Home Office

NON-TECHNICAL SUMMARY

Mechanisms driving regeneration and fibrosis.

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Regeneration, Scarring, Heart Attack, Bone Fracture, Digit Tip

Animal types	Life stages
Mice	Embryo and egg, Neonate, Juvenile, Adult, Pregnant adult

Retrospective assessment

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

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Contains severe procedures

Objectives and benefits

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

What's the aim of this project?

We aim to understand how to prevent permanent scarring after serious injury and instead help tissues like the heart, bone, and skin repair themselves naturally. Our goal is to restore these tissues so they work and look as close as possible to their original healthy state.

A retrospective assessment of these aims will be due by 9 October 2031

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?

Many tissues in the human body, such as the heart, kidney and bones, have only a limited ability to repair themselves after injury. When tissues cannot heal properly, it can lead to serious illness or disability. Fibrosis, or harmful scarring, affects millions of people and contributes to conditions such as chronic kidney disease, liver disease, and heart failure, which is the leading cause of premature death in the UK. As people age, conditions like osteoporosis and slower tissue repair increase the risk of fractures, poor healing, and long-term disability. Current treatments for these problems are often limited and cannot restore normal tissue function. By studying how tissues repair, regenerate, and sometimes fail, this research will generate knowledge that could help develop new treatments to reduce scarring, improve healing, maintain tissue function in older people, and ultimately benefit millions of patients.

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

At the end of this project, we expect to produce new knowledge that will help us better understand how tissues repair themselves, why this ability sometimes fails, and how it links to disease. This work will lead to scientific publications that can be shared with the wider research community, and in the longer term, it may support the development of new treatments.

One key output will be new information on how to reduce harmful scarring (fibrosis). Scarring happens inside the body after many different injuries and illnesses, and it can stop organs, like the heart, from working properly. Our cardiac injury model will help us to see how heart tissue heals and why it often forms too much scar tissue instead of repairing itself well. Understanding this process will point scientists toward new ways of improving healing and protecting heart function.

Another important output will be a better understanding of how bones repair themselves after fractures and why this process is sometimes slow or incomplete. Our bone fracture model will allow us to study how the bone's support structures are built. This will help us to learn why bone healing can fail, especially as we age, and may guide future strategies to help broken bones heal faster and stronger.

We also expect to learn more about why some tissues can repair themselves well, while others cannot. For example, the tip of a mouse digit (the scientific term for finger or toe) can regrow after injury, but this ability fades if the tissue is injured repeatedly, a process similar to ageing. By studying this process, we hope to find out why tissues lose their ability to repair themselves over time. In the long term, this knowledge could help identify new strategies to keep tissues healthier for longer and to slow down age-related decline.

Finally, our research may reveal why tissues that are good at regeneration are also less likely to develop cancer. In many tissues, faulty repair can lead to tumour growth, but in tissues like the fingertip, this does not happen. Understanding how these tissues protect themselves could provide valuable clues for new ways to prevent cancer while encouraging safe tissue repair.

Together, these outputs will provide valuable insights into wound healing, ageing, heart repair, broken bone recovery and cancer. The knowledge gained will be shared through scientific articles and presentations, and in the future, it could support the design of safer and more effective treatments for human disease.

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

This project aims to improve our understanding of how tissues repair themselves, how scarring can be reduced, and how regeneration can occur safely without increasing the risk of cancer. In the short term, the outputs of this work, such as new information about tissue repair and regeneration, will benefit the scientific community. This knowledge will be shared through publications, conference presentations, and collaborations, helping other researchers build on our findings and avoid repeating experiments unnecessarily.

In the longer term, this research could lead to the development of new treatments for a wide range of human diseases. One important area we are studying is the extracellular matrix, which is the scaffolding or support structure that surrounds and holds our cells in place. If we can learn how to control this scaffolding, we may be able to reduce harmful scarring (called fibrosis), which is a big problem in diseases of the kidney, liver and heart. Understanding how regenerative capacity declines with repeated injury may also help develop strategies to maintain tissue repair in aging tissues, potentially improving recovery from injury and degenerative diseases.

