in the project. It is conducted with expert veterinary involvement in the creation of suitable regimes for anaesthesia and post-surgical pain relief.

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

The species used are selected to answer the scientific questions and enable collection of results which will allow continuation of development of the most appropriate new medicine candidates. They are the same species as are used in follow on studies required by government regulators.

Tests are generally of mild severity for animals, and samples are required over a time period which would make continued anaesthesia impractical in almost all cases, would interfere with the outcome in some circumstances.

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

Refinement of on-going procedures is commonly discussed and explored within the animal technical, veterinary and scientific groups, and also as and when any concerns are identified; for example additional assessments may be included based on initial outcomes. The surgery and anaesthesia/pain relief protocols used in the programme undergo regular and routine assessment and refinement to improve outcomes. Habituation of animals to restraint is a routine process, and the schedule can be amended in response to outcomes for individual animals.

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

Dose volume and blood volume limits agreed with the animal welfare and ethical review body are based on the 2001 publication of Diehl et al: A good practice guide to the administration of substances and removal of blood, including routes and volumes. Welfare end-points are developed in general line with publications on the topic, including the NC3Rs document from 2010 on dose level selection for regulatory toxicology studies. Non-human primate housing is in compliance with the NC3Rs document on this topic from 2017.

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

Both our clients and our colleagues working in the same type of work in other countries, are collaborators who can bring ideas as to how to improve how to conduct our animal studies. Various staff at the establishment have been involved with working groups of the UK National Centre for the 3Rs (NC3Rs), over many years. Staff at the site routinely review published papers in the scientific press, some of which propose refined approaches to conduct of work.

A retrospective assessment of refinement will be due by 09 April 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?

30. Plasticity after injury to the nervous system

Project duration

5 years 0 months

Project purpose

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

Key words

Pain, Nerve Injury, Spinal Cord Injury, Plasticity, Neurotoxin

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.

Objectives and benefits

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

What's the aim of this project?

The nervous system undergoes continuous change in terms of the structural connections between nerve cells, the strength of these connections and how readily signals are generated. These changes are termed plasticity and they adjust and regulate the function of the nervous system according to changing requirements throughout life. When the nervous system is damaged, for example if a peripheral nerve is cut or a high velocity impact results in a spinal cord injury, this ability of the nervous system to change can help it to adjust and compensate for the damage, and helps to provide a degree of functional recovery. However, there are limitations to these plastic changes which in turn limit the degree of functional return. We aim to investigate these so that we can better understand how return of function might be optimised. We will also investigate the effect of drugs that increase the plasticity to see if these can promote improved recovery.

Plastic changes after injury to the nervous system can also have negative consequences. After both peripheral nerve and spinal cord injury, there can often be the development of a particularly unpleasant form of continuous pain. Because it is caused by damage of the nervous system, it is called neuropathic pain. This sort of pain is very debilitating and particularly difficult to treat as drugs are often ineffective. We do not fully understand why this maladaptive, pain producing plasticity occurs but we now have tools and approaches that can help us understand the circuits of neurons responsible, and how they change. The signalling of pain by the nervous system also involves several areas in the brain and one

such area has only recently been identified. It will also be important to define the role of changes in these brain areas to the development of chronic pain. There are now also agents that have the potential to interfere with signalling in spinal cord and brain circuits and these may prove useful in preventing or blocking the pain signal. One group of such substances are nerve toxins. This project aims to provide a better understanding of the changes in spinal cord and brain circuits that lead to pain conditions and test whether these substances can successfully block pain. We will also investigate whether these neurotoxins can prevent the over-activity in the nervous system that leads to dangerously high blood pressure (autonomic dysreflexia) and to painful muscle stiffness (spasticity) in spinal cord injured patients. The toxins will be used at levels well below those that cause muscle weakness or other adverse effects.

A retrospective assessment of these aims will be due by 17 January 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 it's aims and if not, why not?

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

What are the potential benefits that will derive from this project?

The aim of the project is to improve understanding of the plastic changes in the nervous system that occur after it is injured. The potential benefits of the work are that this understanding could provide the basis for developing better treatments for restoring sensation and movement; and improved drugs for controlling the debilitating conditions of chronic pain, dangerous increases in blood pressure and painful muscle stiffness.

Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

The project will use rodents, both rats and mice. It is estimated that the experimental programme will require approximately 1,550 rats and 1,650 mice over the course of 5 years. In addition, 5,350 mice will be used for breeding purposes only.

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?

More than half of the mice will be used to for breeding and maintenance of genetically modified lines, and since the vast majority of these animals will have no behavioural abnormality, this is classified as "mild". A small proportion (estimated at 5%) of the animals (rats and mice) will undergo procedures that are carried out under general anaesthesia,

from which they will not recover, and these are therefore classified as "non-recovery". A further group of animals (estimated at 45%, predominantly mice) will undergo procedures such as injection of harmless tracer substances into the brain or spinal cord, or spinal injections of agents that will activate or inactivate different nerve cell populations. These procedures are performed under general anaesthetic. These animals will receive post-operative painkillers and should experience no more than transient discomfort resulting from the operation. About 50 % of animals (rats and mice) will either have a nerve injury (a moderate procedure) or spinal cord injury operation (a moderate or severe procedure), both of which may lead to a mild form of pain with increased sensitivity to touch or warm stimuli. The animals' ability to eat and drink will not be restricted. After nerve injury and spinal cord injuries that are made with a device that applies an impact (contusion) there may be some lameness but animal's movement is not restricted. In a small proportion of the animals (estimated at 1%, rats only) the spinal cord will be completely cut. This is a severe procedure.

These animals have a temporary inability to empty their bladder and need to be regularly assisted. They also permanently loose the use of their hind limbs. These animals feel no discomfort during the application of stimuli to test for autonomic dysreflexia or spasticity, as these are applied below the injury where there is no sensation.

At the end of the procedure the great majority of animals (estimated to be >95%) will be killed whilst they are under the influence of a general anaesthetic. A much smaller proportion will be killed by another humane method.

A retrospective assessment of these predicted harms will be due by 17 January 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 why you need to use animals and why you cannot use non-animal alternatives.

The objectives can only be achieved by experiments using live animals because the complexity of the biological systems to be examined, and the need for events to develop consequent to manipulations such as injury cannot be reproduced *in vitro.* Neural circuits and changes in connectivity due to plasticity all require investigation in intact animals. Conditions such as pain, spasticity and altered cardiovascular function cannot be modelled in other ways. Where possible, however, our work will be informed by in vitro approaches. This applies in particular to development of agents for neural repair. In addition, we incorporated into our Project Plan, the use of ex vivo preparations.

A retrospective assessment of replacement will be due by 17 January 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 you will assure the use of minimum numbers of animals.

The numbers of animals to be used in each part of the study will be the minimum required to provide reliable results (avoiding as far as possible inter-animal and technical variability). Many of the outcome measures that we obtain are based on observation and are largely qualitative or can only be treated in a semi-quantitative way. For these types of results, we will collect results from a minimum of 3 biological replicates. Where appropriate results will be analysed statistically for significance. In addition, where data is available we will use power calculations which is a method for predicting the numbers of animals that are required to provide statistically significant answers. Experimental design is continually reviewed and our studies carefully executed in order ensure use of minimal animal numbers consistent with the experimental aims and to maximise information obtained from each animal.

A retrospective assessment of reduction will be due by 17 January 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

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

Rodents are the most appropriate species as they are the least sentient animals that could be used. Non-mammalian species show a capacity for nervous system regrowth which does not happen in mammals and also show much greater plasticity. The larger size of rats makes some procedures such as fMRI more feasible. Nevertheless, mice may offer an advantage where genetically altered lines are available because this allows for the targeted manipulation of the responsiveness of particular groups of neurons.
We have experience of all of the models to be used and a good understanding of both their scientific merits and the animal husbandry required to minimise adverse effects. Together with the Veterinary and Biological Services staff, we regularly review the general procedures for animal care including analgesia, fluid replacement, and post-operative maintenance of body temperature. In addition, for more severe injuries such as spinal cord contusions and transections, we have introduced a monitoring chart, which incorporates built in end points. I collaborate widely with others to ensure that we follow the most modern and refined approaches to these severe models of disease and I contribute to published material in this area. All animals will be group housed wherever possible.

A retrospective assessment of refinement will be due by 17 January 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?

31. Pluripotent stem cell derived hepatocytes for treatment of liver failure

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

liver diseases, acute liver failure, mouse model, stem cells, therapy

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

Retrospective assessment

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

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?

Stem cells are special cells in the body from which all other cells develop. The aim of this project is to find out whether stem cell products could be used as a potential treatment for liver failure and inherited liver diseases, by transplanting them into rodents with these liver diseases and assessing whether the cell therapy aids recovery.

A retrospective assessment of these aims will be due by 07 March 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 it's aims and if not, why not?

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

Why is it important to undertake this work?

Liver failure and liver diseases are life threatening human diseases for which currently the only long-term treatment is a liver transplant. Due to a shortage of liver donors, for many patients this life saving treatment is simply not available. Furthermore, there are a number of long-term risks for recipients, including organ rejection and unpleasant side effects from the drugs that have to be given on a permanent basis to prevent rejection. Stem cells afford us the exciting possibility of being able to cure patients with liver failure where otherwise the only proven way of treating them is whole organ transplant. Before such cells can be used in patients it is an essential requirement that their effectiveness is tested in animals which model the human disease, and are proved safe.

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

This research could demonstrate the effectiveness of a potential new therapy for patients with liver failure and inherited liver diseases, and so benefit thousands of patients worldwide. Liver diseases comprise a significant and increasing clinical burden. The current standard treatment for liver failure is whole organ transplant, but the number of patients requiring transplant far exceeds the number of available donor organs making alternative treatment methods an urgent priority/clinical need. The short term benefits of this project would be confirmation that stem cells are potentially an effective, safe treatment for liver failure in rodent models.

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

At the end of this project we expect that we will have proved our idea that these cells are an effective therapy for liver diseases. The new therapy will then be further developed for approval by the Medicines and Healthcare products Regulatory Agency (MHRA)) for clinical trials in humans (medium term benefit). Should Phase 1 trials be successful then these cells could be given to patients in the long term and extend lives of patients with liver failure who are not selected for or cannot have transplantation surgery. In the longer-term, stem cells could replace organ transplants as a more readily available method of rescuing the failing liver without the need for complicated surgery.

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

The outputs from this work will be shared through publication and by presentation at conferences, and through our partner biotechnology company, who will develop this further as a proposal to the MHRA with a view to using them on patients in a hospital setting.

Species and numbers of animals expected to be used

- Mice: 7500

Predicted harms

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

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

Mice have been chosen as the experimental animals of choice because the basic features of their livers are remarkably similar to other mammalian species and of are similar complexity to the human.

Immunocompetent mice (such as C57BL/6) will be used in the study of optimization and identification of therapeutic efficacy of PSC hepatocytes of this project, as they do not reject the encapsulated human cells. Genetically altered mice with liver injury will also be used. Human liver cells are inserted into their livers, providing a means of testing our stem cells against failing human liver tissue.

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

Some animals will be bred to produce animals with immune systems which do not work properly or with liver damage.

Most animals under this licence will undergo liver injury to bring about liver failure. Animals may have surgery to cut out part of their liver tissue or be dosed with chemicals which damage the liver. Some animals with inherited liver disease problems may also be used. Stem cells will be delivered either into various sites on the belly or placed under the skin to try to rescue the failing liver. Blood samples and scanning will take place to monitor how the liver is working and movement of the cells we have introduced.

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

Animals are expected to get sick and some with liver failure might die, they will be monitored very frequently so that they can be humanely killed where possible, to prevent further suffering if their liver failure is not responding to the stem cell implants.
Advice from the Veterinary surgeon will ensure that the welfare of the animals is maintained at the highest possible level for their condition. Any animals that show unexpected side effects of the liver injury or of treatments will be killed to avoid unnecessary suffering.

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

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

The breeding strains are expected to experience mild severity (70% of animals) or moderate (30% of animals) severity on this protocol.

Animals undergoing protocols to generate liver failure are likely to experience severe signs (up to 80% of animals), and some may need to be humanely killed to prevent further suffering. The disease develops very quickly and a proportion of animals may die before they can be given any treatment.

Animals undergoing studies on inherited liver diseases are expected to experience moderate severity (100% of animals on this protocol)

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

- Killed

A retrospective assessment of these predicted harms will be due by 07 March 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?

Unfortunately there are no alternative techniques that could replace the use of animals in this project. In order to demonstrate that stem cells would be effective if used as therapy for liver diseases, they have to be tested in animals with the diseases. It is also a requirement for the techniques to be tested in animals and deemed safe before they can go on to be developed for use in real patients.

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

Our approach is to use experiments in the laboratory as much as possible to replace animal experiments. Initially, laboratory testing will provide information on what cells actually do, thereby decreasing the numbers of animal experiments by screening for the most useful cell lines.

Why were they not suitable?

Laboratory methods are suitable for initial screening of cell lines for treatment potential. These methods cannot however adequately model the all of the things cells would be exposed to in the entire animal, making animal based testing a necessity. Importantly it is a requirement of the Medicines and Healthcare products Regulatory Agency (MHRA) that products from stem cells such as these be tested in animals prior to use in human patients.

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

We will endeavour to use laboratory based methods wherever possible thus limiting the numbers of animals undergoing experimental procedures.

The sizes of experimental groups and the number of repeated experiments will be kept to a minimum while ensuring that reproducible results are obtained with clear biological significance. We use statistical analyses to determine the minimum numbers of animals needed for our studies that will produce robust results. We have estimated our initial group sizes from laboratory studies, which suggest that group sizes of 3 might be sufficient to detect experimental effects. A greater degree of variation is expected in animal studies compared to that observed in the lab and we anticipate that we will need group sizes of 6-12 for our animal experiments. This will be revised as the animal work under this project progresses.

We aim to further refine our techniques through high success rate of surgical procedures and good practices of performing preliminary experiments to establish the minimum number of animals. Furthermore, we will continue reducing the use of animals as much as possible in this licence, in collaboration with the named veterinary surgeon.

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

Advice in experimental design has been taken locally from statistician and scientific colleagues.

Online tool (the NC3R's Experimental Design Assistant) has been referenced.

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

Cells to be used for implantation will be screened in the lab before they are used with animals, to ensure that they are likely to produce the effects which we are looking for. Studies using small numbers of animals will be undertaken in initial work to determine the best methods for cells to be implanted before full scale experiments in animals with liver failure.

Where more animals are bred than we need, surplus animals will be made available to others for tissue sharing where possible.

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

The mouse model has been chosen as the experimental animal of choice as the basic features of their livers are remarkably similar to other mammalian species and have a similar level of complexity to the human liver. Mouse models of liver failure and inherited liver disease are successfully used elsewhere in this area of research, can be reproduced and represent relevant models of the range of human diseases we are studying Immunodeficient (less able to recover from infection) mice are necessary for some studies in order to avoid the human cells/tissues we are introducing from being attacked by the body. These animals will be subsequently bred in house.

The mouse paracetamol and partial liver removal model is the standard model of acute liver failure. Mice are also reliable models for our area of research.

Genetically altered mice provide reliable and relevant models of inherited liver disease which can be brought about as required, animals remaining normal until the disease is 'switched on'. Genetically altered mice which have, or can be made to have liver failure, do not require an initial surgery or toxic dose of chemical before stem cells are delivered. The humanised livers of these mice additional provide a method of testing these stem cells against damaged human liver cells.

For these reasons, mice are the species of choice for our studies. These animals are needed to investigate how human liver cells that have been made from stem cells work and how they can ease liver failure symptoms. The mouse models are important in providing new information and as models in which to study new ways of transplanting cells.

Suffering

All the protocols proposed in this application use well-established and tried techniques that have been refined to involve a minimum of suffering. Sterile surgery will be conducted by researchers with considerable experience in animal techniques; training and competency records will be kept. Attention will be paid to keeping rodents warm and hydrated during surgery to improve outcomes. Anaesthesia and pain relief will be administered to minimise discomfort and the animals will be assessed at least daily for any signs of distress. Experiments with a small number of animals will take place first i to refine methods, identify effective doses and avoid excessive deaths. In all the proposed animal experiments, if animals display signs of distress, advice will be sought from the Named Veterinary Surgeon (NVS) and, if distress cannot be eased, the animals will be humanely killed.

Liver failure is associated with a significant death rate and all attempts will be made to avoid this. Animals will be observed multiple times per day after we have brought about liver failure, and small samples of blood will be taken to check liver function using markers in the blood. Hand held blood analysers, as used in human patient care,will be trialled in an attempt to identify animals whose condition is deteriorating quickly so they can be humanely killed immediately rather than waiting for samples to be processed remotely in the lab.

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

The livers of non-mammalian species do not have the same make up as human livers are not good models of human liver disease as they relate to human patients. In addition, animals which have been bred not to reject the transplanted cells are needed for some studies, and these are generally only available in rodents.

The ability of the liver to grow new cells is less in younger animals, thus younger animals are unlikely to recover from liver injury and are not suitable as models for the human adult patient. The mouse is considered least sentient mammalian species and will be used for the majority of studies.

It is not possible for us to carry out our studies on anaesthetised animals which are killed without waking, as we need to find out how the animal recovers from liver failure.
The ability to genetically alter mice also makes them our species of choice.

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

We aim to develop blood tests to indicate increasing likelihood of severe outcomes such that animals can be identified at an early stage and suffering minimised.

We will use frequent observations of animals throughout the critical phases of each experiment, measuring body weight and condition and observation of eating and drinking. Our staff have considerable experience in normal animal behaviour and we will take advice from our named people on animal welfare.

We will start with small scale experiments to determine the most efficient and best way to carry out our studies, progressing to full scale experiments once we have improved our understanding of the unknowns.

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

We will make use of published information on methods and use resources such as PREPARE and the NC3Rs to help us keep informed about best practice.

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

We will communicate with our named people throughout the project and seek advice on refinement of procedures. We well attend conferences and 3Rs events and implement any improvements to the protocols that are identified.

We will also report back to our local AWERB and our Inspector after our initial experiments to determine doses.

