Home Office

Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2019 that require a retrospective assessment Volume 2 (N to Z)

Contents

Project	**1. Neural Control of Sensorimotor and Autonomic Function in Health and Neurological Conditions**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	We aim to study the mechanisms and effects of different interventions capable of promoting repair of injured nerves in the brain and spinal cord to produce functional recovery. We will specifically investigate changes in stepping behavioural and other functions such as bladder control following injury and recovery through different interventions. **A retrospective assessment of these aims will be due by 04 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?
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The ultimate goal of our project is to provide interventions that can facilitate recovery of function following injuries to the brain and spinal cord. Some of the interventions included in this project, such as epidural electrical stimulation and rehabilitation have already shown promise in clinical application. However, further refinements are required to improve their effectiveness. Other newer potential interventions are in earlier development and require to be tested in animals.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats = 2500 Mice = 1800
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?	Some animals will only reach a mild level in the severity scale. Others, will never wake up from the anaesthetic. For the nervous system injury procedures, most animals will be expected to be in the severe level of severity. This is mostly due to disruption of movement and sensation in some parts of the body. The level of disruption will also vary depending on severity of the injury. Control of bladder function is often compromised in these animals. Therefore, we care for each animal at least twice daily for the duration of the experiment to manual express their bladders and check for any abnormal behaviours. For example, persistent weight loss will be treated with supplemental diet, wet food and if necessary saline injections. However, we do not expect to induce pain in these animals. In severe lesions, the communication between the brain and spinal cord is completely severed and pain sensation cannot be processed by the brain. Because our primary aim is to facilitate recovery of function after lesions, the wellbeing and health of animals is of paramount importance. Ill health and pain are not conducive to such recovery. In the rare occasion when adverse effects exceed expected changes (for example, persistent excessive weight loss, inability to eat or drink, etc.) the animal will be humanely killed using an approved method. Therefore, we individually care for each animal at least twice daily for the duration of the experiments. At the end of all

	experiments, animals receive an overdose of anaesthetic for collection of tissues or are killed using an approved Schedule 1 procedure. **A retrospective assessment of these predicted harms will be due by 04 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?
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The complexity of the events leading to and as a consequence of lesions to the nervous system involves several physiological systems in the body. Unfortunately, reduced preparations such as cell in a dish, parts of brain kept in a dish, cannot provide enough information from all of the systems simultaneously. Our primary objective is to investigate functional recovery, which can only be measured in alive behaving animals. **A retrospective assessment of replacement will be due by 04 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?
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our experimental design has been optimized to use the lowest number of animals required to produce reliable statistical inferences. We combine several different techniques, including behavioural, physiological, anatomical tests and computational modelling. This allows us to reduce the number of animals required by eliminating the need to repeat experiments to collect different types of data. In addition, our experiments can provide very powerful results when looking at different types of measurements (behaviour, physiology, anatomy) from the same animal. **A retrospective assessment of reduction will be due by by 04 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?
3. 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 (rats and mice) are the preferred animal model for these projects for the following reasons. First, given the stage of our understanding a certain number of animals need to be studied, which precludes use of larger animals (pigs, cats, monkeys). Second, the nervous system of rodents have several similarities to humans, including the processes involved in responses to injury. Third, rodent models of nervous system lesions have been prevalent in the last years, and much is known about the disease process of the lesion in those species. All surgical procedures are done under anaesthesia and under aseptic conditions, which minimizes the need to deliver medication such as antibiotics. As mentioned before, each animal is individually taken care of at least twice daily. Also, pain and distress are unwanted outcomes because they are detrimental to functional recovery. Therefore even the smallest changes, for example in skin condition, such as a small sore, are immediately treated. **A retrospective assessment of refinement will be due by by 04 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?