Additionally, insights into how certain tissues regenerate without forming cancer could inform new approaches to promote safe tissue repair in organs that are normally prone to tumours. Over time, this knowledge may guide the creation of therapies that enhance regeneration, reduce scarring, and protect against cancer, ultimately benefiting patients with chronic or life-threatening conditions.

Overall, this work will benefit researchers, clinicians, and, ultimately, patients who suffer from fibrosis, age-related tissue degeneration, heart disease, or conditions with a risk of tumour formation. It also

has potential wider societal and economic benefits by supporting the development of new regenerative treatments.

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

We want to make sure that the knowledge gained from this project benefits as many people as possible, both inside and outside the scientific community. To do this, we will share our results openly. This includes publishing our findings in scientific journals, presenting them at conferences, and running workshops. Importantly, we will also publish results from experiments that do not work as expected, so that other researchers do not waste time or animals repeating the same work.

We will also share our findings with the public in an accessible way, so that people interested in tissue repair, regeneration and disease can learn more about the research and its potential benefits. Wherever possible, results will be shared before or at the same time as formal publication, allowing other scientists to use the information sooner.

Collaboration will be an important part of this project. We will work with other researchers and companies to share knowledge, skills, and techniques, and the tools and models we develop will be made available to others in the field. The data we generate will also be stored in publicly available databases, making it widely accessible to the research community. However, in some circumstances data may have to be kept confidential so that new discoveries can be protected with patents and eventually developed into new therapies.

Species and numbers of animals expected to be used

- Mice: 6500

Predicted harms

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

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

We are using mice in this study because their bodies and genes are very similar to humans, which means they react to injury in ways that closely match human responses. For example, the tip of a mouse’s toe and the tip of a human finger are made up of the same parts including skin, blood vessels, nerves, and bone, and both can regrow after being injured. This makes mice a very good model for studying how tissues repair themselves, without needing to test directly on humans. Mice are also useful for studying illnesses or damage, like what happens in the heart after a heart attack or in bones after a break, because their tissues respond in a similar way. We are using adult mice because most injuries and diseases we want to study happen in adults, so the results will be more relevant to real-life medical problems. Mice are the least sentient mammals suitable for this kind of research, which means we can answer important scientific questions while keeping animal suffering as low as possible.

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

To study regeneration and fibrosis, animals will normally have one of three different surgical procedures. This will be either:

1) a surgery to remove 1-2 mm of the end of the digit, which will fully grow back. In a human this would be the same as removing the tip of your finger starting from midway through your nail. Otherwise, we will remove 3-4 mm of the end of the digit. This type of injury will not grow back but will heal and the mouse will have a shorter digit. In a human this would be the same as removing the tip of your finger up until the first joint. Some animals may have their digits amputated multiple times, and others may have small amounts of tumour cells injected into the toe tip so we can study how repair changes cancer.

2) a surgery on their heart, where a small thread is tied around blood vessels to cause a heart injury. This type of injury will mimic what happens when a person has a heart attack.

3) a surgery on their leg bone, where the bone is broken or drilled to study how it repairs. This type of injury will mimic what happens when a person has an accident and fractures their bone.

All these operations will always be done under general anaesthetic so the animals are asleep and cannot feel pain, and pain relief medicine will be given before the operation and afterwards. A scoring sheet will also be used to help decide when extra pain relief is needed, so the decision is clear and consistent and not based on opinion alone. In addition to surgery, some mice will be given injections of substances, or whenever possible will receive these substances mixed in with their food or water. These may include medicines that switch on or switch off certain genes, substances that label dividing cells, or treatments that may help healing. Mice may also be placed in scanners (like magnetic resonance imaging - MRI, or ultrasound) so we can take pictures of their organs while they are alive. For some scans, the mice may need to have food taken away for a few hours (but they will still have access to water). This short fasting period helps to make the images clearer and more accurate. We will also breed special types of mice that carry changes in their genes (called genetically altered mice). To activate these gene changes, we will inject a drug called tamoxifen into their belly once a day for four days. Sometimes mice will be placed in an incubator at 42^0C for up to 15 minutes. This short warming period helps to turn on certain special genes.