A retrospective assessment of refinement will be due by 07 March 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?

32. Protection against incapacitating agents

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

therapy, incapacitating agents

Animal types	Life stages
Marmosets	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.

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?

Pharmaceutical based agents that can cause incapacitation pose a threat to the UK. This project aims to understand the threat posed by such agents and to assess potential treatments for them.

A retrospective assessment of these aims will be due by 09 January 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 it's aims and if not, why not?

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

Why is it important to undertake this work?

This licence will help to understand the threat posed to the UK from pharmaceutical based agents deployed either as incapacitating agents or as lethal agents. The data from these studies will aid in determining the level of threat posed and the requirements for detectors, diagnostics, physical protection, medical countermeasures, extended medical management and decontamination.

If an agent is determined to be a threat, then existing or novel medical countermeasure will be tested to assess their ability to reverse, prevent, reduce, delay or mitigate the effects of exposure. Effective medical countermeasures will be able to save lives.

These agents pose a potential threat to the civilian population, first responders, the NHS and UK Armed Forces.

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

There will be improved knowledge on the potential threat posed by pharmaceutical based agents as either incapacitating agents or lethal agents. This knowledge will aid in determining the level of threat posed and the requirements for detectors, diagnostics, physical protection, diagnostics, medical countermeasures, extended medical management and decontamination.

There will be improved knowledge on the effectiveness of new and existing medical countermeasures to reverse, prevent or mitigate the effects of exposure to pharmaceutical based agents.

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

Increased knowledge of the threat and new medical countermeasures will provide benefit to the civilian population, first responders, UK armed forces and our international partners (nations with whom the UK formally collaborates).

Outputs from this work will aid in the development of new detectors, diagnostics, physical protection and decontamination strategies for pharmaceutical based agents.

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

Some of the work will be published in the public domain in peer reviewed journals. Outputs not suitable for the public domain will be shared with international partners through formal international collaborations. These comprise regular prearranged collaboration meetings / conferences.

Where the long term output of this work is a variation to the currently licensed indications for use of a therapy (for example to add that the therapy be used in a new way to treat exposure to a pharmaceutical based agent) this will be disseminated via medicinal product information and will be accessible to healthcare professionals.

Species and numbers of animals expected to be used

- Marmosets: 358

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 marmosets as we know that they have a similar response to man for the compounds we will be studying.

We also know that other species such as mouse, rat, rabbit and pig respond differently to these compounds and / or are a poor predictor of the human response.

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

Most animals will receive an inhalation exposure to a pharmaceutical based agent (PBA). This will require they are restrained for exposure which also enables the measurement of respiration during the exposure. Exposure to the PBA will likely cause a range of effects such as: sedation, anaesthesia and respiratory depression. This respiratory depression may become fatal. Animals will be observed following exposure until they recover. Some animals may be exposed to PBA via a different route, including injection.

Some animals may receive potential medical countermeasures to assess their ability to block or reverse the effects of PBA exposure. Medical countermeasures may be administered before or after PBA exposure and will be administered by injection. Some animals may be surgically implanted with a drug delivery device containing medical countermeasures. Some animals may be implanted with a device that enables access to blood vessels via a sampling / injection port.

Animals will have blood samples taken to measure the concentration of PBA and / or medical countermeasures in the blood.

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

Animals exposed to PBAs are expected to experience effects such as drowsiness, sedation, anaesthesia and respiratory depression. This respiratory depression may result in death, however, the animal will be anaesthetised by the PBA if this happens. Animals that survive will make a complete recovery. The effects are expected to last for hours. Medical countermeasures are likely to fully or partially block or reverse the effects of exposure to the PBA.

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

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

The severity limit of all protocols is moderate.
It is expected that across the protocols, 56% of the animals will reach moderate severity with the remaining 44% reaching mild severity.

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

- Killed

A retrospective assessment of these predicted harms will be due by 09 January 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?

Exposure to fentanyls can cause a complex range of effects with respiratory, cardiovascular and neurological changes. It is important to understand the doses required to cause a range of incapacitating effects and to cause death as this provides crucial information on the threat posed. It is also important to understand the window of opportunity in which to treat exposure and how long the effects may last. Such data cannot currently be gained without the use of animals to model the whole body response to fentanyls.

Some data can be gained using *in vitro* assays, such a opioid receptor binding potency, however, whilst fentanyl effects are driven by activity at the opioid receptor, fentanyls also affect different signalling pathways. The efficacy of nAChR partial agonists in a rodent model of opioid induced respiratory depression shows that opioid induced respiratory depression and its mitigation involves complex signalling within the central nervous system. Therefore it is important to understand the effects of exposure and countermeasures in an animal model.

In vitro systems can be used to determine if opioid antagonists are able to displace fentanyls from opioid receptors, however information is still required to determine the length and extent of protection that can be afforded, whether planned doses of opioid antagonists are able to counteract predicted challenge levels and whether they can treat all effects of exposure.

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

We have considered in vitro systems and simple model organisms for this project.

Why were they not suitable?

Such approaches can provide useful data, however, they will not be able to achieve aims of our project and would not provide accurate predictions to man which will focus on one class of chemical agent. However, supporting information on novel compounds will be potentially be provided by in vitro systems, simple model organisms and other non-primate animals.

A retrospective assessment of replacement will be due by 09 January 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?

These numbers are based upon work that is currently planned and group sizes that have been used successfully in previous studies in this area.

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

Advice has and will be taken regarding study design from statisticians with respect to statistical designs of studies and the analysis of data from this project. Advice has and will be taken from pharmacokinetics experts regarding design of kinetics studies.
If multiple treatments are to be tested, then a shared control group will be used.

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

Before treatments are tested, a pilot study will be carried out to assess the selected challenge dose to determine its variability and suitability.
If multiple treatments are to be tested, then a shared control group will be used.

A retrospective assessment of reduction will be due by 09 January 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.

Animals will be exposed to pharmaceutical based agents which will themselves cause analgesia, sedation and anaesthesia and will reduce the potential pain, suffering and distress that the animals may experience. Any adverse effects from the exposure will occur once the animal has become anaesthetised by the exposure. If treatments against exposure are effective, then this will prevent or reduce the effects of the exposure.
Some animals may undergo surgical procedures to either allow monitoring of physiological parameters or to administer treatments over an extended period of time. These animals will be given analgesics during their recovery from surgery.

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

The project is to understand the potential threat from pharmaceutical based agents, many of which have an anaesthetic action. Therefore, it is not possible to do the study in terminally anaesthetised animals.

Lower species have been shown to be a poor predictor of human response for the class of pharmaceutical based agents that will be initially studied.

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

Animals will be continually monitored following exposure until they have made a full recovery or reached a humane endpoint.

For animals undergoing any surgical procedure, appropriate analgesics will be given pre- and / or post-surgery. Animals will be monitored as they recover from anaesthesia and will be regularly checked post-surgery to ensure appropriate level of analgesia and monitor the progression of wound recovery. Sub-cuticular stitching will be used wherever possible to minimise any discomfort due to stitching.

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

Available guidelines on the NC3Rs website regarding blood sampling will be followed. This references "A Good Practice Guide to the administration of substances and removal of blood, including routes and volumes" by Diehl et. al. This will also be followed regarding the administration of substances.

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

Updates on advances in the 3Rs are regularly distributed by our Named Information Officer. The licence holder is actively engaged in our establishments Animal Welfare and Ethical Review Body (AWERB). Any appropriate advances will be discussed with our veterinary staff and, where appropriate and compatible with the scientific aims of the project, these advances will be incorporated. I will maintain my required annual CPD, attend relevant external scientific meetings and have meetings and teleconferences with international collaborators working within this field.

A retrospective assessment of refinement will be due by 09 January 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?

33. Targeting Fibroblast Heterogeneity, Plasticity and Functions in Pancreatic Cancer

Project duration

5 years 0 months

Project purpose

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

Key words

Pancreatic cancer, Tumour microenvironment, Fibroblasts, Cancer therapies, Cellular cross-talks

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

Retrospective assessment

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

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 different populations of non-cancerous cells, called fibroblasts, support pancreatic cancer progression to identify new combinations of treatments that block the tumour promoting cross-talks of cancer cells and fibroblasts.

A retrospective assessment of these aims will be due by 24 January 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 it's aims and if not, why not?

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

Why is it important to undertake this work?

Pancreatic cancer is highly lethal. Drug resistance is one of the main reasons of pancreatic cancer lethality and is largely caused by non-cancerous components, called stroma, that comprise up to 90% of the tumour mass. Within the stroma, fibroblasts are the most abundant cell population and promote drug resistance and cancer growth. No current therapy effectively targets these cells. Although previous studies provide insights into the wide range of fibroblast cells (i.e. fibroblast heterogeneity) in pancreatic ductal adenocarcinoma (PDAC), the extent of this heterogeneity, the roles of distinct fibroblast subtypes and how to selectively target them remain unclear.

The role of cancer-associated fibroblasts (CAFs) in modulating tumour progression is emerging as a strong player in therapy resistance, metastasis formation and poor prognosis. However, whereas the biology of pancreatic cancer cells have been extensively investigated, the roles of pancreatic CAFs are largely unknown. Evidence suggests that distinct fibroblast groups (i.e. subtypes) play different roles in the progression of pancreatic cancer, and that targeting them individually may lead to disparate outcomes. We propose that strategies using combinations of drugs that target tumour-promoting fibroblast populations could be used in the future for pancreatic cancer treatment.

It is therefore important to deeply characterise different fibroblast subtypes to understand how to target them.

The strategies proposed herein will directly address knowledge gaps that could have significant therapeutic implications. Notably, the emerging understanding that fibroblast heterogeneity is not restricted to pancreatic cancer indicates that our findings could be applied to other tumours.

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

The completion of this project will significantly expand the knowledge about fibroblasts and the tumour microenvironment (i.e. the arrangement and characteristics of the different cells in the tumour) in pancreatic cancer and will potentially identify new ways to better group patients for different treatments. Overall, the investigation of fibroblast biology may lead to new strategies to treat and detect pancreatic cancer.

Additionally, the publications originating from our studies could inspire future work in various laboratories across the world. As it is emerging that fibroblast heterogeneity is present in other malignancies and inflammatory conditions, our findings could provide useful insights to other cancer fields and be of interest to the broader scientific community.

Similarly, we will generate and make available to the scientific community new mouse models that could allow to investigate fibroblast functions in other malignancies, diseased states and normal tissues, and could thus be important for the scientific advancement in other fields.

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

In the short term (2-3 years), the scientific community will benefit from our discoveries through peer reviewed (i.e. assessed by other academic experts in our field) open-access (i.e. available to everyone without a journal subscription/fee) publications and open-access, not yet peer-reviewed pre-prints originating from our work. This will promote

further research and rapidly advance our and others field. Papers published on both of these platforms will also be available to the non-scientific community, so that everyone could be informed, if interested, about our scientific progress. Additionally, we will present our work at conferences and workshops. We will also engage the non-scientific community in public events. We will also host college students over the summer, to train and inspire the next generation showing them our research progress and models.

Our main long-term goal is to deeply understand the mechanisms regulating tumour heterogeneity to develop strategies that would benefit patients. Although this output will not be rapidly evident following our work, as it will need to be evaluated in clinical trials, our studies will be a first step towards that direction.

Eventually, pancreatic cancer patients could benefit from the design of selective combinations of drug treatments resulting from our research and specific for their particular tumour.

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

We aim at publishing at least one article per objective. When possible, we will also submit these works before peer-review, as pre-prints, so that the findings of these studies will more rapidly advance research and will also limit potential duplications and unnecessary mouse work in other laboratories. Publications of unsuccessful or non-high impact approaches that would spare unnecessary duplications could also be deposited as pre-prints.

As similarities between fibroblasts in pancreatic cancer and fibroblasts in other cancer types are emerging, we also aim at establishing collaborations with other laboratories. We are also planning to share with other laboratories the new mouse models that we will generate. Of note, these new mouse models could be used in the future to study the roles of distinct fibroblast subtypes in other cancer types, inflammatory conditions, and normal tissues.

Species and numbers of animals expected to be used

- Mice: 18300

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 aim to deeply investigate the biology of fibroblasts in pancreatic ductal adenocarcinoma (PDAC), while keeping in sight a potential clinical output. Using the best models of PDAC is, therefore, a priority. We will use mice for the proposed study. Mice are commonly used as an animal model of PDAC as, more than any other model, they recapitulate the progression of the human disease, the crosstalk between cancer cells and stromal cells, such as fibroblasts, and the response to therapies. Finally, mice are required to test the efficacy of potential anti-cancer therapeutics prior to their testing in clinical trials. For example, the KPC mouse model remains the gold-standard for studying PDAC biology, progression and therapeutic response, as numerous parallels can be made between KPC tumours and the human disease.

This is possible because the KPC mouse model has been genetically altered to express in its pancreas the key genetic mutations that occur most frequently in human pancreatic cancer, hence it develops pancreatic tumours which accurately reproduce the characteristics of the tumours that develop in humans.

Our protocols for 1) breeding and maintenance of genetically altered mice, 2) breeding and administration of substances to genetically altered mice, and 3) production/maintenance of genetically altered mice that develop autochthonous (i.e. spontaneously arising) pancreatic tumours (e.g. KPC mice) will include various life stages. For other protocols, e.g. drug studies with genetically altered mice bearing autochthonous pancreatic tumours and transplantation of pancreatic organoids (i.e. three-dimensional cultures of pancreatic cancer cells) into the pancreas (i.e. orthotopically), we will only use adult (and aged) mice, as we aim to investigate tumour biology and tumour response to treatments.

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

Some mice will be administered drugs in the food or water while pregnant or before and after birth, for up to 6 months.

Some mice will develop pancreatic tumours, either spontaneously if genetically engineered mouse models (GEMMs) or following transplantation of organoids into the pancreas, in some cases after having aged. Some of these mice may be imaged (for example by ultrasound) to detect the presence of a tumour in the pancreas and humanely killed, prior to analysis of tissues.

Some mice will develop tumours and will be imaged by ultrasound and will be administered drugs (i.e. enrolled in drug studies). Mice enrolled in drug studies will be typically alone in the cage to decrease the experimental variability, which will overall decrease the number of mice needed. Mice will be then humanely killed, prior to analysis of tissues.

For genetically altered KPC mice, spontaneous pancreatic tumours develop at 3-6 months of age.

Following tumour formation, the mice can survive approximately for up to 1-2 months. For transplantation models, pancreatic cells will be typically injected into the pancreas at 6-8 weeks of age, unless performed in aged mice around 16-18 months of age. Depending on the cell line and number of cells transplanted, tumour presentation typically occurs between 1 (more common) and 12 (rare) months from surgery. Following tumour formation, the mice can survive approximately for up to 2-3 months.

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

Both genetically altered KPC mice and transplantation models bearing pancreatic tumours recapitulate clinical signs observed in pancreatic cancer patients, including loss of appetite, weight loss, inactivity and ill health. Additionally, KPC mice can develop other clinical signs typical of advanced pancreatic cancer in patients, including ascites (i.e. accumulation of fluids in the abdominal cavity) and bowel obstruction (when the presence of the tumour is blocking a part of the intestine). These symptoms usually occur at a late stage of tumour development in the mice and will normally only be present for 12 weeks prior to reaching a humane endpoint.

During their life time, KPC mice also often develop facial and anal papillomas (i.e. benign epithelial tumour growths) that do not cause discomfort, if not infected, unless they obstruct vital functions, such as eating, drinking or defecation.

Administration of substances via injection or oral gavage (i.e. with a feeding tube that reaches the animal stomach) will cause recurrent (daily or weekly), transient (10-15 minutes) discomfort and distress, due to the restraint and procedure required. Administration of substances to the food may also cause discomfort and loss of appetite due to poor palatability, leading to transient weight loss.

Ultrasound imaging could cause transient distress and discomfort normally associated with the anaesthesia.

Ageing mice may exhibit phenotypes associated with ageing. Common age-related clinical presentations, which are not expected to cause adverse suffering and can be managed are: i) skin lesions, such as loss of hair; ii) ocular lesions, such as conjunctivitis; iii) hearing loss; iv) reduced mobility; and v) body weight changes. Additionally, adverse age-related clinical presentations may be: i) visible or palpable masses, including tumours; and ii) prolonged body weight loss.

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, <2.5% of genetically altered KP mice (i.e. parental mice of the KPC mice), <2.5% transplantation models (i.e. models in which pancreatic cancer cells are injected in the pancreas of mice), <5% genetically altered KPC mice may be in a severe (in terms of level of pain, suffering and distress) category. The majority of mice will experience a moderate severity, and the remaining will be in mild or sub-threshold (i.e. even lower than mild in terms of level of pain, suffering and distress) categories.

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 24 January 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?

Although recent improvements have occurred in the development of novel laboratory platforms for the study of the tumour microenvironment, animals remain the most informative model to recapitulate the cross-talks of cancer cells and fibroblasts observed in patients, because only a tumour in a living animal, such as a mouse, can exhibit all the complex features of a human tumour. To carry out impactful and meaningful research that could lead to the development of new therapies, it is therefore essential to use animals for the investigation of cancer/fibroblast cross-talks.

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

As an additional approach, we will employ research alternatives that do not involve the use of animals. In particular, for some of the biological questions we aim to address, we will employ three-dimensional pancreatic organoid/fibroblast co-cultures.

Although the use of these co-cultures will reduce the number of mice employed in this project, it cannot completely substitute for it as, to date, no system can accurately recapitulate the fibroblast cross-talk with pancreatic cancer cells and the surrounding microenvironment as well as mouse models.

However, we are also actively working on optimising our current co-cultures by including additional cell types, such as immune cells, with the hope to develop more meaningful laboratory models for the study of pancreatic cancer biology.

Why were they not suitable?

Although our co-cultures will allow to reduce the use of animals, they cannot entirely replace the use of these models as they do not fully recapitulate the tumour microenvironment of pancreatic cancer.

Additionally, prior to testing our findings in clinical trials, the relevance of our findings will have to be evaluated in mouse models of pancreatic cancer that have been shown to largely recapitulate the human disease (e.g. transplantation mouse models and genetically altered KPC mice).