Project	**2. Neurorestoration following nervous system injury**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
	X	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Overall Aim: the aim of this Project is to develop new therapies for disorders such as spinal cord injuries, which lead to severe disability and for which there is currently no cure Clinical need: Injuries to the central nervous system (the brain and spinal cord) cause devastating and permanent loss of function such as paralysis, loss of the use of the arms and hands, bladder and bowel incontinence and loss of sexual function. Spinal cord injury is a particularly strong example of a life-changing event that can lead to a lifetime of disability and high-dependence care, devastating the lives of individuals and their families. Over 2.5 million people worldwide are	

	currently living with a spinal cord injury and many new injuries are suffered each year. Healthcare costs to treat and care for individuals with spinal cord injuries are among the highest of any neurological disorder. Spinal cord injury therefore represents a major medical challenge and a huge burden to society. There is no cure and no regenerative therapies available to spinal injured individuals. The only available treatments offered to patients are rehabilitation and medication for managing complications such as pain and depression. Therefore, new regenerative therapies that enable tissue repair and new nerve connections to be made and which would lead to some functions being restored are drastically needed. This could improve quality of life of the many thousands of individuals living with severe disabilities. Key objectives: 1. To advance the clinical development of a promising new gene therapy which degrades spinal injury scar tissue. This scar tissue normally blocks new nerve growth, but the gene therapy enables new growth and new nerve connections and can lead to recovery of important functions such as the use of the hands. A main objective of this project is to work out the best timing and doses of this therapy and to improve its safety so that it can be developed into a treatment for human spinal cord injury. 2. To develop new approaches to improve the ability of spinal nerve fibres to regenerate and to overcome the spinal injury scar tissue. We will use newly developed gene therapies that can be switched on and off and that can be targeted to specific cells, as well as new drug therapies that destabilise the growth-blocking elements of the scar. 3. To determine whether the therapies developed in objectives 1 and 2 will work when the treatment is given some time after the injury (when the scar tissue and pathology is well established). This will determine whether our therapy could be useful to the many individuals living with long-established spinal cord injuries. 4. To study the cellular changes that happen in the

	days and weeks after a spinal injury occurs (called the “secondary injury”). By understanding the molecules that cause pathological inflammation and scarring we can develop new therapies to target these molecules. 5. To use genetically modified mice to identify genes that are important for regeneration and recovery and to develop new therapies which would increase the expression of these genes and improve recovery. 6. Develop rehabilitation methods for improving the function of hand and arm muscles. We will focus on abilities that are top priorities for individuals with tetraplegia (where all 4 limbs are affected), such as the ability to pick up and grip an object. Recovering the use of the hands would give individuals greater independence (e.g. the ability to wash, feed and dress independently) and an improved quality of life. 7. Determining the best treatment combinations which have the potential for maximising recovery of function after injury (e.g. a regenerative gene therapy combined with intensive rehabilitation). **A retrospective assessment of these aims will be due by 09 October 2024** 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?

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The work under this Project has multiple potential benefits, both for the advancement of science and, in the longer term, for improving human health. Novel tools and therapies developed under this project will be made available to other researchers to use, which may benefit both their research programme and the wider field of regenerative medicine, since they may be used for improving our understanding of the basic biology of tissue injury and repair. This could be important for developing therapies for traumatic brain and spinal cord injuries, and for other nervous system disorders. Clinicians and neurologists may also benefit from this work, since novel rehabilitation-based strategies developed here may be implemented in the clinic, and candidate biomarkers of injury that may be discovered here could be evaluated in patient samples. The pharmaceutical industry may also benefit, should they invest in the clinical development of our new gene therapy strategy, enabling safety and toxicity studies to be performed and clinical grade vectors produced. The long-term aim of this project is to have practical application in the development of a clinical therapy that could benefit patients living with spinal cord injuries (as well as other stakeholders such as families, caregivers, charitable foundations). The regenerative therapies that will be developed in this project have a realistic potential for improving the functional outcome for thousands of individuals living with paralysis and other severe disabilities. A small gain in function, such as recovering use of the hands, would dramatically change the quality of life for individuals living with tetraplegia (affecting all four limbs), enabling them to carry out basic everyday tasks such as feeding, washing and, dressing themselves, giving them independence and autonomy and enabling greater participation in society.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use approximately 200 mice and 500 rats per year. For any procedures we will undertake power calculations to estimate the minimum number of animals we need in order to obtain statistically meaningful results.