After surgery or other procedures, animals will usually be monitored for up to seven days, but sometimes longer if tissue samples are collected at later time points (up to 2 -3 months). When procedures are repeated, enough time (usually two weeks) will always be left between them to allow the mice to recover properly. Throughout all experiments, animals will be housed in groups, given pain relief when needed, and carefully checked every day.

At the end of experiments, mice will be humanely killed so we can collect tissue samples that cannot be taken from a live animal. For example, we may take digit samples to study changes in the tissue, such as how cells look under the microscope (histology). These samples are essential for understanding healing and cannot be collected in any other way.

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

The mice in this project will be closely monitored, and we will work to reduce any discomfort as much as possible. Many animals will need surgery where the tips of the middle three toes on one or both back feet are removed. Although this is a moderate-severity procedure, regular checks show that this does not stop the mice from walking, moving around, or cleaning themselves normally. Their front feet will never be touched. All surgeries are carried out while the mice are asleep under anaesthetic, and they are given pain medicine so that they do not feel pain during or after the surgery. The mice usually wake up within 10 to 15 minutes and quickly return to normal behaviour. When 3-4 mm of the toe is removed, it does not grow back. There is often some swelling in the toe for 7 - 14 days after surgery. Because this swelling can last for up to two weeks, the mice will continue to receive pain medication for the full period. We will work with the veterinary team to make sure the medicine is the most effective it can be and helps the swelling go down faster. During daily checks, the mice behave normally and do not appear to be in pain.

In some studies, small numbers of tumour cells are placed into the toe. This can cause extra swelling and a tumour that may last for the whole experiment. We carry out daily scoring of the toe, the swelling and the mouse's ability to walk, stand and move around. These checks ensure that the tumour does not stop the mouse from moving normally or carrying out natural behaviours. The tumour cells stay only where they are placed and do not spread to other parts of the body.

Some genetically altered mice will be given a drug called tamoxifen. These mice may lose up to 10% of their body weight in the first one to two days after treatment. They usually regain the lost weight within 5 days and do not show other harmful effects.

In some cases, mice will have more complex surgeries. If we need to make a small bone fracture, the mouse will not be able to put weight on the operated leg immediately afterwards. This usually improves within 3 days. The mice are given pain medicine and are watched closely until they recover. For surgery to cause a heart attack, the risks are much greater. Some of the mice will not survive. Around half of the deaths happen during the operation itself, when the mouse is under anaesthetic and does not feel discomfort. The others may die suddenly at different times after the surgery. When sudden death occurs, the mouse becomes unconscious very quickly and dies within minutes, which means suffering is not prolonged. In humans, heart attacks can cause chest pain, breathing problems, nausea and other symptoms. We cannot completely rule out the chance that mice may also feel some of these effects. Signs of heart failure in mice may include a blue colour to the skin, swelling in the feet and ankles, and trouble breathing. If these signs appear, the mouse will be put to sleep humanely to prevent further 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)?

20% Mild, 70% Moderate and 10% Severe.

What will happen to animals used in this project?

- Killed
- Kept alive at a licensed establishment for non-regulated purposes or possible reuse

A retrospective assessment of these predicted harms will be due by 9 October 2031

The PPL holder will be required to disclose:

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

Replacement

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

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

We need to use animals to achieve the aims of this project because they provide a living system that is essential for studying how tissues repair, regenerate, and sometimes fail to heal. While cells in a dish or computer models can provide some information, they cannot replicate the complex interactions between different cell types, tissues, and organs, or show how cells behave over time. For example, the mouse digit tip can fully regenerate after amputation, allowing us to study why this ability declines with repeated injury and to identify factors that support tissue repair, a process that occurs over several months and relies on signals from surrounding cells that are difficult to reproduce in a dish. Animals are also needed to study fibrosis, which is harmful scarring that occurs in diseases such as kidney, liver, and heart conditions, because healing involves interactions with the immune system that cannot be replicated properly outside an animal. They are also necessary for studying cancer, as tumour formation depends on interactions between multiple cell types and their surrounding tissues that only occur accurately in a whole organism. Most techniques that work with cells outside living animals do not fully reflect their normal behaviour over long periods, making animal studies essential for understanding these complex biological processes.