A retrospective assessment of replacement will be due by 24 January 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?

These numbers are an approximation as the effective size will depend on the results obtained from the pancreatic cancer organoid/fibroblast co-cultures and initial smaller-scale (i.e. pilot) studies, when relevant. Indeed, animal usage may be reduced by performing pilot studies (with 4-5 animals) prior to the full studies with a larger number of mice. If biological and statistical significance is reached in a pilot study, a full study may not be required and this will reduce the number of animals used.

Additionally, the results gathered from experiments performed in the co-cultures will inform the design of the animal studies.

For transplantation models of pancreatic cancer, the cohort sizes have been initially determined based on our previous studies. Previously, n=5-10 per group has been sufficient to reach biological and statistical significance.

Similarly, for therapeutic studies in genetically altered mouse models (e.g. KPC mice), we have performed pilot studies to characterise growth rate and variability of tumours to inform subsequent studies. Previously, n=5-10 per group has been sufficient for monitoring the anti-tumour effect of drugs. Finally, based on our experience, for survival studies (i.e. studies in which animals are humanely killed only when they show severe clinical signs at humane enpoints), 10-12 mice per cohort have been sufficient to reach biological and statistical significance.

Moving forward, we will re-assess these numbers when more information will become available with our future studies. To do this, we will work closely with our Bioinformatics core facility who will provide advice on the study design and statistical analysis to determine the number of animals needed.

Additionally, our Bioinformatics core facility uses a variety of statistical methods to analyse different types of datasets.

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

At all times, we attempt to reduce, replace and refine our animal models. We are committed to limit the use of animals in our research. To this end, when scientifically meaningful, instead of using genetically engineered mouse models (GEMMs), we will establish pancreatic transplantation mouse models in which pancreatic cells or organoids are injected into the mouse pancreas. These models will reduce the number of mice needed to address our experimental questions, as they do not need the breeding required to generate the GEMMs. Although useful, these transplantation models cannot entirely replace GEMMs, such as KPC mice, as they bypass the early stages of tumour formation. The combination of both mouse models will be necessary for deeply understanding pancreatic cancer fibroblast biology and for evaluating new therapeutic strategies.
To further reduce the number of animals in our research, whenever possible and scientifically meaningful, we will use co-cultures of previously established murine and human pancreatic cancer organoids and fibroblasts.

These analyses will help indicate how and whether an animal study should take place. Additionally, each therapeutic study will include all necessary controls, thus increasing the reproducibility and robustness of the study, and decreasing the variability and, therefore, the number of mice per group.

Additionally, we will employ ultrasound imaging to reduce the number of mice used in drug studies. Indeed, this technique allows to identify, and exclude, mice without pancreatic cancer or mice with other pathologies. Additionally, as the time needed for the development of a tumour is quite variable across animals (between 3-6 months), the use of imaging allows to start treating mice with similar tumour sizes (6-8 mm in diameter), reducing the variability during studies and therefore, reducing the number of mice needed. Finally, imaging techniques allow to follow tumour growth at different time points, reducing the number of animals required to observe tumour growth at various stages.

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 (with 4-5 animals) will be performed to characterise tumour growth rate and experimental variability to inform the design of subsequent studies and also to potentially reduce the number of animals used. Indeed, if biological and statistical significance is reached in a pilot study, a full study may not be required.

For genetically altered mouse models, whenever possible, we will maintain parents already with the required phenotype to reduce the overall number of mice needed, as all mice born from such breeding strategies will have the required genotype. Whenever this will not be possible, mice carrying inappropriate alleles will be humanely killed.

Although we do not expect surplus animals produced from our breeding strategy, if more mice than what we can use are born, the surplus may be transferred to other project licences to limit wastage. We will also share with other laboratories the tissues and organoid/fibroblast lines we will generate, to limit the use of additional mice elsewhere.

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

For our research, we will use mice. In particular, we will use genetically engineered mouse models (GEMMs), such as KPC mice, and, when scientifically meaningful, mouse models with organoids/cells transplanted in the pancreas. These transplantation mouse models appear healthier than KPC mice (i.e. develop less clinical signs during tumour progression) and likely experience less distress and pain during tumour formation compared to GEMMs, as evident by reduced signs of ill health. Subcutaneous transplantation models of pancreatic cancer, which would further decrease the occurrence of clinical signs in the

animals, and the associated pain, do not faithfully recapitulate the cancer/fibroblast interactions that are at the centre of our research, and we therefore cannot consider them as relevant and scientifically meaningful substitutes.

We will minimise suffering by adhering to best practice guidance, in accordance with the "Guidelines for the welfare and use of animals in cancer research" by Workman et al. 2010, BR J Cancer and the National Centre for the 3Rs. Every procedure proposed has been refined in order to cause the minimum distress, pain and discomfort to the animals.

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

The mouse remains the most relevant species with the least sentience that we can use to predict the biology and therapy response of pancreatic cancer. In particular, KPC mice are the gold-standard for pancreatic cancer research, as they recapitulate many aspects of the human disease. Not only they recapitulate the characteristics of human pancreatic tumours, but they also recapitulate many cancer associated clinical signs typical of pancreatic cancer patients with advanced disease, including weight loss, jaundice (i.e. yellow skin colour), and ascites (i.e. accumulation of fluids in the abdomen).
KPC mice develop pancreatic tumours at 3-6 months of age and their analysis over the following few weeks is pivotal for the understanding of tumour progression and testing of novel therapeutic approaches. Transplantation models of organoids or cancer cells in the mouse pancreas also largely recapitulate the human disease and associated clinical signs.

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

Environmental enrichment (i.e. material that is added in the cage to improve the psychological and physical well-being of animals, such as small tunnels) will be provided to improve animal welfare and promote the expression of species-appropriate behaviour and mental activities. More material that can be used to make a nest will be provided to mice that have been housed alone, for example, during a therapeutic study. Animals will be housed on an appropriate light/dark cycle with control of room temperature and humidity. Animals purchased and transferred to our facility will be allowed at least 7 days to adapt to their new environment and will be handled prior to any procedure or surgery to minimise their distress.

All mice will be monitored at least once a day by qualified, competent and trained staff. Post-operative care will occur following every pancreatic surgery (e.g. transplantation of tumour cells/organoids into the pancreas) and imaging procedure. Mice that show any clinical sign will be more closely monitored and, at all times, we will attempt to reduce any sign of discomfort, distress or pain (e.g. providing gel cups and food at the bottom of the cage, if possible). If long-lasting pain is experienced and no sign of improvement is observed from any measure that we will undertake, mice will be humanely killed.
We will use anaesthetics and analgesics during procedures that require physical restraint (e.g.
ultrasound imaging), and during invasive procedures, such as pancreatic surgeries to minimize the animal distress and/or pain.

When mice are to be given certain agents in the diet, it is well documented that mice refuse to eat the diet for a few days due to its unpleasant taste. This results in a transient weight loss, which is typically recovered after a few days, when the animal starts eating again. However, if possible, to try limiting the weight loss by making the food more appealing, Nesquik or condensed milk may be added to the regular mashed diet. If this

were to be the route of administration, mice would be exposed to Nesquik or condensed milk mashed food prior to treatment to acclimatise them to the diet. During the course of this licence, we will regularly monitor and try to reduce the percentage of mice that experience transient weight losses associated with this type of drug administration.
All protocols will be annually reviewed based upon records of severity kept under the Animals Scientific Procedures Act (ASPA) requirements to ensure that any new advances that can minimize the severity (in terms of pain, distress and discomfort) will be incorporated.

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

We are committed to following the LASA Guiding Principles for Preparing for and Undertaking Aseptic Surgery in our surgical protocols. We will also follow guidelines in accordance with Workman et al 2010, Br J Cancer and the National Centre for the 3Rs, which are considered the best current practice. We will also regularly review and implement any updated guidance from the National Centre for the 3Rs.

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

We currently follow any update provided by the National Centre for the 3Rs and, whenever possible, we will implement any new advance identified in term of replacement, reduction and refinement. We will also attend conferences and keep up-to-date with the current publications on themes related to the animal work carried out in our laboratory (e.g. conferences on animal models). These approaches will allow us to be exposed to the newest advances in the field which, whenever scientifically meaningful, we will start implementing in our research.

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

34. The Development of Animal Health 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
  - Improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes
- Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)
- Protection of the natural environment in the interests of the health or welfare of man or animals

Key words
Animal Health, Veterinary, Feed additives, Farm animals, Companion animals

Animal types	Life stages
Rats	adult
Mice	adult
Beagles	adult,juvenile
Cattle	juvenile, adult, pregnant
Sheep	juvenile, adult, pregnant
Pigs	juvenile, adult, pregnant, neonate
Broiler Chickens, Laying Hens, Turkeys	neonate, juvenile, adult, 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.

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 intention of the work covered by this licence is to determine the safety and efficacy of animal health products. Animal health products generally fall into three categories: veterinary drugs, vaccines or medical feed products, however novel technologies such as monoclonal antibodies may also be developed for the treatment of sick animals. The work carried out under this licence will be designed to meet the requirements of government regulators in Europe and elsewhere, who must agree to the sale and use of these materials in society.

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

The work in this project will be performed to meet the requirements of regulatory authorities responsible for the authorising the marketing of veterinary medicines and medicated feed-stuffs. In doing so, the work enables the development of safe new medicines for use in animals. In addition, the work helps to assure the safety of human consumers who may be exposed to residues of veterinary medicines in the edible tissues and other products of food-producing animals.

As well as assuring the safety of animals and human consumers, the successful conduct of tests will help bring to market materials which improve the health and welfare of animals in which they are used.

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

Data collected will be information on how animals are affected by potential new veterinary medicines; or how much and for how long the animals retain the medicines in their body; or how much of the medicines are in animal tissues, eggs or milk which might contribute to human food.

Outputs will include simple measures like changes in behaviour, food consumption, growth rate and weight retention or loss. Samples will commonly be taken, particularly of blood, but also other excretions such as urine to assess any changes over time.

Post mortem examination can demonstrate change in function or structure of body organs, including examination at a microscopic level.

The data will be collected to the standards required by government regulators in the UK, Europe and elsewhere, for identifying and excluding inappropriate medicines due to safety concerns, and enabling further development of successful veterinary medicines.

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

Our clients, typically commercial animal health companies, will benefit from the provision of high quality data. This will help them in their work to produce safer and more effective medicines which can be safely made and used without increasing health risks for animals or for people who could potentially be exposed to them through the eating of animal-based food.

Enabling development of successful veterinary medicines will have a general benefit for animal welfare in society, through diagnosis, treatment or prevention of disease.

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

By conducting the work to the expected quality standard (Good Laboratory Practice) and by following relevant internationally-agreed guidelines, the outputs of the work should be readily accepted in all markets of the world.

Collaborations and information exchange with others within the organisation and with our clients, helps to identify and spread information on successful and unsuccessful approaches, and on product development.

Species and numbers of animals expected to be used

- Domestic fowl: No answer provided
- Beagles: 250
- Cattle: 800
- Sheep: 100
- Pigs: 500
- Mice: 150
- Rats: 250

Predicted harms

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

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

Most of the animals and life stages used in the project are examples of species which will be given the test items in veterinary practice; the target species. Some laboratory rodents are used to help consider safety of test items which would be given to animals which contribute to human food.

They are also species included in relevant guidance accepted by government regulators in the UK, Europe and elsewhere.

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

Animals will be given a possible new veterinary medicine or other animal health product, by the same way that this would be done in veterinary practice. Some studies will be to check for any effects of the medicine; some studies will be to measure how much of the medicine is absorbed into the blood, or into the milk, eggs or meat of animal species which contribute to human food. Urine and faeces, and the excreta of birds, are commonly collected, which requires housing the animals singly, although next to each other, in a small cage which allows the urine and faeces to fall through a grid, typically for about a week.
Animals are normally humanely killed after the collection period, and tissues may be taken from the animals post mortem, and analysed.

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

The process of dosing animals or taking blood samples may cause minimal discomfort during conduct; no lasting effects are expected.
The medicines are not normally expected to cause any significant harm for the animals.
Confinement for collection of excretions may cause a degree of discomfort and/or reduced activity; a degree of reduced food consumption and/or weight loss may be noted, but this is not expected to be significant based on experience of studies conducted over many years.

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)?

No significant harms are normally identified by examining the animals. This and controlled limits on blood sampling are likely to result in no more than mild severity outcomes in most cases. The single housed confinement of animals for several days, particularly if it includes restricted movement, results in a consideration that this is of moderate severity. This is likely to be required for about half of the animals used in the project, because of the need to collect the excretions of individuals for a sufficient time to complete the scientific aims.

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 19 April 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?

While non-animal methods are used in some aspects of the programme of safety assessment of new materials, they are currently not able to predict effects on whole body systems or to provide information on how much of a material is absorbed, or how animals change and excrete materials. Animal products which contribute to food (meat, milk, eggs) need to be assessed for presence of drug residues which could cause harm to people.

This work must be done using the same animals which will be given the medicines, some of which also contribute to human food.

The protocols described in this project are conducted according to internationally-agreed guidelines, to meet the requirements of UK and European law, and are expected to be performed before government authorities will authorise the marketing of new veterinary medicines for animals.

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

The organisation conducts non-animal tests as part of the many programmes of safety assessment of new materials. However as noted above, it is still considered essential by scientists and government regulators, to also do work using animals, which this project describes.

A potential *in vitro* approach to comparative metabolism is described in the relevant guideline, and will be considered as and when such studies are requested.

Why were they not suitable?

In vitro comparative metabolism will be used where it is deemed to be an appropriate scientific alternative, and acceptable to government regulators.

A retrospective assessment of replacement will be due by 19 April 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 estimates are based on analysis of use of animals in an existing licence authorising work for the same purpose, combined with anticipated upcoming studies.

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

The animal numbers for studies required by government regulators typically follow those identified in internationally-accepted guidelines, as expected to provide sufficiently significant outcomes.

In situations where there is no definitive guidance on the numbers of animals to be used, the applicant and colleagues will use their extensive experience of related programmes,

taking account of statistical significance and scientific advice as necessary, to use sufficient animals for studies to provide robust results.

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

All studies are performed according to the principles of Good Laboratory Practice (GLP), which is an international standard for the quality of experimental study conduct with animals, and is overseen by an independent agency of the government. Following this practice should ensure the quality of studies and acceptance of results by government regulators worldwide, and also reduce the potential for error.

By fully understanding the needs for testing in each case, and by following internationally-agreed guidelines and GLP, the same study should be acceptable to government regulators in all parts of the world where it is needed, removing any need to do the similar work more than once, and use more animals, to meet the needs of all markets where the medicines may be used.

Pilot studies may be used to confirm appropriate elements of study design in advance of full studies, for example, the best timing for taking samples of various body tissues or products to investigate the how much and when a drug is excreted or retained by the animals.

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

Materials are given to the animals by the same way in which they would be given in veterinary practice, most commonly by mouth, in food, or by injection. The dosing methods used are all very well established and common for the animals to be used, and known to cause minimal discomfort based on extensive experience at the site.
Blood sampling is a common need. We follow published guidance on methods and suitable volumes which can be taken while minimising harms to animals.

Restraint or confinement of animals is a common need, to allow collection of samples, generally urine and faeces. Methods used are those with which staff have extensive

experience, and the duration of time is minimised wherever possible while allowing completion of the process.

Milk-producing animals are milked regularly using the same processes as are used on farms.

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

The species used are in most cases the same species, and at a stage of life when they would be given the medicines in veterinary practice; and so are the most appropriate scientific models. Where laboratory animals (rodents) are used, they are to help confirm the suitability of study outcomes for people who could potentially be exposed to the medicines by presence in products of animals which contribute to human food. They are the species whose use is expected by government regulators.

The time required to study the outcome of giving the medicines, and for samples to be taken means that continued anaesthesia is impractical, and could interfere with the scientific outcomes.

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

Refinement of procedures is an on-going consideration at the site, and new opportunities are particularly assessed if any concerns are identified during or after studies; for example new methods or additional assessments may be included in future study designs as a result of a review.

Methods to enrich the housing are routinely considered, and changes are commonly introduced to seek to improve the housing. A standing group regularly considers potential refinements at the site, and reports to the animal welfare and ethics review body routinely on this topic.

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

Where required, blood volume limits are within those proposed in the 2001 publication of Diehl *et al*: A good practice guide to the administration of substances and removal of blood, including routes and volumes.

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

Both our clients and our colleagues conducting the same type of work in other countries, are collaborators who can bring ideas as to how to improve how to conduct our animal studies. Various staff at the establishment have been involved with working groups of the UK National Centre for the 3Rs (NC3Rs), over many years. Staff at the site routinely review published papers in the scientific press, some of which propose refined approaches to conduct of work.

A retrospective assessment of refinement will be due by 19 April 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?

35. The role of adiponectin in equine endocrinopathic laminitis

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

laminitis, adipose-tissue derived hormones, insulin, endocrinopathy

Animal types	Life stages
Ponies	adult
Horses	adult

Retrospective assessment

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

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 further elucidate the role of specific adipose tissue derived hormones in the pathogenesis of equine endocrinopathic laminitis.

Retrospective assessment

Published: 28 November 2023

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

No

Did the project achieve its aims and if not, why not?
The aim of the project was to further elucidate the role of the adipose tissue derived hormone adiponectin in the pathogenesis of equine endocrinopathic laminitis. In order to achieve this aim, firstly the effect of high blood insulin concentrations (induced by insulin infusion) and the induction of tissue insulin resistance (using corticosteroids) on circulating adiponectin concentrations and adiponectin receptor expression were investigated in six

insulin-sensitive, never laminitic ponies. Next, these six ponies plus three additional insulin-sensitive never laminitic ponies (9 in total) underwent pasture-induced weight gain over 22 weeks to elucidate the effects of spring/summer pasture consumption and obesity on insulin sensitivity and adiponectin concentrations. Experimentally induced hyperinsulinemia and tissue insulin resistance did not affect circulating adiponectin concentrations. However, the induction of tissue insulin resistance was associated with upregulation of adiponectin receptor 1 (AdipoR1) and insulin-like growth factor 1 receptor (IGF- 1R), two receptors linked to adiponectin signaling, within the circulation. Additionally, pasture- induced weight gain was associated with a gradual decrease in adiponectin concentrations and increase in circulating AdipoR2 expression in all ponies. All animals developed insulin dysregulation (ID) at one or more time-points during the pasture study, although insulin sensitivity status varied throughout, possibly in response to changing pasture conditions and did not appear to be closely associated, or caused by, changes in insulin sensitivity or the development of insulin resistance. These results therefore support the theory that insulin dysregulation does not directly cause hypoadiponectinemia in insulin-sensitive never-laminitic ponies and instead other factors determine adiponectin concentrations.