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?	1. Cell culture work involves removal of tissues after humane killing; no additional adverse effects are expected. Severity level is mild. 2. Nervous system injury work, including spinal cord injuries, involves surgeries performed with anaesthesia and analgesia. We aim to use injury models that cause partial damage to precise areas of the brain and spinal cord as well as more “clinically-relevant” models that replicate the typical pathology of human spinal cord injuries. In both cases we aim use the most minimal injury that will enable us to assess muscle and limb weakness and dysfunction, but that will keep the animals in as best health as possible. For example, none of our protocols induce complete paralysis in the experimental animals. Following injury, animals will show varying levels of impairment depending on the severity of the injury they have received. Typically, for the first two or three days after partial brain or spinal cord injury (or up to two weeks after clinically relevant injuries), rodents require special care because they may be transiently weak or partially paralysed, may feed and drink less, and appear unkempt. We provide them special intensive care including soft bedding, additional fluids (by injection), pain relief medication, additional food (feeding by hand if necessary). One of our procedures is classified as “severe” because the loss of mobility is greater than other injury models. However, this is the most clinically relevant model and therefore important to use in some cases for assessing and developing new therapies. General well-being of the animals will recover quickly following injury (within the first week) and typically this will be followed by dramatic functional improvements such that animals will be able to support body-weight on their affected limbs by 2 weeks post-injury. We continuously strive to improve the welfare of our animals and to reduce their suffering. Animals that do not recover with this additional special care will be humanely killed. However, we have considerable experience caring for animals of this kind and the majority recover as expected. At the end of the studies, animals will undergo euthanasia and their tissues will be used for further analysis of treatment effects; making maximal use of tissue will reduce the number of animals. **A retrospective assessment of these predicted harms will be due by 09 October 2024**

	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?
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Our work aims to develop novel therapies for promoting recovery following spinal cord injury. This work requires the demonstration of robust effects in clinically relevant animal models. Additionally, many mechanistic questions and experimental strategies require invasive techniques that are not possible or feasible at present in humans. Cell culture and computer simulation techniques are not significantly advanced to model the integrated actions of the nervous system, in particular they cannot mimic the complex inflammation and scarring processes and cellular reactions that lead to destructive tissue pathology within a central nervous system injury environment. For this, there is no alternative to using in vivo systems, therefore animal models are required. **A retrospective assessment of replacement will be due by 09 October 2024** 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?
2. Reduction Explain how you will assure the use of minimum numbers of animals	Animal numbers will be kept to a minimum by carefully designing and planning all studies to ensure that the group sizes are kept to the smallest possible size at which a meaningful effect can be detected. Additionally, we aim to measure many different variables in each animal, thereby reducing the numbers used. Typically, after a nervous system injury (performed under anaesthesia) a therapy will be administered, and simple tasks (e.g. griping a bar and reaching for sugar cubes) will be used to assess any improvements in function. At the end of the study, tissue will be used to obtain molecular and anatomical data from the same animal. **A retrospective assessment of reduction will**

	be due by by 09 October 2024 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?
3. 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.	Rats will be used for the majority of studies as they closely mimic the pathology of human nervous system injury. Mice will be used in some instances however, specifically when the use of a particular gene can reveal valuable information. these species represent the lowest sentient mammalian species, and a great deal is known about their anatomy, neurophysiology, genetics and behaviour. The models we will use will either be discrete injuries of nerve fibre pathways to study how they respond to injury and regenerative therapies. Or, we will use clinically-relevant models which closely mimic the pathology, disease progression and functional readouts observed in human patients. In these cases, we can test promising therapies in these valuable pre-clinical models as a first step towards translating a therapy to the clinic. We have experience of all the techniques detailed in this project. All animals are subject to regular inspections by the scientists, NACWO and veterinary surgeon and mild health problems are dealt with accordingly. In the event of any unexpected adverse reaction during experiments the animal will be humanely killed. **A retrospective assessment of refinement will be due by 09 October 2024** 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?