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

For our project we thought about different ways to studying regeneration and scarring without using animals. One exciting new method is called “organoids”. These are tiny 3D cell cultures that can be grown from human or mouse stem cells. They act a bit like mini organs such as the brain, kidney, lung, or liver, and can live for a long time in the lab. When looking at the heart, we can also make special heart muscle cells, called cardiomyocytes, from stem cells. These cells are useful because they behave like young heart cells and let us study how the heart works and responds to damage. For bone healing, we can grow bone cells such as osteoblasts, osteoclasts, and stem cells to study how bone is made and repaired. In our lab, we are also developing a non-animal model for digit tip regeneration using donated human embryonic tissue. This ‘ex vivo’ system keeps the tissue alive outside the body so we can watch how it repairs itself. It gives us the chance to study the same kinds of signals and processes that happen in people when fingertips regrow, which makes it a powerful alternative to animal experiments.

Why were they not suitable?

Although non-animal models are exciting and helpful, they are not always enough on their own. For example, scientists have not yet made an organoid that copies the human digit tip. These models also lack blood vessels and immune cells, which are important for guiding regeneration. For the heart, stem cell-derived heart cells behave like very young cells, not adult ones, so they do not show the same limits in healing that adults have. Adult heart cells, on the other hand, do not live long in culture, making them hard to study. For bone healing, cell cultures can teach us how certain bone cells work but they cannot copy the full environment of a broken bone inside a living body, where many types of cells including blood vessels and immune cells interact. In general, cells grown in the lab live in an artificial environment with unusual levels of oxygen and without the many signals they would normally get inside a body. Because of this, these models cannot yet replace animal studies. However, they are still very useful, and we can use them alongside animal models to give us more information while helping reduce the number of animals needed.

A retrospective assessment of replacement will be due by 9 October 2031

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 carefully planned the number of animals we will need, to make sure we ask for enough to complete the work but do not use more than necessary. To do this, we have spoken with a trained statistician to help us design experiments properly, and we have based our numbers on the amount of work we expect to do each year.

We have also asked experienced researchers for advice and used our own past experience to guide our estimates. When working with genetically altered mice, we have thought about how best to keep the number of different strains as low as possible while still making sure we have enough animals for the experiments.

The natural breeding patterns of the mice we use have been taken into account. For example, some strains normally have smaller litters of around 6–8 pups, while others can have 10–15. To avoid producing more animals than we need, we will also buy in many of the mice from suppliers, as this gives us better control over numbers.

In some cases, we will first run small test studies (called pilot experiments) to find the lowest safe amount of a substance that still has an effect. This helps us plan later experiments with the right number of animals and avoids unnecessary use.

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

We have carefully designed our experiments to make sure that we use the smallest number of animals possible while still collecting reliable and meaningful results. To support this, we used the NC3Rs Experimental Design Assistant, an online tool that helps researchers plan studies in the most efficient way. We also sought advice from a qualified statistician, who reviewed our plans to make sure the experiments are set up correctly and the data will be interpreted fairly.

Where possible, we have designed experiments to gather the maximum amount of information from each animal. For example, in some studies we can treat digits on one side of the body with a drug, while digits on the opposite side receive a control treatment. In this context, the control refers to an injection containing on the substance in which the drug is dissolved, but without the active drug itself. This allows us to compare the treated and untreated sides within the same animal. Because each animal provides both treated and control samples, fewer animals are needed overall.

We will also make use of non-invasive imaging methods, such as ultrasound and magnetic resonance imaging. These techniques allow us to take images before any treatment is given and then compare these 'before treatment' images with those taken afterwards. In this way, each animal acts as its own control. This reduces the natural variation that exists between animals and means fewer animals are required to detect meaningful changes.

The number of animals in each experimental group has been based on pre-existing data, ensuring we only use what is required. In many cases, especially when using imaging methods, each animal can act as its own control. This approach avoids repeating experiments unnecessarily and helps us keep numbers as low as possible.