Human metabolic syndrome (HMS) is very similar to equine metabolic syndrome (EMS) in terms of the metabolic alterations that occur and it is associated with an increased risk of certain cardiovascular diseases. Dietary manipulation and pharmacologic agents are used to increase circulating adiponectin concentrations in people with HMS and this in turn reduces the associated cardiovascular disease risk. Thus, this project also aimed to determine whether similar approaches can be used in Equine Metabolic Syndrome (EMS) by evaluating the effect of weight loss with or without dietary supplementation and/or pharmacologic agents on circulating adiponectin concentrations. Potential pharmacologic agents were to be screened in vitro through evaluation of their effects on equine fat tissue (adipocyte) adiponectin production after developing a suitable model. In vitro studies were performed to optimise an equine adipocyte model by differentiating equine adipocytes from bone marrow derived stem cells. Promising results were obtained, although further work is required before this can be used to screen potential pharmacologic agents. Additional funding would be required in order to pursue this part of the aim further.

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?

Laminitis is a painful condition of the equine foot that affects approximately 4% of the horse and pony population in the UK and worldwide. In addition, it is frequently recurrent, with up to 70% of animals suffering from repeated episodes. There are three forms of laminitis, namely sepsis-associated, endocrinopathic and supporting limb laminitis. Endocrinopathic laminitis is the commonest form, accounting for up to 90% of cases and encompasses laminitis associated with the endocrine (hormone) diseases equine metabolic syndrome (EMS) and pituitary pars intermedia dysfunction (PPID). The key feature of EMS is insulin dysregulation (ID), which is abnormal insulin metabolism in response to a normal physiologic process, such as eating. In horses, this manifests as high blood insulin concentrations (hyperinsulinaemia) and/or an excessive insulin response to ingested carbohydrate and/or resistance to insulin at the level of the tissues. Additional features include obesity, high blood fat concentrations (hypertriglyceridaemia) and abnormal fat tissue metabolism (adipose dysregulation) manifesting as abnormal

plasma adipokine (hormones produced by fat tissue) concentrations. It is well established that high blood insulin concentrations for a prolonged period (48-72 hours) can induce laminitis, but the underlying mechanism remains unclear. Current research has focused on insulin binding to and inappropriately stimulating the receptors for the hormone insulin-like growth factor-1 (IGF-1) which are found in the equine foot. Some adipokines have anti-inflammatory and insulin-sensitising actions and low circulating concentrations of some adipokines, as well as high blood insulin concentrations, is a risk factor for endocrinopathic laminitis. In other species, specific adipokine and insulin signaling pathways within cells converge at the level of the adaptor protein APPL1 and there is emerging evidence of cross talk between specific adipokines via its receptors and both the insulin and IGF-1 receptors, resulting in increased and decreased signaling respectively. This project will firstly investigate the effect of high blood insulin concentrations, induction of tissue insulin resistance (using corticosteroids) and obesity (via pasture-induced weight gain) on circulating concentrations of specific adipokines *in vivo*. Human metabolic syndrome (HMS) is very similar to equine metabolic syndrome in terms of the metabolic alternations that occur and it is associated with an increase risk of certain cardiovascular diseases. Dietary manipulation and pharmacologic agents are used to increase circulating concentrations of certain adipokines in people with HMS and this in turn reduces the associated cardiovascular disease risk. Thus, this project will also determine whether similar approaches can be used in EMS. The effect of weight loss with or without dietary supplementation and/or pharmacologic agents on circulating concentrations of specific adipokines will be evaluated. Potential pharmacologic agents will first be screened *in vitro* through evaluation of their effects on equine fat tissue (adipocyte) adipokine production.

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

This project seeks to further elucidate the role played by specific adipokines in endocrinopathic laminitis and to identify potential pharmacologic agents and management interventions that will increase circulating concentrations of these adipokines. This in turn may reduce the risk of endocrinopathic laminitis in high risk animals. This new information will be disseminated in the form of presentations at suitable equine veterinary and research conferences and publications in suitable journals.

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

The project has a potential beneficial welfare impact for horses and ponies worldwide and an economic impact for their owners through reduced veterinary expenditure and athletic performance loss. This impact will not be fully realised until the project is completed.

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

New knowledge gained will be disseminated through presentation at suitable conferences to researchers working in this field, veterinarians and horse caregivers and publication in suitable journals and lay articles. This will include publication of unsuccessful approaches.

Species and numbers of animals expected to be used

- Ponies: 25
- Horses: 25

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 not possible to achieve the objectives of this project without using animals, as we are studying complex metabolic pathways and physiological responses, that cannot with our current state of knowledge, be modeled using isolated tissues, cells or computer simulations.

Laminitis is a disease which affects adult horses and ponies and it is therefore most appropriate to undertake these studies in these animals. Whilst we can model some aspects of digital vascular physiology and fat, muscle and caecal function *in vitro, t*he unique metabolism of the horse is central to the pathophysiology of the endocrinopathic laminitis. Thus, our studies to further elucidate the role of specific adipokines in the pathogenesis of the disease require *in vivo* experiments.

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

Typically, animals will undergo each of the procedures a maximum of twice in this project.
Protocol 1 involves placement of intravenous catheters in each jugular vein under local anaesthesia and infusion of glucose and infusion via one catheter and collection of blood samples via the second catheter. It also involves a single intramuscular injection of corticosteroid followed by blood sample collection via jugular venepuncture.
Protocol 2 involves consuming sufficient pasture to promote natural weight gain. Blood samples will be obtained weekly by jugular venepuncture until each animal becomes overweight. This will be followed by weight loss achieved through consuming a hay-based diet with or without supplementation using nutritional supplements or pharmacologic agents administered orally. Blood samples will be obtained weekly by jugular venepuncture until each animal reaches ideal weight.

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

Studies conducted under this licence should not induce long term adverse effects in the animals.

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

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

All protocols will be mild for all animals.

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

- Kept alive
- Rehomed
- Used in other projects

Retrospective assessment

Published: 28 November 2023

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

Six adult ponies underwent protocol 1 which involved animals experiencing mild discomfort associated with intravenous catheter placement under local anaesthesia into the jugular veins on two occasions a minimum of 14 days apart. Blood samples were collected from one catheter and an infusion of glucose and insulin administered via the second catheter over a 12-hour period on one occasion. Dexamethasone was was administered and blood samples collected over a 3-hour period via a single catheter on one occasion. All animals were allowed free access to hay and water throughout and none of the animals experienced any adverse effects associated with the procedure.

Five out of these six ponies plus three additional ponies underwent protocol 2 which involved animals consuming sufficient pasture to gain weight over a 9-12 week period to reach obesity (body condition score 7/9) and then maintain their weight for a further 12 weeks. Laminitis, a known potential adverse effect associated with the pasture-induced weight gain, was experienced by two animals (one after 3 weeks and one after 20 weeks). Both animals were immediately withdrawn from the study and received appropriate treatment under the guidance of the Named Veterinary Surgeon. Once every 2 weeks, animals experienced mild discomfort associated with two single blood samples 30 minutes apart being obtained by jugular venipuncture and insulin being administered intravenously via the same needle insertion (insulin tolerance test [ITT]). All animals experienced only mild short-term discomfort on all occasions apart from one pony on one occasion. On this occasion, the animal experienced an adverse effect (hypoglycemia; moderate severity) associated with the insulin administration and this was reported to the Home Office. The animal recovered uneventfully and did not experience any long term adverse effects. In addition, an oral sugar test was performed once every 2 weeks on a different day to the ITT in all animals. These involved animals experiencing mild discomfort associated with two single blood samples being obtained by jugular venipuncture 60 minutes apart and oral administration of a commercial corn syrup. None of the animals experienced any adverse effects associated with this.

The pony that developed laminitis after 3 weeks recovered completely with appropriate veterinary management. However, the animal also suffered from insect bite hypersensitivity and severe equine asthma which are both recurrent conditions that can be managed but not cured. The management of laminitis and these two addition chronic conditions are frequently opposing and so the animal was euthanased at the request of her owner.

The second pony recovered from the laminitis and was returned to her owner.

The remaining seven ponies were all kept alive at the end of the procedures and rehomed.

Replacement

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

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

It is not possible to achieve the objectives of this project without using animals as we are studying complex metabolic pathways and physiological responses, including some that are influenced by season, that cannot with our current state of knowledge, be modelled using isolated tissues, cells or computer simulations.
#
Which non-animal alternatives did you consider for use in this project?

Isolated equine tissues or cells and computer simulations were considered for use in this project. *In vitro* studies will inform which drugs to use in protocol 3 and help understanding of the mechanisms involved in adipokine release from cultured adipocytes. The combination of *in vitro* and *in vivo* approaches is more powerful scientifically than either on its own.

Why were they not suitable?

Whilst *in vitro* studies will be used to inform the choice of pharmacologic agents used in protocol 3, they are not suitable as they do not take into account the complex physiological responses and complex metabolic pathways that all interact in the pathogenesis of endocrinopathic laminitis.

Retrospective assessment

Published: 28 November 2023

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

No non-animal alternatives became available or viable after the project licence was granted and so it was not possible to replace any of planned animal use.

Reduction

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

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

The number of animals to be included in this project has been based on sample size calculations and on the assumption that animals will be re-used between protocols in order to reduce the overall number of animals used. Data from our previous studies involving measurement of circulating adipokine concentrations in healthy ponies as well as weight gain and weight loss studies have been used to inform these studies. In each case, group sizes of six animals are sufficient. In addition, in previous *in vivo* equine studies, we have found that group sizes of six animals have been sufficient to produce robust results. Protocol 3 requires three groups of animals equating to a total of 18 animals. Thus, it is estimated that 25 animals will be used in total to allow for illness, accidental injury and the need to repeat a study in an individual animal for experimental reasons.

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 to be included in this project has been based on sample size calculations and on the assumption that animals will be re-used between protocols in order to reduce the overall 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?

The numbers to be used in this project are based on sample size calculations and previous experience.

Retrospective assessment

Published: 28 November 2023

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

The licence allowed for the use of 25 horses and 25 ponies; however only 9 ponies were used in total.

The number of animals to be included in this project was based on sample size calculations and on the assumption that animals would be re-used between protocols to reduce the overall number of animals used. Data from our previous studies involving measurement of circulating adiponectin concentrations in healthy ponies as well as weight gain and weight loss studies was used to inform these numbers. In each case, group sizes of six animals were sufficient. In addition, in previous in vivo equine studies, we have found that group sizes of six animals have been sufficient to produce robust results. Thus, six ponies underwent Protocol 1. The study plan was then altered (and Protocol 2 in the project licence amended) to allow for the chronic effects of obesity to be investigated. In total, 9 animals (6 animals reused from Protocol 1 and 3 additional animals) were used in protocol 2 total to allow for illness, accidental injury and adverse effects over the 6-month study period to ensure that final results were available from 6 animals.

The effects of weight loss with and without dietary manipulation and/or pharmacologic intervention on insulin sensitivity and circulating adiponectin concentrations (protocol 2; step 2) were not studied and the studies were not repeated using horses rather than ponies. Thus, reducing the overall number of animals used from 50 to 9.

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.

Protocol 1

The methods used to induce hyperinsulinaemia (high blood insulin concentrations) and tissue insulin resistance have been previously validated for ponies. The duration of the hyperinsulinaemia chosen is much shorter than that which has been previously reported to induce laminitis in healthy ponies. In addition, there is no scientific evidence to link corticosteroid administration with the development of laminitis in healthy animals, only in those with other laminitis risk factors. thus, only healthy, ideal weight animals with no history of previous laminitis will be used. Jugular catheters will be placed using local anaesthetic in order to minimise any pain and distress potentially associated with repeated blood sampling.

Protocol 2 and 3
The pasture-induced weight gain will be undertaken in consultation with an equine nutrition specialist to ensure that there is a gradual gain in weight over 9-12 weeks. Previous studies using this approach have not resulted in any adverse effects. The weight loss will be induced by feeding a diet low in non structural carbohydrate (<10% dry matter) at 1.25% (dry matter intake; DMI) of body weight. This is standard dietary change that is recommended by veterinary surgeons for weight loss in clinical cases of equine obesity when owners have allowed their animals to become obese. Thus, this will mimic somethings that happens in the real world. In those animals with weight loss resistance (animals that fail to lose weight despite dietary restriction), the diet may need to be reduced to 1% (DMI) of body weight, but no lower as lower percentages are associated with gastrointestinal disturbances such as gastric ulcers and the development of stereotypies (repetitive abnormal behaviours). The methods that will be used to assess the effect of the weight gain and subsequent weight loss on insulin metabolism (i.e. oral sugar test and insulin tolerance test) are methods that are commonly used in equine clinical practice.

Thus, all of these methods proposed have been previously validated for use in ponies in either the clinical or the research setting. Animals will be returned to their normal management regime between protocols which will involved continuous access to pasture. All of the protocols are mild in severity and none of the methods will cause lasting harm to the animals.

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

As previously explained, all of the experiments proposed need to be performed in horses and ponies. As equine metabolism differs significantly between the foetus, neonate and adult, and laminitis is a disease that only affects adult horses and ponies, we are unable to use a less sentient stage of life.

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

All animals will be habituated over several days to the room and the stocks in which the insulin infusion will be undertaken prior to commencement of the protocol.
All animals will be constantly monitored for the duration of all protocols and for the next 3 days to ensure that no adverse effects develop. If any adverse effects are noted, appropriate treatment will be provided immediately in consultation with the named veterinary surgeon.

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

We will follow the NC3Rs advice on the 3Rs for project licence holders.
The NC3Rs General Principles for blood sampling applicable to horses will be followed as well as the recommendations relating to use of vascular catheters.

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

We will stay informed of advances in 3Rs via the 3Rs Liaison, as well as via the NC3Rs website and attending relevant seminars/talks. Wherever we find an opportunity to improve our technique/experimental design to minimise animal numbers and/or suffering, we will rapidly incorporate it into our protocols. We will closely work to ensure that our animal care is always optimal, which ultimately ensure high quality results.

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

36. Toxicology of Pharmaceuticals

Project duration

5 years 0 months

Project purpose

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

Toxicology, Safety, Regulatory

Animal types	Life stages
Mice	juvenile, adult, aged, embryo, neonate, pregnant
Rats	juvenile, adult, aged, embryo, neonate, pregnant
Rabbits	adult, pregnant, neonate, embryo, juvenile
Pigs	juvenile
Beagles	juvenile, adult
Cynomolgus macaques	juvenile, adult
Minipigs	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.

Objectives and benefits

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

What's the aim of this project?

The aim of the project is to evaluate the safety of various types of potential new human drugs when given to test animals. The work is required for new drugs, for the safety of the human volunteers and patients who will take the drugs, and it is designed to meet the requirements of regulatory bodies in Europe and elsewhere, who must agree to the sale and use of drugs.

A retrospective assessment of these aims will be due by 09 April 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?

As well as assuring the safety of human volunteers and patients, the successful conduct of tests will help bring to market those materials which are safe and are subsequently shown to be effective in the treatment or prevention of human diseases. Without these studies, progression of new medicines to early human studies and to patients could not occur in the current regulatory framework.

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

Data collected will be information on how animals are affected by potential new medicines. This will include efforts to identify systems within the body or particular organs of the body that may be affected by short term or accumulated exposure to the new medicines. Outputs will include simple measures like changes in behaviour, food consumption, growth rate and weight retention or loss. Samples will commonly be taken, particularly of blood, but also other excretions such as urine to assess any changes over time, as well as to assess how much of a medicine has been absorbed or excreted. Post mortem examination can demonstrate change in function or structure of body organs, including examination at a microscopic level. Some studies will be to check if there is any effect on ability of animals to breed, or any effect on the development of the young in the uterus. The data will be collected to the standards required by government regulators in the UK, Europe and elsewhere, for identifying and excluding inappropriate medicines due to safety concerns, and enabling further development of successful medicines.
Improved methods of conduct of specific data collection processes may be developed during the course of the project.

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

Our clients, typically commercial drug companies, will benefit from the provision of high quality data. This will help them in their work to develop new and better medicines, to discontinue development of inappropriate medicines or to understand and manage the risks of new medicines given to people. Work on this project may also provide data to inform ongoing human clinical trials.
Enabling development of successful medicines will benefit society through diagnosis, treatment or prevention of disease.
Identification of adverse effects can prevent future harms to human volunteers or patients by resulting changes to medicine development programmes.
The wider scientific community may benefit from publication of refined approaches to animal use.

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

Our organisation has colleagues with extensive experience of such work in different parts of the world. Collaborations and information exchange with others within the organisation helps to identify and spread information on successful and unsuccessful approaches. Collaboration with clients (knowledge gained on products).

On-going collaborations with NC3Rs on various aspects of regulatory safety studies, over many years.

Presenting outputs at scientific conferences and contributing to publications in the scientific literature where relevant.

Species and numbers of animals expected to be used

- Mice: 20000
- Rats: 45000
- Rabbits: 4000
- Beagles: 4000
- Cynomolgus macaques: 3500
- Minipigs: 900
- 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.

Many scientific studies have been conducted to demonstrate that the types of animals to be used in the project will provide results which reflect the likely effects in humans. The way in which each new medicine works in the body will be known, and the animal type(s) to be used will be chosen based on an understanding that the medicine will work in a similar way.

The stage of life of the animals to be tested reflects the age/stage of life of people who would receive the medicines.