Project	**3. New treatment strategies for myocardial infarction and aortic aneurysm**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Blood vessel obstruction is commonly caused by Atherosclerosis - a ‘furring up’ of the inside of a vessel. This leads to heart attacks and loss of heart muscle, when blood flow is interrupted in the arteries supplying blood to the heart, and to strokes when blood flow to the brain is interrupted. Alternatively, weakness of the artery wall leads to a ‘ballooning’ of the vessel, also known as an aortic aneurysm: devastating if this tears or bursts. REDACTED is an example of this kind of disease.	

	Under this llicence we will: 1. Determine the origins of the cells that repair the heart following heart attack and how these cells are activated and switched on when needed. 2. Establish whether stem cell-derived cells can be used to regenerate the damaged heart muscle following a heart attack. 3. Identify the signals that trigger aneurysms in large blood vessels using human stem cells, test whether the same signals cause aneurysms in animal models of these conditions and develop new treatments for aortic aneurysm. **A retrospective assessment of these aims will be due by 28 July 2024** 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?
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	1. Understanding how heart and blood vessels develop and their responses to injury and disease – new scientific knowledge. 2. Developing a way to regenerate injured heart muscle after a heart attack, using stem cells. 3. Identifying new treatments for aortic aneurysms.
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 8600 mice and 650 rats over 5 years.

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?	For objective 1, genetically modified mice in which we can track heart cells will be used to identify which cells contribute to repair after a surgically induced heart attack and what genes may control this repair process. Some animals will be treated with medication to see if we can alter the repair response. For objective 2, we will try and regenerate the damage done to the heart after a surgically induced heart attack by treating rats or mice with stem cells aiming to restore heart function. For objective 3, we will use mice that develop aortic aneurysms either due to a mutation in their genes or caused surgically or by a drug treatment. The work will identify exactly how aneurysms develop and new treatments to prevent this. The majority of animals on this licence will be under mild or moderate protocols, and suffer minimal adverse effects. However, to test and develop new treatments for heart attacks and aortic aneurysms, that could one day be used in patients, we need to use some animals that also suffer a heart attack or aortic aneurysm. These are serious conditions and frequently lead to death in patients, so these animal protocols are severe in category. However, death usually occurs suddenly with only transient suffering. If the animals are suffering, they will be given suitable treatment and if this does not alleviate the suffering promptly, they will be killed humanely. All animals will be killed humanely at the end of the studies. The enormous burden and severity of heart attacks and aneurysms in patients warrants the use of severe category animal protocols in order to find new treatments. **A retrospective assessment of these predicted harms will be due by 28 July 2024** 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?

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	My group is a world leader in using human stem cells generated from patients to develop cell culture based models of human disease that can replace the use of animal models in some circumstances. Together with collaborators, we have replaced the use of a mouse with a genetic abnormality that predisposed to heart disease REDACTED with a human stem cell model instead. However, it is not possible to model many of the complexities of cardiovascular disease such as interactions of different cell types, immune response and blood flow in culture. Some aspects of disease including assessment of new treatments still require animal studies. Indeed it is usually not possible to take new treatments forward to patients without comprehensive animal studies. **A retrospective assessment of replacement will be due by 28 July 2024** 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?
2. Reduction Explain how you will assure the use of minimum numbers of animals	Using the human stem cell disease models that we are pioneering, many aspects of understanding disease mechanisms and testing new treatments can be carried out in cell culture so greatly reducing the number of animals required for final validation. As an example, we have developed a human stem cell model of REDACTED- a genetic abnormality which is passed down in families and results in REDACTED. Using just patient derived stem cells, we identified a new disease causing signal, and published the results in a prestigious scientific journal REDACTED with no animal usage at all. Testing of new treatments and final validation of new mechanisms does however require animal models, although these are minimised by the extensive cell culture work already carried out.