Where it is possible, we will also use randomisation and blinding. Randomisation means that animals are assigned to treatment groups by chance rather than by any characteristic of the animal or researcher, which prevents unintentional bias. Blinding means that the person assessing the images or analysing the data does not know which treatment group each animal belonged to. This avoids expectations influencing the results. Both practices increase the reliability of the findings and reduce the risk of needing to repeat experiments, which helps to keep animal use to a minimum.

We have also followed recognised standards such as the PREPARE guidelines, which give advice on how to plan animal research responsibly. Using these tools and recommendations helps us avoid wasting animals, minimise the numbers required, and ensure that every study provides useful results.

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

We have planned several steps to make sure we use the smallest number of animals needed while still carrying out meaningful research.

We will manage our breeding programmes carefully so that animals are not wasted. For genetically modified strains that are not currently in use, we will freeze embryos for future work instead of keeping live colonies. For strains with complex genetic backgrounds, we will plan breeding strategies so that the maximum number of suitable experimental animals and their control littermates are produced from each litter.

Where possible, we will carry out small test studies (pilot experiments). These will help us check the safety and effectiveness of treatments, such as the lowest amount of a substance needed to have an effect, before moving on to larger studies. We will also use information from substances and approaches that have already been tested by other researchers, which avoids unnecessary duplication.

We will increase the amount of information we gain from each animal. For example, non-invasive imaging techniques will allow us to monitor the same animal over time. When tissue is needed, we will divide samples so that one organ can be used for more than one type of test. Wherever possible, tissues will be shared within the research group, reducing the need to use additional animals.

We will also work with other scientists to share tissues, for example from mice used in digit removal studies, so that fewer animals need to be bred overall. In addition, we will stay up to date with new methods that may replace or reduce the use of animals in the future.

A retrospective assessment of reduction will be due by 9 October 2031

The PPL holder will be required to disclose:

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

Refinement

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

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

This project will use mice because they are the least sentient mammals in which heart, bone, and tissue repair can be studied, and their genes can be easily controlled to understand how healing happens. Three models will be used including regrowth of the digit tip, heart attack (called myocardial infarction), and bone fracture.

For **digit tip studies**, the very end of the toe is removed. This area if known to heal well in mice, and depending on how much is removed, the digit can either fully regrow or form a small scar. Care is

taken to minimise the impact of this procedure, including the use of anaesthesia, pain relief, and regular monitoring to make sure the mice are comfortable and recovering normally.

For the **heart model**, a controlled heart attack is carried out under full general anaesthesia. This is the best established method for understanding how the heart repairs itself, and it cannot be studied safely in humans. Pain relief is given before and after the procedure, and animals are monitored closely during recovery.

For **bone healing**, a single surgery is used to create a small, controlled break in the thigh bone. This is also done under general anaesthesia, with pain relief and careful follow-up to ensure the animal's well-being.

Across all models, several refinements are used to minimise suffering. These include modern imaging methods, such as CT scans, which help researchers track healing in the same animal over time, reducing the need for extra procedures. Genetically altered mice also help limit interventions because genes can be switched on or off without additional surgery. Together, these refinements ensure that important questions about tissue repair can be answered while keeping discomfort and lasting harm to the lowest possible level.

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

Less sentient species such as worms, fish, or amphibians cannot be used because the way they regenerate is very different from mammals. For example, animals like zebrafish and newts can repair their hearts throughout life using mechanisms and proteins that humans simply do not have. Studying these animals would not give us answers about why regeneration is so limited in adult humans, particularly in the heart and skeleton.

We also cannot use very young animals or animals under terminal anaesthesia. Young animals naturally repair injuries much more easily than adults, so they would not give results that match adult human healing. Animals under terminal anaesthesia are also unsuitable because regeneration and scarring take place slowly over many days or weeks, and these processes cannot occur normally in an animal that is not awake. Long periods of deep anaesthesia would also change how the body works and prevent normal healing from happening. For these reasons, adult mice are the simplest and least sentient mammals that can still model the adult human healing process.