Another big advantage of using the listed animal types is that these animal types may be recommended by specific guidelines on how to do this work, and the results of tests are known to be acceptable to the government agencies responsible for authorising use of the medicines in human volunteers and patients. Development of new medicines cannot currently be achieved without this approval by government agencies in the UK, elsewhere in Europe and in other parts of the world.

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

Animals will be given a potential new human medicine by the same method that people would be exposed to them - most commonly by mouth, but may be by injection, application to the skin, or by inhalation. Inhalation of materials generally requires that animals are accustomed to close restraint in a purpose-made device while breathing the medicine, and/or wear a mask while breathing the medicine. If applying medicines to the skin, some form of covering or temporary restraint is required, to stop the animal or cage-mates

interfering with it. Dosing of the medicines is for at least as long as people would be asked to take a medicine. A small number of studies involve giving the medicine to rodents for an estimate of their lifetime, to check if it might cause cancer.
A small number of studies involve surgery, to allow dosing of medicines intravenously for an extended period daily, or continuously. Surgery may also be conducted to take tissue samples, to assess a change in effect over time or to allow collection of information such as ECGs without restraint of animals. All surgery is conducted under anaesthesia and with use of post-surgical pain relief, under veterinary guidance.
Other samples such as blood samples are commonly taken to assess any effect and/or to assess how much of the medicine is absorbed. Behavioural tests may be conducted to check for effects. Other specific examinations may be undertaken, including examination of the eyes. Some studies are to assess if the material has an effect on the unborn, or on the development of young animals.
Animals will usually be used once only, and then will be humanely killed to check for effects in the body, including by examining the tissues microscopically.

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

The process of dosing animals or taking samples can cause a degree of discomfort during conduct, particularly if animals have to be restrained to enable the work. Behaviour and health may be affected by the materials being given, and reduced health can be measured, e.g. by reduction in food consumption, weight loss, changes in blood results. Some studies may have effects on the ability to breed or on development of the young. In lifetime studies, adverse effects are usually those seen in ageing animals, such as reducing function of the body's organs, resulting in reducing quality of life over time. Surgery can cause some discomfort in the immediate post-surgical period, but this is prevented or minimised by use of appropriate anaesthetics drugs and pain relief.

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

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

The vast majority of animals which may experience harms as described above are expected to be considered as mild severity. This includes 85% or more rodents and 70% or more non-rodents expected to be used in the project. It is expected that about 80% of animals to be used in the project will be rodents.

Most other animals may experience harms categorised as moderate. Severe outcomes are not anticipated in most studies; about 1% to 1.5% of rodents may be used in a severe protocol for testing of anti-cancer medicines intended for use in patients with late-stage cancer. Some of these animals may experience harms which are categorised as severe, including lack of appetite, significant weight loss, diarrhoea; such animals would be humanely killed. Some animals may experience no apparent harms.

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 09 April 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?

Non-animal methods are routinely used in some aspects of the development programme for new human drugs, but they are currently not able to sufficiently predict effects on whole body systems or to provide information on how much of a medicine is absorbed. It is not currently possible to acquire all of the information on how the body systems such as the heart, brain, liver and kidneys may be affected by new medicines, without using animals. This information is essential, to protect human volunteers and patients. The protocols described in this project are conducted according to internationally-agreed guidelines, and are expected to be performed before government authorities will authorise giving new experimental medicines to people.

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

The organisation does conduct various non-animal tests as part of the development programme for new medicines, but as noted above, it is still considered essential by scientists and government regulators, to also do work using animals, which this project describes.

Why were they not suitable?

There currently remains general scientific agreement, and agreement of government regulators, that to protect human volunteers and patients, non-animal alternatives do not, as yet, provide enough information to replace all animal studies.

A retrospective assessment of replacement will be due by 09 April 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 estimates are based on analysis of use of animals in an existing licence authorising work for the same purpose, combined with anticipated need for use to a similar extent.

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

There are various guidelines issued by government regulators on how to conduct the various study types described in the licence. While giving general information on study design, most of these do not give specific information on the numbers of animals to be used in a study. Sufficient, but minimum numbers are expected to be selected. In the case of reproduction toxicity studies, specific information on animal numbers is provided. Where there is no definitive regulatory guidance on numbers of animals, the applicant and colleagues will use their extensive experience of related programmes, taking account of statistical significance and scientific advice to use sufficient animals for studies to provide robust results, known to be acceptable to government regulators. In general, longer-term studies will use larger group sizes of animals.

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

Pilot studies will be used to investigate the potential of new designs or processes to improve outcomes, before being used in larger numbers of animals. Initial screening studies, using small numbers of animals, are designed to identify and eliminate materials with undesirable results, and so reduce the numbers of animal which are then used in the studies required by government regulators. Short-term studies are conducted, and results assessed, before starting longer-terms studies in the same programme, to ensure the need for the follow-on study, and to help maximise the study design opportunities.

A retrospective assessment of reduction will be due by 09 April 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.

Dosing of potential new medicines is by the same way as they would be given to people; most commonly by mouth, but including various injection methods, dosing by inhalation and by application to the skin. The methods used are generally very well established and

commonly used by experienced staff at the establishment. Volumes of drugs to be given are in line with published guidance on minimising discomfort, and/or are known to cause minimal discomfort based on extensive experience at the site. Any novel volumes would be tested to confirm lack of discomfort before further use; he Animal Welfare and Ethical Review Body must approve any such requests. The amount of a drug, and the amount of time dosing is continued, depend on how much and how long people might be expected to take a drug.

Blood sampling is a common need. We follow published guidance on methods and suitable volumes which can be taken while minimising harms to animals.

Restraint or confinement of animals is occasionally needed to allow conduct of dosing or sampling processes. Methods used are those with which staff have extensive experience, and the duration of time is minimised wherever possible while allowing completion of the process so that tasks do not generally have to be repeated.

Surgery is conducted with expert veterinary involvement in the creation of suitable regimes for anaesthesia and post-surgical pain relief.

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

The species used are selected based on known standards of outcome which will answer the scientific questions. They are also known industry and regulatory standards which will meet expectations of government regulators internationally. The particular species chosen for a programme may also allow comparison with other data which as been generated using the same species with the same medicine, or similar medicines, to assess which might be the best for people to take.

Response to tests is assessed over a time period which would make continued anaesthesia impractical in almost all cases, and would interfere with the outcome in some circumstances.

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

Refinement of on-going procedures is commonly discussed and explored within the animal technical, veterinary and scientific groups, and also as and when any concerns are identified; for example additional assessments may be included based on initial outcomes. The surgery and anaesthesia/pain relief protocols used in the programme undergo regular and routine assessment and refinement to improve outcomes. Habituation of animals to restraint is a routine process, and the schedule can be amended in response to outcomes for individual animals.

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

Dose volume and blood volume limits agreed with the animal welfare and ethical review body are based on the 2001 publication of Diehl *et al*: A good practice guide to the administration of substances and removal of blood, including routes and volumes. Welfare end-points are developed in general line with publications on the topic, including the NC3Rs document from 2010 on dose level selection for regulatory toxicology studies. Non-human primate housing is in compliance with the NC3Rs document on this topic from 2017.

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

Both our clients and our colleagues working in the same type of work in other countries, are collaborators who can bring ideas as to how to improve how to conduct our animal studies. Various staff at the establishment have been involved with working groups of the UK National Centre for the 3Rs (NC3Rs), over many years. Staff at the site routinely review published papers in the scientific press, some of which propose refined approaches to conduct of work.

A retrospective assessment of refinement will be due by 09 April 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?

37. Training in complex surgical procedures

Project duration

1 years 0 months

Project purpose

- Higher education or training for the acquisition, maintenance or improvement of vocational skills.

Key words

No answer provided

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.

Objectives and benefits

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

What's the aim of this project?

This project will train surgeons in advanced, therapeutic, minimally invasive, surgical procedures.

Retrospective assessment

Published: 07 April 2022

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

Yes

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

We did manage to carry out some education and training for practising surgeons. However due to the pandemic, and the associated movement restrictions and increased demand upon clinician's time, the numbers were considerably below what we originally anticipated.

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.

What are the potential benefits that will derive from this project?

In many cases minimally invasive (keyhole) surgical procedures are significantly better for patients than open procedures as they are associated with less post-operative adhesions, less time in hospital, faster recovery, less pain, easier post-operative care and much faster return to active life. Consequently, many new minimally invasive procedures are being developed to replace larger, open procedures - particularly in response to the Governments new screening programmes for bowel cancer and aortic aneurysm among others. These screening programmes are identifying 30-40% more patients requiring surgical intervention for their conditions and the number of surgeons qualified in the new procedures is very limited. Un- or insufficiently trained use of these new procedures results in unacceptable death rates and long term side effects. We aim to teach surgeons these new, complex procedures, in terminally anaesthetised animals, to ensure rapid competency and safety. These courses will ensure an adequate supply of appropriately trained surgeons who will be able to fulfil the needs of our increasing numbers of patients using new minimally invasive procedures safely and effectively.

Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

- 51 pigs and 8 sheep over the course of the licence

Predicted harms

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

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?

As all protocols are non-recovery, and animals are given an anaesthetic overdose whilst under anaesthesia, no adverse effects are envisaged. Also, at the end of the procedures, all possible tissue and organs are harvested for use in other studies as well as for use in other training courses.

Retrospective assessment

Published: 07 April 2022

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

All five animals used under this licence were used under deep, general anaesthesia in non-recovery procedures. Therefore, with the exception of the initial injection and the subsequent anaesthesia, they should not have experienced any pain, suffering or discomfort.

Replacement

State why you need to use animals and why you cannot use non-animal alternatives.

As yet, there are no simulators that truly represent the full physiological state necessary to teach these procedures. Current simulators are unable to replicate the blood and lymph flow of tissues and are also not able to replicate tissue responses to stimuli, muscular activity in bowel, effects of surgery affected by temperature, or tissue changes relative to procedures. We will endeavour to develop better simulators as these courses progress.

Retrospective assessment

Published: 07 April 2022

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

Whilst it is possible to do some basic training in dead tissue and using simulators, it is not yet possible for complex/advanced training of this nature. The reason for this is that, for advanced training, the model needs to be as close as possible to the whole, human patient conditions e.g. bleeding, breathing movement, muscle/tissue tension, bowel movement, etc.

We will continue to look for non-animal alternatives but it is not currently possible.

Reduction

Explain how you will assure the use of minimum numbers of animals.

By carrying out a number of procedures in one animal we can reduce the number needed and, as all animals will be deeply and terminally anaesthetised, there will be no suffering or adverse effects. Using 2 animals per 3 or 6 surgeons depending on the course also reduces the number of animals needed.

Retrospective assessment

Published: 07 April 2022

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

The use of large animals (60-80kg) makes them more comparable to adult humans but also allows for more sites within each animal to be used for training. this leads to the need for less animals to be used overall.

Also, by working on both the lower bowel (e.g. colon and rectum) and the upper part of the digestive tract (i.e. the stomach and oesophagus) in some animals, it is possible to reduce overall animal usage.

Refinement

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

The pig and sheep have been chosen for these courses as we need to represent the same size and physiology as humans, in particular with regard to blood system, lymph system, tissue response and general anatomy. Principally, animals are terminally anaesthetised and therefore insentient throughout.

They are carefully monitored using staff trained, skilled and experienced in ensuring effective prolonged anaesthesia in these species.

Retrospective assessment

Published: 07 April 2022

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

We have been using this model for a number of year's and whilst we continue look for ways to improve the model, we did not make any modifications this time.

The only animal welfare modifications we did make was in the way in which we gave the initial anaesthetic to the animal to try and further reduce any stress at the start of the procedure - giving anaesthetic gas before placing in a transfer crate.

38. Training in Murine Polio Intraspinal Inoculations

Project duration

5 years 0 months

Project purpose

- Higher education and training

Key words

Training, Polio, Vaccine, Batch release, Research and development

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.

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 plan of work is to enable a team of prospective and existing practitioners to develop and maintain manual skill in the inoculation of substances into discrete areas of the mouse spinal cord with sufficient accuracy and reliability to satisfy established World Health Organization (WHO) criteria for the polio vaccine safety test using transgenic mice expressing the human poliovirus receptor.

A retrospective assessment of these aims will be due by 27 April 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?

Skills gained and maintained as a direct result of the procedures applied in this project will be utilised in the successful conduct of definitive the transgenic mouse polio vaccine safety test (Tgm test). The most immediate benefit of the proposed training programme will be that it will be possible to retain a pool of appropriately qualified and competent practitioners able to perform the Tgm test in-house.

The benefits arising are:

Implementation and use of the Tgm test will continue to significantly reduce the use of nonhuman primates for batch-release testing of monovalent poliovirus vaccine bulks both in UK (if still required) and in Europe.

The Tgm test is now included in the European Pharmacopoeia (EP) and there have been strong ethical, practical and financial imperatives upon the vaccine manufacturers to take up the test as they are increasingly doing. The lack of an Official Medicines Control Laboratories (OMCL) in Europe adequately qualified and competent to complete the test or comment on its execution would severely compromise the public acceptability of the test within Europe.

The WHO will continue to have access to a suitably qualified reference laboratory to which matters of global health significance relating to neurovirulence of poliomyelitis vaccines can be referred.

The parameters detected by the Tgm test compared to other possible assays will be better established giving greater confidence in its reliability and meaning for product safety. New strains to be used for live-attenuated oral polio vaccine (OPV) and inactivated polio vaccine (IPV) production and antivirals will be evaluated early in their development giving some confidence in their safety and likely protective efficacy before their use in human subjects.

Ability to oversee training programs and advise new manufacturers, national control laboratories, and contract research organizations (CROs) globally in the testing procedures which is imperative to the quality control of new vaccine production and testing of clinical trial materials from new vaccine trials.

Failure to obtain this licence application would compromise the ability to train new staff or retrain existing staff if there is a break in their execution of the test of sufficient duration, leading too loss of skill. With inability to perform the test, in the medium to long term (3-10 years), this would significantly affect the UK's, Europe's and the World's ability to effectively control the release of OPVs. It will also halt the development of new improved vaccine seeds for OPV/IPV production.

How will course attendees use their knowledge or skills in their future careers?

Staff completing this training will then move on to performing work under the main testing licence that covers the intraspinal inoculations required for the WHO Tgm test. This is a very unique skill and requires a high level of dexterity. Being able to perform these procedures means that they can perform quality control testing for polio vaccines and also be involved in testing new vaccine candidates for the polio eradication initiative.

What are the principal learning outcomes from the course?

The objective is to inoculate the dye into the grey matter of the spinal cord. This training stage is considered complete when the inoculator reaches >90% success rate in three consecutive tests of at least 30 animals each.

How are these learning outcomes important to the people on the course?

Progression to the next stage cannot occur until this step is completed.

Who or what will benefit from the transfer of knowledge, or acquisition of skills that this course will deliver?

The World Health Organization uses designated laboratories to provide training in this test globally.

European national authorities and medicine regulators rely on this animal model for the OPV safety testing for quality control of vaccines manufactured in Europe.

New manufacturers requiring safety testing for new vaccines in development.

Clinical trial programmes using this test to test material from the trials. These tests provide data on how successful the trials are and can inform new vaccine development. New novel vaccine strains are being developed for all three polio serotypes, clinical trials for these are currently on-going and they are set to run for a least the next 5 years.

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

An annual workshop with all laboratories performing the test globally is held annually. This is an open forum for all laboratories to discuss issues and achievements in the testing and encourages consistency in the test methodology. This opportunity gives the opportunity to discuss refinements in the test methodology and animal welfare.

Species and numbers of animals expected to be used

- Mice: 4000

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 including genetically altered are used. Transgenic mice with the human polio virus receptor are used in the main testing licence. Genetically altered mice surplus from this main testing licence can be used for this project. For training with the ink inoculations mice are not required to be used within a certain age range although only adults are used, both males and females can be utilised depending on availability.

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

Surgical exposure of the injection site. (AC).
Intraspinal injection of substances (inert pigment). (AC).
Animals will be killed by a Schedule 1 method.

Experience with previously trained inoculators indicates that training speed will vary. Therefore, inoculators will be monitored throughout this important phase of the training, with particular attention in the initial sessions in order to quickly identify trainees who will have difficulty developing the necessary skill: inoculation results will be assessed after each training session. As long as an inoculator is making steady progress towards the target success rate of 90%, training will continue. If progress is poor a full review of the training schedule and results will be initiated, and the trainee may be withdrawn from the programme. Any such review of poor progress will ideally be done early in the programme (typically after about 6 sessions).

As the role of the co-inoculator is simpler, progress is expected to be more rapid though would still normally require on average 3 sessions over 2-3 months, and “loss” of skill less likely. However, progress will still be monitored in the same way as for the inoculator.

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

The procedure is non-recovery so any adverse effects would be related to anaesthesia only and minimised by following best practice and veterinary advice, any animals showing any deviation from the norm that is not immediately rectified by adjustment of anaesthesia level 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)?

Expected severity is non-recovery. Only mice are used is this procedure.

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

- Killed

A retrospective assessment of these predicted harms will be due by 27 April 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?

The initial training in identifying the anatomical landmarks can be carried out by looking at pictures and diagrams and watching experienced operators and videos. The initial training in making the skin incision can be obtained on cadavers
The aim is to train the prospective operator(s) to develop the required manual skills to make the inoculation into the correct region of the spinal cord, i.e. into the grey matter.

Correct inoculation has been shown to correlate well with two reactions in the test animals: First, a twitch in one or both of the hind limbs as the needle reaches the grey matter of the spinal cord. Second, as the inoculation is made, a distinct tremor/spasm is produced in one or both of the hind-limbs.

Why can't your aim be met by observing or by participating in ongoing research or clinical procedures?

The purpose of this of this project is to train new staff in a procedure in a manual skill which cannot be learnt from just observing. Participation in ongoing procedures is not an option as the operator needs to reach a certain level of skill prior to participation in such procedures.

In a wider context, there are current research and development efforts to replace the WHO Tgm test with molecular assays using next generation sequencing analysis of polio vaccine products. While these methods might take some time to be fully established and validated, replacement of the WHO Tgm test with molecular methods will help reduce the need for this licence in the long-term.