	Similarly, we are generating heart cells from human stem cells and making engineered heart tissues from these in culture. We can test many combinations of cells and materials in culture this way in order to optimise how we regenerate damaged hearts. This strategy of testing engineered heart tissues in culture will again reduce the number of animals finally used in the definitive tests of this approach. In addition we also aim to use noninvasive imaging such as ultrasound (recently purchased for £300,000) that can be used repeatedly in the same animal with minimum discomfort, so needing fewer animals to obtain information from multiple time points. **A retrospective assessment of reduction will be due by by 28 July 2024** 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?
3. 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.	Animals are housed according to the best recommendations in an appropriate and enriched environment. We collaborate extensively with experts including those in the USA to obtain the benefit of their experience in refining the protocols; so we minimise the effects on the mice and rats and subsequently pain, distress and suffering. **A retrospective assessment of refinement will be due by by 28 July 2024** 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?

Project	**4. Novel therapies for pancreatic cancer**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
What's the aim of this project?	To evaluate the pharmacology and antitumour activity of experimental therapies (alone or in combination) in mouse models of cancer, focussing on pancreatic cancer. **A retrospective assessment of these aims will be due by 28 May 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?	

Why is it important to undertake this work?	Less than 1 in 20 people with pancreatic cancer survive more than 5 years, and the majority live less than 12 months from diagnosis, because currently available treatments are largely ineffective. Thus there is a need to (a) understand the underlying reasons why pancreatic cancer does not respond to therapies that are effective in some other cancer types and (b) to develop and test new therapies for treatment of pancreatic cancer. Before novel therapy can be tested in patients we need to identify the best way to administer that therapy (e.g. how often to treat, for how long, and at what dose level, and in what schedule when combined with other therapy), and identify the best ways to measure the effect of the therapy. Ultimately, we need to generate evidence that it works in the most relevant cancer model systems (i.e. mouse models of pancreatic cancer) without being too toxic, before planning clinical trials.
What outputs do you think you will see at the end of this project?	The outputs will be data demonstrating the effectiveness of novel anticancer therapies, identification of which particular tumours (with specific genetic features) are most likely to respond, and identification of the best biomarkers that can subsequently be used in clinical trials to measure response. It will also generate new information on the underlying biology of pancreatic cancer. These data will lead to scientific publications and may generate Intellectual Property. These data will enable Go/No Go decisions on whether to progress the therapy into clinical trials in patients. In addition, the data will aid design of the dosing regimens for clinical trial protocols.
Who or what will benefit from these outputs, and how?	In the short term we anticipate scientific publications to arise from these studies, building a package of data to justify translation of the best treatments into the clinic. There will also be benefit from identifying those treatments that are not effective or have a poor therapeutic index, avoiding future patients from being exposed to non-beneficial, harmful, treatment. In the medium term (2 – 5 years) we anticipate some of our therapies to be made available to patients on phase 1 clinical trials. Longer term, these animal studies may contribute to patient benefit by identifying more effective cancer treatments.
Will this work be offered as a service to others?	No
How will you look to maximise the outputs of this work?	The proposed studies require collaboration with pharmaceutical/biotech companies who are developing the drugs we wish to test, and we have long-standing and productive collaborations that will continue to give us access to the best therapeutic agents that are commercially viable (and therefore developable). Most of the companies will not have pancreatic cancer as their primary tumour of