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

All methods in this project will use techniques that reduce animal stress and make sure the animal does not suffer as detailed below:

- **Planning and review:** Before each study begins, we will work closely with the veterinary team to plan the procedures and make sure the care and monitoring of the animals are as refined and safe as possible.
- **Anaesthesia and pain relief:** All surgeries, including digit tip amputations, heart procedures, and bone fractures, will be performed under anaesthesia so that the animals do not feel pain. When

performing procedures on juvenile mice, a custom-made anaesthetic mask designed for improved fit for animals of this age will be used. All procedures will be done using clean techniques in line with Home Office Minimum Standards and LASA guidance (2017). Pain relief will be given before surgery and afterwards if needed. We will always consult the Named Veterinary Surgeon to make sure we are doing this correctly.

- **Monitoring and humane endpoints:** Animals will be carefully observed before and after procedures using health checks and scoring systems. If an animal becomes unwell or shows signs of distress that cannot be relieved, it will be humanely killed. Although a small number of animals may reach a higher level of illness due to the nature of the models, we will take every possible step to recognise problems early and act quickly so that animals do not remain in severe discomfort.

- **Non-invasive techniques:** Where possible, we will use non-invasive methods, such as imaging, to gather scientific information. This reduces the number of procedures and the total number of animals required.

- **Housing and enrichment:** Mice will be kept with other compatible animals and provided with bedding, nesting material, and shelters (such as cardboard tubes or small houses). This encourages natural behaviours, allows them to explore, and gives them a safe place to hide or rest.

- **Acclimatisation and handling:** Animals will have at least seven days to settle into their new surroundings and get used to handlers before experiments begin. We will use gentle handling techniques, such as tunnels and cupping, to reduce stress during procedures.

- **Palatable treatments:** Flavoured drinks, jelly, or spreads will be used to make medications and gene-activating substances easier for animals to take.

- **Least invasive routes and combining procedures:** When injections are required, we will use the least harmful route, the smallest needle possible, and only use the needle once. Where possible, more than one procedure will be carried out under a single anaesthetic to reduce repeated handling and recovery.

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

To ensure that all experiments are carried out to the highest possible standards, we will follow established guidance for planning, reporting, and performing animal research. At the planning stage, we will use the PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) guidelines, and for reporting results we will follow the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines.

For all surgical procedures, we will follow the Home Office Minimum Standards of Aseptic Surgery and the Laboratory Animal Science Association (LASA) Guidance on Preparing for and Undertaking Aseptic Surgery (2017). For the breeding and use of genetically altered mice, we will follow the Home Office guidelines and the NC3Rs resources on genetically altered mice, including the GAA Framework (2018) and guidance available at the NC3Rs website.

To ensure that substances are administered in the least harmful and most effective way, we will refer to recognised resources, including the BVAAWF/FRAME/RSPCA/UFAW report on refinement of substance administration, the article by Turner et al. (2011) on administration routes, the LASA Procedures with Care guidance, and recent publications on safe administration techniques. Following these guidelines ensures that experiments are conducted consistently, safely, and with the least possible impact on the welfare of the animals.

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

I will stay informed about advances in the 3Rs (Replacement, Reduction and Refinement) by regularly consulting trusted resources such as the NC3Rs and Norecopa websites, signing up to the NC3Rs e-newsletter, and attending relevant workshops or events. I will also use my Institute's 3Rs search tool, which provides up-to-date information on ways to reduce animal numbers, replace animal use where possible, and refine techniques to minimise stress. In addition, I will keep up to date with the published scientific literature (in particular consulting the Alternatives to Laboratory Animals Journal) to identify new approaches that could improve animal welfare or reduce reliance on animal experiments.

To ensure that new methods are implemented effectively, I will discuss 3R opportunities in regular meetings with local experts in animal welfare, veterinary matters and information sourcing, and animal care technicians. Where new techniques are identified, I will work with colleagues who already have the expertise to ensure proper training and safe introduction of the method. Any refinements will be trialled first to confirm they do not cause additional harm. This approach ensures that any advances in the 3Rs are rapidly adopted while safeguarding both animal welfare and scientific quality.

A retrospective assessment of refinement will be due by 9 October 2031

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?