A retrospective assessment of replacement will be due by 27 April 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?

There are a number of assumptions that can be made which aid in setting totals for the 5 year life of the licence:

- Given the nature of changes to currently trained operators, it is reasonable to assume the need to train up to 2 new operators in 5 years (to maintain the current figure of 4 fully trained operators). These will train in both roles. Up to 100 mice are likely to be needed to train for the co-inoculator role. From previous experience, up to 1000 mice will be needed to train for the inoculator role. (2200 mice for 5 years).
- Required annual proficiency testing will require a minimum of 40 mice per trained operator per year. (800 mice for 5 years).
- Each of the 4 trained operators may require up to 2 additional ink sessions annually to retain/regain proficiency. Each session would require a minimum of 40 mice each. (1600 mice for 5 years). With current testing levels this option would probably not be required. However, testing frequencies may change during the life of the licence, making this contingency option necessary
- A conservative estimate for 5 years based on these assumptions suggests 4000 mice will be required (with a reasonable margin of error factored in).

What in silico or ex vivo techniques will you use during training?

Some aspects of initial training such as identifying the anatomical landmarks and making the skin incision can be carried out by looking at pictures and videos or using cadavers. New methods such as ultrasound guidance have been considered to augment the training process.

Will these techniques reduce animal numbers? If so, how?

The trainers will make sure the trainee is aware of the requirements of the procedures and is as knowledgeable as possible about the requirements prior to performing the procedure. This awareness should reduce the numbers required to be used once performing the manual training themselves.

What other measures will you use to minimise the number of animals you plan to use in your project?

The process is repeated until a satisfactory percentage positive score is reached. This has taken 750 to 1000 mice under the previous training licence. In contrast the training previously carried out required over 1500 mice for each trainee because of irregular intervals in training associated with the need to liaise with another site. Training therefore reduces the total number of animals used.

Having full control of the training process means that potential trainees can be selected based on genuine experience of proficiency in Regulated Procedures and can be monitored closely and constantly by experienced operators to determine genuine rate of progress. Training can be planned carefully to maximise success in a reasonable time frame. Unnecessary repetition of aspects of training can be avoided.
We also use animals surplus from other protocols.

A retrospective assessment of reduction will be due by 27 April 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.

Mouse model is used including genetically altered, these are the methods used:
Surgical exposure of the injection site. (AC).
Intraspinal injection of substances (inert pigment). (AC).

Animals will be killed by a Schedule 1 method.
This is a non-recovery severity procedure so that opportunities for further refinement are limited to good induction and maintenance of general anaesthesia. It is not necessary for animals to recover from the anaesthesia and they are killed immediately following the inoculation. The vertebral column and spinal cord are then dissected out to allow for precise assessment of the accuracy of inoculation by viewing the distribution pattern of the dye.

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

The main reason for using mice in this training PPL is to prepare for the use of genetically altered mice in the polio studies. Mice are necessary because the main animal model, makes use of genetically modified mice that are susceptible to poliovirus. Our experience, supported through discussion with other organisations performing this test, strongly indicates that it is not possible to observe these required reactions on non-sentient (freshly-killed) animals. In order to elicit the reactions, it is essential that the animals are alive and correctly anaesthetised, but it is not necessary for animals to recover from the anaesthesia - they are killed immediately following the inoculation. The vertebral column and spinal cord are then dissected out to allow assessment of the accuracy of inoculation.

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

This is a non-recovery severity procedure so that opportunities for further refinement are limited to good induction and maintenance of general anaesthesia. However, the programme of training and competency is maintained to ensure that the full number of animals under the full protocol is kept to a minimum. In the absence of this training, more animals would be used as tests might fail and need repeating due to suboptimal inoculation. Suffering could also escalate for a test because staff competency would not be as high.

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

As this training licence is aimed at preparing for the main licence, we carry out the surgical procedures aseptically and to the same high standards. Therefore, and although there is no post-op care, we follow the general LASA guidance: Guiding Principles for Preparing for and Undertaking Aseptic Surgery. Training protocols were optimised under WHO supervision through experience in different laboratories and discussions in training workshops.

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

Staff attend conferences on these subjects and are continually researching new methods. The applicant's expert knowledge in this area and he himself keeping abreast of any new developments. Annual meetings are organised for this forum where presentations are given on the 3Rs and how they have been implemented.

A retrospective assessment of refinement will be due by 27 April 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?

39. Understanding the interplay between different B cell subsets and the gut in immunodependent 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

Key words

Arthritis, Autoimmunity, B cells, The gut, Bacteria

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

Retrospective assessment

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

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 the gut-microbiota and gut "microenvironment" influence the differentiation of B cells, in particular of a subset of B cells called regulatory B cells that can control the development of autoimmunity, as well as to understand more widely how the gut and bacteria contribute to the causes and severity of autoimmunity.

A retrospective assessment of these aims will be due by 12 April 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?

This work is important because it has the potential to improve the management of disease in patients with rheumatoid arthritis and systemic lupus erythematosus, firstly by obtaining a greater understanding of the causes and development of these complex diseases, and secondly by providing new microbiota or gut-targeted therapeutic strategies for the treatment of these diseases.

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

The primary outputs of this project will be publications relating novel basic findings about the outcome of the interaction between gut-bacteria, B cells activation and arthritis. We expect to provide new information on firstly how regulatory B cells develop and how the gut, and the gut bacteria composition influence their development and function in healthy mice, and secondly how changes in gut-bacteria composition observed during the development of arthritis and consequent inflammation in the gut affects their differentiation and the severity of arthritis. In the longer term, we hope our research will have an impact on the way patients suffering from autoimmune conditions are treated. For example, our work will investigate how maintaining gut health (making sure the gut doesn't leak contents into the body) affects the severity of arthritis. The drugs we will use to maintain gut health (either by preventing it from becoming leaky, or by preventing the recirculation of inflamed cells to and from the gut) are currently being tested for their efficacy in gut related diseases and could be repurposed for the treatment of arthritis. Thus, our outputs may include novel treatment strategies.

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

Academic beneficiaries: Given that the work in our proposed project is looking at basic molecular and immunological interactions of a type of anti-inflammatory white blood cell called regulatory B cells (Bregs), and the potential genes and gut-derived metabolites regulating these interactions in the context of autoimmune disease, the main short-term beneficiaries of our work will be researchers working in the field of immunology, mucosal immunology, rheumatology and autoimmunity.
Industry beneficiaries: As the proposed research is to be carried out in animal models there will be no initial commercially exploitable results. However our results will be used to inform work developing therapies for patients (e.g. cellular therapy using Bregs, or manipulation of the metabolites in the gut to alter arthritis symptoms) that may, at a later point, result in commercially exploitable findings.
Non-academic beneficiaries: In the longer term, we hope our research will have an impact on the way patients suffering from autoimmune conditions are treated. If our hypotheses are confirmed by the experiments proposed under this licence we will be able to transfer this knowledge into patients with autoimmunity. Bregs are vital in restraining excessive inflammation and in regulating immune responses including those developing in autoimmunity and in cancer. Thus, for example, our extensive profiling of murine Bregs may identify molecules or genes that can be used to better define Bregs in both mice and humans. These markers they could then be used to better identify patients with abnormally low, or high, Breg numbers, possibly stratifying patients into correct treatment groups. Similarly, strategies to enhance butyrate production (butyrate is a metabolite produced by gut bacteria that has been shown to reduce symptoms of arthritis in mice, partially by enhancing Breg function) by gut bacteria may be a non-invasive way of aiding tolerance in

patients with inflammatory disorders. Manipulation of gut bacteria directly (via administration of antibiotics or probiotics) may lead to an enrichment of Bregs over pathogenic B cells. In addition, other biological therapies aimed at restoring potential gut-barrier disruption or recirculation of gut-resident inflammatory cells into the joints could also explored in clinical trials. We anticipate that we need a maximum of 4 years before we are ready to translate some of our results to the clinic.

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

In order to inform these beneficiaries of our work we will present our data at local departmental and divisional meetings as well at national and international meetings, in particular at meetings such as the
British Society for Immunology, the British Society for Rheumatology, the European Congress of Immunology and the American College of Rheumatology and Keystone Symposia. We have the opportunity to present our findings at least 2-3 times a year at internal meetings, and we hope to present our data at least once a year at international meetings from the second year of the proposed work. Once completed, we intend to publish our data as open access articles in the highest possible impact journals, so that our findings are available to the widest possible audience, and make our data available on widely used public repositories such as GEO, while programming codes will be published via GitHub.

Species and numbers of animals expected to be used

- Mice: 8500

Predicted harms

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

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

Unfortunately, at present we are unable to model systemic diseases such as arthritis *in vitro*, as autoimmunity affects multiple organs via complex crosstalk between different cell types. Answers that are more representative of human disease are obtained when we study directly the immunobiology of kidneys, liver, spleen, blood, lymph nodes and joints in diseased mice. This allows us to gain an overview of the potential of therapeutics. Presently we cannot do this either *in vitro* or *in silico*.
Mice and humans are genetically and physiologically very similar. The protein coding regions of the two genomes are on average 85% identical, and as much as 99% for some genes. In terms of the study of autoimmunity, this means that mouse models of disease have similar physiological characteristics and clinical symptoms to their human counterparts. As such, using mice as a model system for human autoimmunity has been proven to be very useful in research.
There is a wealth of experience using mice to measure immune responses. The availability of well described inbred, congenic and genetically altered strains, as well as well described spontaneous and inducible models of autoimmunity makes them currently the most appropriate species in which to carry out this work. In addition, it is now straightforward and affordable to generate genetically altered mice to allow the precise dissection of the contribution of individual molecules to the immune system and disease. Thus, mice provide an excellent experimentally tractable system to investigate autoimmune disease development, immune responses, and therapies.

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

Typically, a mouse used in our project will be induced to have a type of experimental arthritis. We will then compare the effect of different treatments, or the effect of changing the gut bacteria, on symptoms of arthritis and the activation of the immune system. The mice we use will typically be 7-8 weeks old, with fully adult immune systems, and the duration of experiments will typically be 2 to 3 weeks from first treatment to culling.
To induce arthritis, we typically first give a fully anesthetised (using inhaled anaesthetic) 7-8 week old mouse an injection of the molecule that the immune response is to be directed against to cause arthritis. This molecule will be in emulsion with an oil-based adjuvant, which is a substance that causes the mouse's immune system to recognise that molecule as dangerous, and to become specifically active against it. This injection is in a small volume (0.05-0.1 ml) and is given just under the skin, in the fatty tissue on the rear of the mouse near the base of the tail. The mouse is then allowed to wake up and is returned to its housing. The mice will be unconscious for less than 5 minutes. This injection can cause some irritation, like a strong reaction to a BCG immunisation, for the first few days until the adjuvant is absorbed.
One week later we will anaesthetise the mouse again and, using a very fine needle, we will inject the same molecule in a very small volume of saline like solution (0.01 ml) directly into one knee. In the other knee we will inject the same volume of saline like solution with no molecule so that we can compare an arthritic knee to a healthy knee in the same mouse. Again, the mice will be unconscious for less than 5 minutes. These injections will cause some discomfort, however the mice are able to walk and feed as soon as they wake up and are returned to the cage.
Over the course of the next week mice will develop swelling in the knee that was injected with the molecule. This swelling starts on the first day and reaches a peak by 3 days, after which the swelling subsides and usually completely disappears by day 7. Although mice will undoubtedly feel discomfort at the inflammation in their knee, it is not sufficient to stop them moving, eating, or grooming. While the mice have arthritis we will measure the degree of swelling of both knees daily using callipers. On day 7 mice will be sacrificed so that we can investigate their immune system cells. Alternatively, we may sacrifice the mice at day 3 to investigate the immune cells at the height of disease.
To test whether different interventions can affect the severity of disease, or the function of cells of the immune system, we will usually begin treating mice before we give the first injection that causes disease. Treatment can be simply adding substances to their drinking water, however more commonly it will be given by oral gavage, or by intraperitoneal or intravenous injection. In oral gavage, a stainless-steel bulb tipped gavage needle, or a flexible cannula or tube, is attached to a syringe and used to deliver the compound into the stomach. For an intraperitoneal injection, a fine needle is used to inject the substance into the peritoneal space. Both these procedures are carried out while the mice are awake and being gently scruffed in one hand. Both procedures cause minimal distress when performed competently and properly, and may be performed daily for the course of the disease. For an intravenous injection the mice will be gently warmed up in a heating box to dilate the vein, which should cause minimal discomfort, and substances or cells injected into a vein in the tail. Generally, we will only give one intravenous injection.

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

The major impact from the procedures in our licence will be the joint pain associated with arthritis-like disease.

We have three models of inducible arthritis with slightly different durations of disease. Our most commonly used model involves joint swelling that peaks after 3 days and resolves after 7 days. Our second most commonly used model involves joint swelling that peaks after 6 days and resolves by 1518 days. Our third, and least used, inducible arthritis model can have joint swelling that lasts for a month, however we would typically cull these mice before that stage, as soon as we see differences between groups.
We will also use mice that display spontaneous arthritis which more closely models human disease. These mice can show joint swelling from 4 weeks of age that never fully recovers. We will generally cull these mice shortly after the peak of disease once differences between groups have been established (7-8 weeks of age).

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 the majority of animals that undergo arthritis in this licence to experience a moderate level of severity. The majority (~80%) of mice will be used for inducible arthritis protocols and these mice will experience a moderate level of severity or lower.
Mice that develop spontaneous, chronic, arthritis (~20% of mice used) will experience a moderate to severe level of severity due to the size and duration of joint swelling.

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 12 April 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?

Part of the work in our group is human-based *in vitro* studies using peripheral mononuclear cells and serum from rheumatoid arthritis and systemic lupus erythematosus patients, which always informs our murine work.
Unfortunately, at present we are unable to model systemic disease *in vitro*, as autoimmunity affects multiple organs via complex crosstalk between cell subsets, as well as cross talk between different physiological systems such as the mucosa of the gut and the joint. Answers that are more
representative of human disease are obtained when we study directly the immunobiology of intestines, kidneys, liver, spleen, blood, lymph nodes and joints in diseased mice. This allows us to gain an overview of the potential of therapeutics. Presently we cannot do this either *in vitro* or *in silico*.

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

Whenever possible we will use human patient samples and healthy controls to inform our animal work. Unfortunately, the only samples we can reliably obtain from humans are from the blood (peripheral mononuclear cells and serum) and therefore we cannot fully investigate the crosstalk of cells and the interaction between organs throughout the body. To study the role of the gut in arthritis we have considered using cell lines such as the widely studied Caco-2 cell line, colonic cells that are derived from a colon cancer patient. However, we have found that the small intestine is more affected than the colon during arthritis, and thus the relevance of findings using Caco-2 cells to arthritis is unclear and would need to be confirmed using mice. However, if our future experiments determine that colonic cells are also of interest in arthritis we will, whenever possible, use this as an *in vitro* alternative to mice.

Why were they not suitable?

While human peripheral blood immune cells can be used to investigate very basic interactions between cells, or basic responses of cells to different substances, *in vitro* systems do not reflect the tissue specific behaviour of cells during autoimmunity. Cells recovered from the small intestine, colon, lymph nodes, or spleens contain different populations of white blood cell from those found in blood, and they can behave in quite different ways. In addition, although steps have been taken to try and model the anatomical structures where cells would interact *in vivo*, this work is still at a relatively early stage and the dynamics and duration of how cells may interact with each other *in vivo* in a lymph node, or in the gut, are quite different from how they will interact in liquid growth media in a test tube or well of a plastic plate. Thus, we still need to examine cellular interactions in a system that is biologically relevant to humans.
In terms of addressing basic interactions between white blood cells and the epithelial cells that make up the lining of the gut by using gut cells lines, the major problem is the origin of the cell lines. The majority of intestinal cell lines derive from colonic cancers and do no accurately reflect how the small intestine, the part of the gut that is most immunologically active, would respond. In addition, similarly to work with human white blood cells, growth of colonic cell lines in liquid on plastic does not reflect the anatomical structures these cells would comprise *in vivo*.
Moreover, as joint swelling in human arthritis is the result of multiple immune phenomena, there is no specific readout (changes to a particular cell, or levels of a particular molecule) that can act as a surrogate of disease. Thus, to test the effects of a particular intervention on the development of arthritis we need to look at what happens to symptoms of disease in a complex biologically relevant system such as a mouse.

A retrospective assessment of replacement will be due by 12 April 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 large numbers of mice included in this project reflect the work of the 7 researchers employed in our research group. Careful consideration is given to minimise the number of mice needed to give statistically significant results.
These numbers also reflect the fact that we are maintaining ~15 different strains of mice and as such have multiple breeding colonies.
Previous work in our group has provided pilot studies for most of our protocols and we have used them for power calculations. For instance, in the AIA model the power calculations showed that an experiment with at least 3 mice per group can reach statistical significance. We will perform similar calculations for all models in order to reduce the number of mice used overall for each protocol.

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

The size of the group has been decided after consultation with our departmental statistician and we will continue consultation for optimisation of the experiments throughout the life of the licence.
We will continue to consult with the NC3Rs' Experimental Design Assistant, as well as with the PREPARE guidelines: Planning Research and Experimental Procedures on Animals: Recommendations for Excellence.

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 pilot studies to provide data for power calculations so that we can use the minimum number of animals necessary per experiment. In particular, when investigating novel treatments, we will use small pilot studies to determine the potential efficacy of the treatment so that no mice get used in the future if the treatment proves unsuccessful.
Where possible, we will combine experiments so that one control group can be used for multiple experiments and to allow for sharing of tissues from the same mouse between researchers.
We will also use inducible models of autoimmunity such as AIA or STA where possible, as these models have 90-100% incidence, minimising the number of animals needed per experiment.

A retrospective assessment of reduction will be due by 12 April 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.

The highly complex nature of human autoimmune diseases requires *in vivo* experimental models, and in order to better understand their heterogeneity we have included multiple murine models.

Arthritis models:

Antigen-induced arthritis (AIA) – moderate severity.