	interest for their drug(s) and so it is mutually beneficial for us to test their drugs in our pancreatic cancer models. We will have access to their scientific expertise and unpublished data regarding the agent and the best ways to measure its effects. We will continue our existing multi-centre collaborations: REDACTED which is coordinating large scale efforts to make precision medicine a reality for patients with pancreatic cancer across the UK, by molecular phenotyping of each tumour to enable matching of patients to appropriate clinical trials – our preclinical mouse study data will feed into the portfolio of therapies REDACTED. We are also leveraging novel technology for maximising drug delivery via our multi-disciplinary, multi-Institution, collaboration REDACTED. We will disseminate new knowledge via publication. We would like to publish unsuccessful approaches if there were an appropriate route, as long as the data were not restricted as part of a commercial agreement.
Explain why you are using these types of animals and your choice of life stages.	The majority of animal models of cancer have been developed in mice, and there is a longstanding literature on cancer biology and pharmacology based on mice, which provides the background to our studies. Studies of the effects of drugs on the body and on the whole tumour in its complex microenvironment have to be performed in live animals. Studies on pancreatic cancer require adult mice in order to match the biology of the human disease. We will use some mice that are genetically modified to develop spontaneous pancreatic cancer. We will also use some mice as recipients for implanted tumours which are genetically modified, to either alter a natural gene of interest or to express a “marker” gene that enables detection of certain cell types.
Typically, what will be done to an animal used in your project?	Approximately 70% of the mice will be used in breeding, producing mice with the correct genes for use in experiments. The majority of the experimental mice (the other 30%) will either develop a pancreatic tumour or be implanted with a tumour (or occasionally 2 tumours). The most common site of implantation of tumours will be under the skin, with a very small number of mice having a tumour implanted surgically into the pancreas. Most of the mice on experiments will be treated for a period of up to several weeks with one or more anticancer drugs and/or radiotherapy, and a small number of mice may have the tumour under the skin removed surgically. Drugs may be administered either by mouth or by

	injection. Internal pancreas tumours will typically be monitored by ultrasound imaging under anaesthesia weekly. Typical duration of study for an individual mouse, from the time of tumour implantation, would be 3 to 6 weeks. The mice will be killed humanely at the end of the experiments, with blood and tissues taken for subsequent analysis to provide data on the pharmacological effects of the drugs.
What are the expected impacts and/or adverse effects for the animals during your project?	The majority of experimental mice will experience transient discomfort from drug dosing. Many will undergo short (<30 minute) periods of general anaesthesia (e.g. for imaging or surgical tumour implantation or radiotherapy). They may experience illness due to the presence of cancer (particularly those with pancreatic tumours), which may include weight loss, jaundice, build-up of fluid in the abdomen. They may also experience some adverse side effects from their anticancer drugs or radiotherapy (usually seen as weight loss, diarrhoea, and/or reduction in their normal activity levels).
What are the expected severities and the proportion of animals in each category (per animal type)?	Overall, 56% of the mice are expected to be subthreshold, with 17% experiencing mild severity. 27% are expected to experience moderate severity, with no more than 2% severe. **A retrospective assessment of these predicted harms will be due by 28 May 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?
What will happen to animals at the end of this project?	killed
Why do you need to use animals to achieve the aim of your project?	We are developing drug therapy for cancer. By definition, pharmacokinetics (what the body does to the drug) and pharmacodynamics (what the drug does to the body) require the use of live organisms (animals or man). The Regulatory Authorities (FDA, MHRA, EMEA) will not allow agents to be tested in man until they have been shown to be safe and effective in animals. In addition, a large part of our research program involves exploiting the immune system to aid in anticancer therapy, and this requires the whole organism.
Which non-animal alternatives did you consider for use in this project?	We do perform many experiments in cancer cells grown in culture dishes, to screen different drugs and combinations to identify those most likely to be effective in mouse/man, and to investigate the drug mechanisms. We also use