AIA animals are injected with methylated bovine serum albumin (mBSA) in complete Freund's adjuvant (CFA) subcutaneously followed one week later by an intra-articular injection of mBSA. This results in a self-remitting mono-articular knee swelling which resolves within 7 days. This will be our main model used for arthritis as it is probably the least severe model of this disease. In addition, AIA only affects one joint and the disease course is very short, both aspects limiting the suffering of the animal. AIA is an acute model of disease rather than a chronic model and therefore we require further arthritis models to investigate the chronicity of arthritis, such as the CIA inducible model or the K/BxA^{g7} mice which develop spontaneous arthritis.

Collagen-induced arthritis (CIA) – moderate severity.

CIA animals are immunised with type II collagen in CFA and rarely boosted with type II collagen in incomplete Freund's Adjuvant two weeks later. In contrast to the acute, short-term AIA model that we will use most often, CIA persists for up to a month and affects multiple limbs and joints within the same limb. These features make CIA a closer match to human disease than AIA and will provide us with a more representative system to confirm the potential therapeutics that we can initially test in the AIA model.

We will generate initial data in AIA and use the CIA model for confirmation so as to limit the time that mice experience arthritis.

Serum transfer arthritis (STA) – moderate severity.

STA animals are injected with serum from arthritic K/BxA^{g7} mice. This arthritis model is a good combination of the two above protocols as it is relatively short term (resolution by 15-18 days after serum injection) yet it induces inflammation in both rear and front paws, which is similar to human rheumatoid arthritis. Another advantage of this model is that it does not require the use of immunostimulatory adjuvants, such as CFA, which can cause localised discomfort for mice. However, the disadvantage of this model, which gives us the need for the adjuvant-induced models above, is that STA only activates part of the immune system and as such does not fully represent the human disease, which involves most cells in the immune system.

K/BxA^{g7} mice – severe classification.

Unlike the inducible models of arthritis, K/BxA^{g7} mice develop a spontaneous arthritis involving a loss of both T and B cell tolerance and production of autoantibody. The K/BxA^{g7} model will complement the AIA, CIA and STA models in our study as its spontaneous nature obviates the need for bacterial adjuvant or any other transferred substance to induce disease.

In terms of duration, K/BxA^{g7} mice will not recover from arthritis and we will humanely kill the mice as soon as we see reproducible differences between treatment groups.

As well as using arthritis models we will perform experiments in lupus and multiple sclerosis models to confirm whether our results are arthritis specific or can be generalised to autoimmunity more widely.

Systemic lupus erythematosus models

MRL/*lpr* mice – moderate severity.
This is a well-established spontaneous model of lupus that develops around 8 weeks of age caused by a mutation in a gene that controls cell death (*fas)*. We anticipate the development of autoantibodies and clinical symptoms, notably alopecia, proteinuria and rash, similarly to the clinical features seen in humans. Similarly to the arthritis models above, unfortunately we cannot replicate the complexity of lupus *in vitro* without the interaction of cells and physiological systems. We have used this model extensively and have shown that it is sensitive to changes in the B cell population, the cells we are studying.
NZBWF1/J mice - moderate severity.
NZBWF1/J develop an autoimmune disease resembling human systemic lupus erythematosus.
Autoimmunity is characterized by high levels of antinuclear antibodies, hemolytic anemia, proteinuria, and progressive immune complex glomerulonephritis. The incidence and severity of symptoms is more pronounced in females. NZBWF1/J mice have been used as a model for autoimmune disease since the early 1960s elucidating not only the complex immunobiological responses and mechanisms but also the genetic basis for the complex multifactorial disease.
Multiple sclerosis model - Experimental autoimmune encephalomyelitis (EAE) – moderate severity.
EAE has been used by a number of research groups to study the role of Bregs in controlling autoimmunity. This model appears to be sensitive to both Breg control and the modulation of gut bacteria. Thus, it will allow us to investigate mechanistic aspects of regulatory B cell function and the role of microbiota in disease induction. Extending our work to a model of a third autoimmune disease will determine whether our findings on B cells, microbiota, or the gut itself are disease specific or whether they may apply across the autoimmune spectrum. EAE can induce total bilateral paralysis in mice, however on average most mice will only experience at most bilateral hind limb paralysis and should experience no pain. Mice showing any signs of front limb paralysis will be humanely culled before the disease can progress. EAE in mouse is the gold standard animal model for human MS as, similarly to human RA, it is mediated by both B and T cells, and induces myelin-specific antibodies.

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

Mice and humans are genetically and physiologically very similar. In terms of the study of autoimmunity this means that mouse models of disease have similar physiological characteristics and clinical symptoms to their human counterparts. As such, using mice as a model system for human autoimmunity has been proven to be very useful in research. Unfortunately, animals at a more immature stage of life, or less sentient animals, are not as genetically or immunologically similar to humans and cannot therefore replicate human clinical symptoms as reliably. Furthermore, our models are carried out over several weeks and thus we cannot use terminally anaesthetised mice.
The availability of well described spontaneous and inducible models of autoimmunity makes mice currently the most appropriate species in which to carry out this work. In addition, it is now straightforward and affordable to generate transgenic mice to allow the precise dissection of the contribution of individual molecules to the immune system and disease. Thus, mice provide an excellent experimentally tractable system to investigate autoimmune disease development, immune responses, and therapies.

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

All protocols proposed in this application are well-published models that have been tried and tested to minimise discomfort. Anaesthesia and analgesia will be administered to minimise discomfort where appropriate, and animals will be assessed daily for any signs of distress including piloerection, hunched appearance, lethargy and loss of weight. In all the proposed *in vivo* models, if animals display any signs of distress, advice will be obtained from the named veterinary surgeon and, if distress cannot be alleviated, the animals will be humanely killed. Furthermore, we have chosen specific humane endpoints in order to limit the severity of disease models.
Where appropriate, we will carry out pilot experiments to determine earliest endpoints of experiments so as to minimise suffering while generating reproducible data.
For instance, if we want to investigate the presence and function of long lived memory B cells (cells that are generated 2-3 weeks into an immune response) in one of our inducible autoimmunity models we will run initial experiments to determine when memory B cells first arise in that model. We will then design experiments that run just long enough to investigate these cells.
Lastly, all animals will be kept in well maintained, individually ventilated cages with sufficient bedding and nesting material with good quality food and water, which will also reduce experimental variability caused by environmental stresses. Animals will be monitored for weight loss and signs of toxicity, pain or distress following the mouse grimace scale. In the event of unexpected adverse effects, we will use the humane endpoints written under every protocol.

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

We will follow the PREPARE guidelines: Planning Research and Experimental Procedures on
Animals: Recommendations for Excellence, as well as any other useful material, such as the Experimental Design assistant, from the NC3R website to ensure experiments are conducted in the most refined way. Where applicable we will also follow the refinements of arthritis protocols as outlined in Hawkins et al. *Applying refinement to the use of mice and rats in rheumatoid arthritis research*.
Inflammopharmacology. 2015; 23(4): 131–150.
(https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4508365/), and have incorporated a number of their refinements into our experimental protocols.

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

We will continue to consult with the NC3Rs website for any advances, as well as with the animal welfare officer, named veterinary surgeon and technicians from the animal facility.

A retrospective assessment of refinement will be due by 12 April 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?

40. Zebrafish: an alternative model for drug safety & efficacy

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

Drug toxicity, Central nervous system disorders, Organ regeneration, Heart and kidney failure, Anaesthesia

Animal types	Life stages
Zebra fish	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.

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 use the zebrafish as an alternative, non-mammalian, model for assessing the safety and efficacy of new human drugs and human drug targets with a view to understanding how they might act in humans. This is achieved by studying the effects of drugs and drug target modification on the development, structure and function of major organs systems of the body, including nerves, heart, skeleton, kidneys, liver and digestive systems.

A retrospective assessment of these aims will be due by 13 April 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?

A very high proportion of new human drugs fail to reach patients as they show some toxicity or are relatively ineffective. Moreover, many of these failures occur after a considerable amount of time, money and animals (usually mammals) have already been used to test their safety and effectiveness. Consequently better approaches for measuring drug toxicity and effectiveness are needed, which can be deployed early in drug development so that more effective and safer medicines reach the market, without wasting these valuable resources on drugs that subsequently fail. This is where the zebrafish, in particular the embryonic and larval forms (<14 days old) is proving invaluable for several important reasons. These reasons include: good genetic and physiological similarity to humans; transparent development allowing visualisation of organ function (e.g. heart rate and blood flow); ease of genetic manipulation allowing us to test the effects of altering gene function; and a small size (a few mm's) meaning we only need to use very small amounts of drugs for testing. This project aims to utilise these attributes to develop and apply approaches in which we can measure the toxicity and effectiveness of new drugs, and test the effects of potential drug target modification on major organs system of the body.

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

Overall, the outputs from this project will include: new information regarding drug safety, efficacy and target validation; new fundamental knowledge regarding the endpoints under investigation; new methodology to complement or in some cases replace the use of higher vertebrates for testing drug safety and efficacy; new data on the translational power of the zebrafish as an alternative model to mammals for these research areas; and publications, presentations and information for educational purposes.
Objective 1) Outputs will primarily be data on the safety profile of new candidate drugs with respect to seizure liability, developmental toxicology, cardiovascular, kidney toxicity and hearing and visual impairment. These data will be used for internal decision making processes in order to drop compounds with unacceptable liabilities, allow alterations in compound chemistry to be undertaken to avoid such liabilities, and to prioritise the further development of compounds showing a good side-effect profile. In the absence of such data, the first stage of *in vivo* testing (in which the integrated whole organismal biological activity of new drugs can be adequately assessed) would be in traditional mammalian toxicological and safety pharmacological assessments, and the selection of compounds for testing in such systems would be made in the absence of any other *in vivo* data. Consequently, the data provided in this project will enable better decision making regarding the progression of compounds into higher vertebrate testing programmes and reduce unnecessary testing in higher vertebrates on compounds destined to fail later in development
Objective 2) Outputs will primarily be the provision of new *in vivo* methods for the assessment of gastrointestinal toxicity, liver toxicity and neurobehavioral toxicity that may be deployed in the early stages of drug development provided sufficient levels of translation to higher vertebrates including man are achieved. Subsequently these methods

may be used in the same way as the established assays in Objective 1 leading to gains in the efficiency and effectiveness of the drug discovery and development process. In all cases, assays are developed and validated for only high impact and/or high incidence adverse drug reactions and only in the absence of an appropriate existing approach to address a specific safety liability in a whole animal context early in the drug development process. This means in all cases that at present the first stage of *in vivo* testing is undertaken in mammals at a relatively late stage of development after a considerable amount of resources have already been invested (animals, money and time). Using kidney toxicity as an example, the ultimate impact on organ function constitutes the extremely complex culmination of the influences of many different organs systems and initiating pathways. Firstly, delivery of the drug to the kidney (or relevant indirect target), is clearly dependent on the Absorption Distribution Metabolism and Elimination of the drug in question (e.g. protein binding, partitioning, metabolism etc). Once at the target the drug may exert its toxic influence via many different mechanisms, for example: physical occlusion via altered local haemodynamics through vasoconstriction or microthrombosis; crystal nephropathy caused by tubular uric acid accumulation; cellular injury for example due to rhabdomyolysis, acid/alkylosis or localised oxidative stress; or altered tubular membrane conductances or impaired solute transporter activities at multiple sites along the kidney nephron(s). This complexity simply cannot be recreated *in vitro* and as the most important consequence is an adverse impact on renal function, the development of *in vivo* measures of functionality are crucial.

Objective 3) Outputs will primarily be data on the efficacy profile of new candidate drugs with respect to the treatment of epilepsy, diabetes, kidney and hearing regeneration, alongside other general research and educational outputs. In common with Objective 1, data will be primarily used for internal decision making processes in order to drop compounds showing insufficient efficacy against the disease phenotype in question, compared with other compounds within that specific discovery project. Again, in common with assessment based upon safety liability, in the absence of zebrafish data the first stage of *in vivo* testing would be in a mammalian non–clinical efficacy model, for example a mammalian genetically-modified mouse model that recapitulates the disease phenotype of interest. Consequently, the selection of compounds for this *in vivo* efficacy assessment would be made in the absence of any prior *in vivo* data and as such the inclusion of zebrafish efficacy data allows better decision making regarding the progression of compounds and reduces unnecessary testing in higher vertebrates on compounds destined to fail later in development. For the specific disease models being used here, there are no appropriate *in vivo* approaches suitable for early stage efficacy assessment available. Using epilepsy as an example, as epilepsy is a complex multifactorial neurological disorder, *in vivo* models have been crucial in gaining a better understanding of ictal and inter-ictal processes, as well as for testing the effectiveness of antiepileptic drugs (AEDs). Traditionally the assessment of AED efficacy has focused on the use of pharmacologically or electrically-induced rodent seizure models, or rodent genetic models of specific epileptic syndromes such as Dravet syndrome (caused by a mutation in a voltage-gated Na^{2+} channel). The zebrafish has emerged over recent years as a viable lower vertebrate model for assessing AEC efficacy and unlike rodent models can be deployed early in the drug development process when compound numbers are high, and compound availability is low. In addition to its value as an efficacy assessment model, our use of video tracking and our development of the functional imaging approach promise the potential for identifying novel mechanisms of antiepileptic action by providing mechanistic data following specific pharmacological intervention. For example, using functional imaging we have already started to identify specific neural circuits that are more frequently activated during pro-convulsive compound exposure (e.g. the cerebellum and associated circuitry) and other circuits that appear particularly activated during exposure to specific pharmacological classes of compound (such as monoaminergic and cholinergic agents).

Consequently, the data outputs from such work are helping us to understand which circuits could be novel targets for AEDs, what drug-induced seizures look like (thus improving their detection during drug development), and what the neural-network processes (such as altered functional connectivity between certain brain regions or the recruitment of specific circuits associated with specific neurotransmission systems) leading from the resting state to the ictogenic state might be. Importantly regarding these 3 points: most AEDs target a limited number of ictogenic mechanisms, and as such there is still a massive unmet need for AEDs to treat refractory epilepsy; seizure liability still presents a major cause of CNSrelated attrition during drug development; and finally the exact mechanisms that lead to ictogenesis, or the transition from local excitation to widespread hyper-synchronicity typical of a seizure are still poorly understood. Collectively, therefore, our work will further the fundamental understanding of the development, progression and treatment of seizures and the chronic seizurogenic syndrome epilepsy in humans.

Objective 4) The main output from this objective will be the provision of *in vivo* data on the link between genotype and phenotype in zebrafish in relation to high impact and/or incidence human diseases. Generation of these data will serve three main purposes: firstly they will allow drug discovery scientists to make data-driven decisions about which genetic targets to prioritise for further evaluation in genetically modified mouse models (currently decisions are made using only *in silico* and i*n vitro* data meaning only a limited number of targets can be pursued *in vivo*, and some targets subsequently fail due to poor *in vitro* to *in vivo* translation); secondly, in some cases they could provide zebrafish-based disease models that can be used as alternatives to mouse-based disease models and allow assessment of multiple novel drugs at a much earlier stage of discovery; and thirdly, these data will help to improve our understanding of the genetic basis of the targeted human disease for example by allowing exploration of the link between a gene mutation identified from clinical assessments of patients, and an *in vivo* phenotype that has not yet been described in a non-clinical model. Demonstration of a similarity between the clinical and non-clinical phenotypes supports the involvement of mutation of this gene in the disease in question. Specifically, no CRISPR-based knockout and subsequent systematic organ development and functional data in zebrafish exists for any of the genes being proposed here. For those genes identified in patients suffering from heart or renal failure, for example, there are no published studies in which gene knockout followed by detailed phenotypic analysis and measurement of cardiovascular (e.g. heart rate, blood flow, vasodilation/constriction) or renal function/pathology (e.g. glomerular filtration rate, podocyte number) are available. Consequently to date there are no data to support a functional link other than that from *in silico* or *in vitro* assessments, and therefore limited data to support their further development in mammalian models as potential targets for therapeutic intervention, or to support further research into the molecular mechanisms involved in the transition from gene mutation to altered protein function. This project will provide these data. For those genes identified as associated with ADHD and ASD, there is still a lack of fundamental data on which brain structures and circuits, or which neurotransmitter systems underpin these multifactorial diseases, and how alterations in these results in the human disease phenotype. Using our functional imaging approach we will generate data on altered brain circuitry associated with mutation of several risk genes compared with non-mutated animals, and assess the resultant effect on dopaminergic signalling in the vertebrate brain. This will provide an insight into the factors linking genotype with phenotype in these conditions, providing further knowledge of the aetiology of these diseases as well as potential targets for further therapeutic intervention.

Objective 5) The work undertaken under this objective will provide researchers with the first definitive data on appropriate anaesthetic and analgesic agents and treatment regimes in embryo-larval zebrafish (the most widely used species/life stage of laboratory fish), improving understanding of appropriate anaesthetic doses and induction/recovery times, as well as providing information on post-surgical analgesia. The data generated will

also help to refine the accuracy of visual indicators of anaesthetic status for in-procedure monitoring, which are routinely used during such procedures. These data will be generated in zebrafish, but will provide a foundation for refining anaesthetic protocols in other species of fish. We will provide the first detailed data on the effect of agents on specific zebrafish neural circuits allowing CNS researchers to select the most appropriate agents for their research. These data will also provide fundamental information on fish (un)consciousness and pain perception, and in addition, the functional imaging component will provide fish CNS researchers with a potential non-invasive replacement for implanted electrode techniques in CNS functional studies.

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

Industry will benefit from the provision of techniques, data and improved knowledge of their drugs to enable faster and more effective decisions to be made about the potential of certain projects. This is turn will lead to economic and animal savings destined to be used for studies on drugs that subsequently fail. The wider public will benefit through the generation of safer more efficacious medicines, more efficiently and rapidly than would otherwise be possible using traditional *in vivo* testing approaches.
The scientific community will be benefit from improved knowledge, development of techniques and access to publications and presentations of our research findings.
Objective 1) These data will allow safety assessment scientists within our collaborator laboratories to make decisions regarding the further development of certain compounds based on their safety profile. The data generated will allow them to drop compounds, redesign chemistry or prioritise additional safety assessment tests this leading to increased efficiency (including saving animals, time and money used on compounds with safety liabilities) and success in the development of new medicines for patient use. These gains are expected to be immediate. These gains are expected to be within the life span of the current project, as has been the case with previous licences.
Objective 2) It is anticipated that these outputs will be used by collaborator and other laboratories both for drug screening purposes, but also for other purposes as has been the case in the past for example with the use of cardiovascular screening approaches in the assessment of the effects of environmental chemical exposure in fish.
Objective 3) These data will be used by discovery biologists within our collaborator laboratories to make decisions regarding the further development of certain compounds based on their efficacy profile. This data will allow them to assess many more compounds than is currently possible using traditional i*n vivo* model systems with the aim of selecting the most efficacious for further testing and development, for example in mouse disease models. These gains are expected to be immediate in the case of current models, and within the time scale of the current project in the case of models that require additional development time.
Objective 4) Discovery scientists within our collaborating laboratories will use these data to contribute towards the discovery of new drug targets for high impact human diseases. This information is likely to be used alongside *in silico* and *in vitro* data on new drug targets to justify the generation of mouse models of the most promising gene targets thus allowing more effective evidence-based triaging. In addition, in some cases new screening models may be produced in which new candidate drugs can be tested at a higher throughout than is currently possible with mouse models used in target validation and early drug testing. Data generated from this objective are likely to be the most commercially sensitive, however wherever possible general research and educational outputs will be provided to increase learning about the function of these genes and their roles in human disease aetiology. These gains are expected to be within the life span of the current project, as has been the case with previous licences.

Objective 5) Researchers using fish models will gain the first definitive data on appropriate anaesthetic and analgesic agents and treatment regimes in embryo-larval zebrafish (the most widely used species/lifestage) and zebrafish neuroscientists with data to assess compatibility with their work as well as fundamental information on fish (un)consciousness and pain perception. Although focused on zebrafish, it is anticipated that this project will also provide valuable translational data for aiding decision making in other fish species for example by helping to refine the accuracy of visual indicators of anaesthetic status for in-procedure monitoring by veterinary surgeons, or by providing a starting point for large-scale fish anaesthesia for transportation, vaccination, tagging etc. in the ornamental fish industry.

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

We are a highly collaborative group and always seek to exploit opportunities for collaborative working.
This is illustrated by a number of publications in which some of our in house developed techniques have been used for studies outside of our core aims of assessing drug safety and efficacy (e.g. for assessing the physiological impact of environmental contaminants).
In addition, we are active presenters at scientific meetings and other universities, for example delivering presentations at international conferences, specialist meetings, local seminars and outreach events.
Despite a close working relationship with pharmaceutical companies, as a group it is our policy to publish (as evidenced by our collectively strong publication records, given our industry focus), wherever possible in peer-reviewed journals. This approach maximises the benefit of developments made under this project licence to the scientific community, and contributes towards the body of evidence supporting the usefulness (and ultimately regulatory acceptance) of the zebrafish as a mammalian alternative for drug safety and efficacy testing.

Species and numbers of animals expected to be used

- Zebra fish: 104500

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 zebrafish has emerged as a credible vertebrate model for the assessment of human drug safety and efficacy. In particular, the embryo-larval possesses a number of important attributes that infer advantages over more traditional, higher vertebrate (e.g. mammalian) models. These include: rapid and transparent development meaning biological processes can be easily be seen under a microscope; good genetic similarity with humans (a reported 83% of human disease-related genes are found in the zebrafish); and a small size meaning they do not require a large amount of space or large amounts of drug for use in tests.

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

Procedures used are: modifying a gene and assessing the effect this has on an animal or its organs; exposing zebrafish to drugs by immersion or injection and assessing the effect

this has on an animal or its organs; assessing whether drugs are effective in treating fish in which the development or function of an organ has been compromised.
Apart from the provision of adult broodstock, in the vast majority of cases, these procedures will be undertaken in young zebrafish embryos and larvae (typically <10 days old and a few mm's in length). In many cases, the duration of the experiments is short (e.g. 1 hour-2 days) with the longest part of all procedures associated with exposure to the drugs to ensure sufficient absorption into the animal.

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

In many cases, the effects of genetic modification, drug exposure or specific procedures will be mild with little more than transient effects on the welfare of the animals used. For example, over the past 4 years 46% of the animals used have been classified as of mild severity. The following adverse effects are based upon the worst case scenario where a new drug is being tested in larval zebrafish (<14dpf) without prior knowledge of its toxicity or a new disease model has been created:
Death
Morphological defects (estimated duration in protected animals 8 hours)
Seizures (<2 hours)
Moderate water retention (lifespan of the animal)
Abnormal posture or loss of balance (estimated duration 2 days)
Any of these observed in adult animals would lead to those animals begin humanely killed immediately.

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 range from mild through to severe across all protocols. The numbers expected within each category can be estimated from previous figures (2016-2019) as an average across all protocols:
Mild ~45%
Moderate 42%
Severe ~11%
Non-recovery ~2%
All animals used are zebrafish and in the vast majority of cases will be embryos and larvae of <14 days old. Specifically, >99% of exposures will be undertaken in animals of <14dpf, with adult animals only used under specific circumstances (e.g. for the assessment of later-stage endpoints for developmental toxicity), or for the production of brood stock to supply embryos and larvae for subsequent drug testing.

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 13 April 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?

Drug safety assessment and a true representation of human disease characteristics require intact animal models to accurately recreate a full organism response. For example, seizures and epilepsy are complex neurological processes and, as such, *in vivo* models with intact nervous system structure and function are crucial for furthering our understanding and for confidently detecting seizures as a side effect of new drugs. Similarly the overall aim of the target validation work is to use the zebrafish as the first *in vivo* model for
assessing the likelihood of new drug target working. Cell-based approaches are suitable for understanding basic function, but the complexity of a whole animal is vital to fully understand the structural and functional effects of gene alteration and interactions with new drugs. Normally mice are used for this but this project aims to use the zebrafish as a precursor in order to ensure only the most scientifically-appropriate and relevant mouse models are then generated for further study.

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

The ultimate purpose of this project is to understand the effects of drugs and the biology of drug targets in humans. With increasing knowledge through the project, there is the potential for replacement of protected with non-protected animals. A good example is with our recent advances in brain imaging in which we may be able to detect and measure seizures at 4dpf instead of 7dpf. Similarly, in the target validation work our strategy is to use non-protected life-stages of zebrafish initially, and only use protected life stages when and where is absolutely necessary in order to answer the specific biological question being posed. This approach makes sense as the specific properties of the embryo-larval zebrafish allow target validation to be undertaken more simply and rapidly than in older animals, or in mice.

Why were they not suitable?

The endpoints selected represent those for which no appropriate alternatives exist to bridge the gap between cell based and traditional (e.g. mammalian) animal testing models. For example, the emphasis in the drug safety assays is on functional assessment of side effects, and thus these types of assessments in their entirety cannot be recreated using cell based assays. Using seizures and epilepsy as an example, although some invertebrate models have been proposed (e.g. fruit flies) these lack the level of nervous system complexity and human translatability that the zebrafish offers and the larval zebrafish is currently the most refined animal in which the detection of, and mechanistic studies into, seizures can be undertaken. In this respect, the overall aim of this project is reduce or replace the use of rats and mice for this purpose. Our approaches, therefore, offer the best level of refinement for this process and this is supported by existing published and recently derived data.

Similarly with the target validation work, although some invertebrate models have been proposed, these broadly lack the level of physiological complexity and human translatability that the zebrafish offers, and again the zebrafish offers the most refined vertebrate species for use in this context. In common with the drug safety and effectiveness tests, the aim of the target validation work is to use the zebrafish as a lower vertebrate bridge from cell based to whole animal translation to ensure evidence for the link between gene and target is strong prior to investing in mouse models.

A retrospective assessment of replacement will be due by 13 April 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 numbers estimated reflect our average annual returns over the last 4 years with a 30% increase added in anticipation of an expected increase in demand, especially with respect to the target validation work which is in its relative infancy in our group.
In mitigation, our data reflect the fact that embryo-larval zebrafish have higher throughput amenability, that we use individual animals as the experimental unit in our work rather than using the tank as the experimental replicate measure, and that we use the most appropriate age of animal to meet the requirements of the endpoint under investigation. For example, method development work and model validation exercises suggest that 7 days is the earliest age for assessing particular behaviours, although our proposed work to validate the use of brain imaging for seizure detection may mean this is reduced to 4dpf. Adult fish are only used in drug exposures under rare circumstances (e.g. <1% of animals used), for example where the investigation of a specific endpoint requires analysis of an older animal (e.g. for developmental toxicity assessment).

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

Despite the relatively high numbers proposed, for every endpoint investigated the numbers used will be minimised by employing the following control measures:
Careful experimental design (e.g. utilising shared control groups and/or intra-individual controls where possible, power calculations, and previously generated datasets) has allowed the development of methods to obtain usable data with the minimum number of animals.
To reduce embryo-larval numbers used in established assays (seizure liability and developmental toxicology), the assessment of general toxicity (via the Maximum Tolerated Concentration or MTC assessment) is integrated into the main assessments rather than being conducted as a separate study. For any adult fish exposures, and during larval fish assay development, however, a pilot study is undertaken using a reduced number of

animals to establish an appropriate exposure regimen, prior to commencing the main study. For non-proprietary drugs, this is supplemented with information on toxicity from the published literature. The overall aim is to minimise inappropriate drug-exposure related adverse effects in the definitive assessments.
Where appropriate, power calculations will be used, although when assessing drugs with unknown toxicological and pharmacological properties, the magnitude of response is less predictable. In such cases, experimental experience will be used to judge an appropriate number of animals to use in the first instance, after which standard operating procedures will be used.

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

A key strategy is to maximise the amount of information extracted from each animal used. Examples include using the same animals for morphological assessment and histopathology in the target validation work, use of toxicity data for multiple assays employing the same test compound, and the measurement of multiple endpoints in the same animals. The latter approach is important; as well as providing more scientifically-relevant and robust data, this also serves to increase the throughput of any assay developed which is central to the success of any work that is undertaken using the zebrafish as an alternative model.
Ultimately, the overall purpose is to reduce the number of tests undertaken unnecessarily on mammals with drugs that will eventually fail due to a poor side effect profile or low efficacy, or for which the evidence for target translation to humans is poor.

A retrospective assessment of reduction will be due by 13 April 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.

All of the work contained within this project uses the zebrafish, and in the vast majority of cases, embryo-larval fish will be used in exposures (>99% expected to be <14dpf). Only under very specific circumstances will older animals be used, for example for the investigation of a specific endpoint requiring analysis of an older animal (e.g. for developmental toxicity), or when genetically-modified fish are maintained and bred to provide embryos and larvae for subsequent drug testing. With regards to the specific models and techniques to be used:
Basic brain structure and function of relevance to seizures and epilepsy are highly conserved between mammals and zebrafish. The actual measurement of seizures in

zebrafish are non-invasive (behaviour and imaging) in contrast with the use of implanted electrode recording in the brains of rodents.
The Zebrafish developmental toxicity assay has shown high predictive value against rats and rabbits traditionally used for developmental toxicity testing. Assessing development defects in zebrafish is noninvasive (imaging) and can be undertaken without the need to sacrifice pregnant rats or rabbits.
Zebrafish embryo-larvae possess an anatomically simple kidney that is easily accessed and functionally comparable to mammals. The actual measurement of renal function in zebrafish is noninvasive (imaging), and due to *ex vivo* development can be observed without the need for invasive surgery.
Due to external and transparent development, cardiovascular endpoints (e.g. heart beats, blood flow and vessel diameter) can be observed without the need for invasive procedures as is the case in mammalian models.
The lateral line hair cells of the zebrafish are structurally and functionally comparable with those of the inner ear of mammals, and have been shown to be sensitive to the action of ototoxic drugs. These can also be observed without termination of the animal and sectioning of the head, as is required in mammals.
The zebrafish visual system shows good comparability with humans having similar retinal structure and an abundance of cones providing rich colour vision. The measurement of visual acuity in zebrafish is non-invasive (imaging), and can be measured using passive assessment of behavioural responsiveness without the need for restrictive experimental chamber containment, for example in the case of rat based assays.
The zebrafish gastrointestinal system shows anatomical and cellular architecture that is similar to that of the human tract. Crucially the transparent nature of the body wall in larvae allows non-invasive visualisation of contractions following drug treatment.
The zebrafish liver is functionally comparable with that of the mammalian liver, even in larval life stages. In common with other endpoints, observation of liver structure and function can be undertaken noninvasively in embryo-larvae due to their optical transparency.
The zebrafish pancreas is structurally and functionally comparable with mammals and can be observed non-invasively through the use of imaging in transgenic zebrafish models in which specific cell types are fluorescently-labelled.
In addition to the study of seizures and epilepsy, the zebrafish is emerging as a valuable model for other neurological and behavioural disorders, for example into the genetic basis of autism spectrum disorder (ASD) and attention defect hyperactivity disorder (ADHD). The use of non-invasive brain imaging can provide better insights into potential therapeutic targets.

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

Fish are arguably the least complex vertebrates that are generally regarded as an appropriate surrogate species for the assessment of mammalian (including human) drug safety and efficacy. More specifically, the zebrafish, particularly in the embryonic and larval stages, possess a number of features that make them particularly valuable for use in assays that address drug safety and efficacy. In particular they possess functionally comparable major organ systems; they exhibit good genetic comparability with mammals in key regions; they are easily genetically manipulated; and they are transparent and small meaning organ function can be easily visualised, and small amounts of compound can be used to undertake small-scale studies which are amenable for use early in the development process of any given drug.

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

In addition to the use of generic indicators of adverse effects as humane endpoints, information is gathered to provide more specific humane endpoints for use on both existing and future models.
Protocol 5 is rated as severe as many of the new candidate drugs begin tested have very few if any available information on their toxicology. Consequently, there is the possibility of severe adverse effects including death the first time these drugs are tested. For this protocol only immature life stages (no adults) will be used. As the larval zebrafish is the first 'whole animal' in which any testing will be undertaken it is difficult to predict the likelihood of adverse effects in advance of testing. Where prior data are available (rare), the concentration range used initially will be refined accordingly. Where not available, standard concentration ranges are used for each assay which are further refined based on compound solubility. In addition, exposure periods are either very short and the animals are under almost constant observation with the animal begin humanely killed as soon as the aims of the experiment are achieved (e.g. to assess seizure liability), exposure is commenced on day 0 (before) protection and the animals are terminated on day 5 when aims of the experiment are achieved (developmental toxicity), or for longer exposure in protected animals, frequent checks for adverse effects and limits of severity (e.g. hourly for the first 6 hours of exposure), extending to a standard regime of at least 2 times in any 24 hour period with no more than 14 hours between any two checks are undertaken. In all cases the Maximum Tolerated Concentration (MTC) of the drug will be ascertained allowing adjustment of the dose regimen in subsequent experiments.
For genetic modification, animals will be sacrificed as soon as the experimental aims are reached. in many cases, these are fluorescent reporter lines showing no adverse effects of genetic modification. For target validation, phenotypes that are considered sufficiently mild to grow on for further assessment will only be grown on when the specific requirements of the experiment necessitate it. For example: to assess for a phenotype that emerges later in development; to allow phenotypic analysis in adults without the influence of active development or to provide sufficient tissue for detailed histological analysis; to provide brood stock for drug efficacy screening; or to assess trans-generational effects. It is estimated that this will apply to less than 10% of the genes investigated.
The use of fin clipping is widespread for the purposes of genotyping fish, but a growing number of publications are proposing skin swabbing as a more refined method, especially in older animals. Despite this, there is still some debate around which method is less stressful for the fish and if skin swabbing is appropriate in earlier life stages. As part of this project we will further investigate the appropriateness of skin swabbing for the purpose of genotyping individual zebrafish, particularity at younger life stages (as well as a very new method involving skin sampling from larvae via non-contact collection of sloughed cells). Provided adequate comparability in terms of DNA quality, contamination and sample size between skin swabbing and fin clipping are achieved, we will move to skin swabbing for all genotyping work under this licence. These data will also inform existing projects within our facility that still routinely use fin clipping for the purposes of genotyping zebrafish.
The use of individual animals as our experimental replicate often necessitates the housing of animals in individual wells of micro-plates during drug exposure. Prior to commencing the experiment, embryolarval stocks are held in groups of around 50 animals in Petri-dishes (circa. 35 ml volume) until experimental use where they may be transferred to micro-plates. Typically 24 (maximum volume ~ 3 ml) or 48 well (maximum volume ~ 1.6 ml) plates are used for animals up to 8dpf, and larger volume wells used for older animals such as 12 well plates (maximum volume ~6 ml) or Petridishes up to 14 dpf.
With full validation, and assuming good concordance with clinical outcome, much of this work could lead to the a reduction or even the replacement of mammalian *in vivo* techniques, thus replacing animals of higher neurophysiological development with those of lower sentience, or replacement of protected with non-protected (pre free feeding) embryo-

larval forms. Indeed progress made under previous licence(s) has meant that the use of established assays in drug safety screening programmes within industry allows, in some cases, deselection of drugs with poor safety profiles prior to testing in mammals, and in other cases prioritisation of endpoint-specific studies earlier in development than would otherwise be the case. This is expected to be expanded further with the addition of genetic target validation work into our experiential portfolio.

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

We adhere to the Home Office, UKRC and NC3Rs guidance on the application of the 3Rs to our research. We also adhere to the PREPARE guidelines for animal experimentation and ARRIVE guidelines for the publication of our *in vivo* experimental findings.

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

As a laboratory we are highly active in the promotion and application of the 3Rs to our work as evidenced by some of our recent publications and presentations including at fora promoting the use of alternative models in toxicology and pharmacology.
Moreover, some of work we undertake is directly funded by the NC3Rs and we have been active in the development of refined techniques throughout the tenure of previous licences, such as the development of the non-invasive neural imaging approach in non-protected larvae.
We will continue this approach throughout the tenure of this licence by keeping up to date through publications, conference attendance, collaboration and funding body interactions.

A retrospective assessment of refinement will be due by 13 April 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?