	more complex mixed cultures– mixing cancer cells with other cell types that in the body may support the growth and drug resistance of tumours.
Why were they not suitable?	We do use cancer cell cultures and complex mixed cultures, but once we have identified the best therapy options we have to test them in the context of the whole animal. It is very easy to kill cancer cells in a dish, but we have to identify those therapies that will not also be toxic to the normal body. **A retrospective assessment of replacement will be due by 28 May 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?
Enter the estimated number of animals of each type used in this project.	mice: 23,355
How have you estimated the numbers of animals you will use?	For each study that we will perform, the number of mice per group will be determined by power calculations using knowledge of the effect size expected or desired, using data on variability in drug response, tumour growth, etc. In previous studies n=10 per group has typically sufficed for monitoring antitumour effect of agents in most xenograft models, but it depends on the variability in each model. Pilot studies are usually performed to characterise growth rate and variability of new models, to inform subsequent studies. For Pharmacokinetic (PK) studies (where we measure drug uptake and what the body does to the drug), 3 mice per timepoint usually suffices, but more may be required for Pharmacodynamic (PD) studies (where we measure what the drug does to the body), when biomarker expression is variable. In studies identifying the maximum tolerated dose (MTD) only 2 animals are used per group for initial dose-finding, to expose the minimum number of mice to potential substantial toxicity, expanding to a larger cohort (typically n=5) to confirm the tolerated dose. We have used these estimates of a typical number of mice per study, multiplied by the maximum number of each type of study we expect to perform over the 5 year period, to estimate the total numbers of animals to be used for the

	experimental studies in this programme of work (Protocols 3 to 8: 5,855 mice). We have then calculated the expected number of mice on breeding protocols 1 and 2 likely to be used to provide the required numbers of experimental mice, based on our previous 2 years' data on breeding these same mouse lines for the same purposes (total 17,500).
What steps did you take during the experimental design phase to reduce the number of animals being used in this project?	Because the speed of pancreatic tumour development in the genetically engineered mouse model can be variable, imaging (e.g. ultrasound scanning or MRI) allows recruitment of animals with pancreas tumours of a particular size, reducing variability and the number of animals per group. Imaging also permits measurement of certain features (such as pancreatic tumour size) at multiple different times in a single mouse, reducing the numbers required. Microsampling (taking tiny volumes) of blood permits multiple samples to be taken from each mouse for pharmacokinetic (PK, drug uptake), so that measurements of drug can be made at multiple times, reducing the number of mice required. For complex transgenic mouse lines with multiple genetic alterations (alleles), as many alleles as possible will be carried homozygously - with both gene copies the same, so that each offspring definitely inherits the genetic alteration. This reduces the breeding required to generate sufficient offspring carrying all the required alleles for completion of the goals. The tumour microenvironment is the environment around the tumour cells, including blood vessels, immune cells, fibroblasts, signalling molecules and biological matrix, with which the tumour cells interact constantly, and which plays a key role in therapeutic response of tumours. Characterisation of the microenvironment of tumours implanted under the skin (using either cell lines or tumour fragments), in genetically matched mice with complete immune system, has identified that these are indistinguishable from KPC tumours, and are suitable alternatives to KPC mice for many studies, so reduced numbers of KPC mice will be needed, with reduced breeding.
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 to characterise growth rate and variability of new tumour models, to inform subsequent studies. A Bioinformatics/statistics core facility will provide advice on the power calculations, study design and statistical analysis. If new breeding colonies are established, we will calculate

	the numbers of mice required to produce the number of mice with the correct genotype for the studies, and check this against the estimate for that protocol. At annual review the total numbers used on each protocol will be reviewed in relation to the number permitted on the licence. **A retrospective assessment of reduction will be due by 28 May 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?
Which animal models and methods will you use during this project?	We will use only mice. Rodents are the species with the lowest neurological sensitivity likely to produce data predictive of the effect in man. Also, the majority of animal models of cancer have been developed in mice, and there is a longstanding literature on cancer biology and pharmacology based on mice, which provides the background to our studies. We will grow tumours in mice by a number of different routes – injecting cancer cells under the skin, into the pancreas, into a vein to simulate metastasis, or via genetic alteration to cause mice to develop spontaneous pancreatic cancer (the “KPC” mouse). We select the tumour models most similar (biologically, genetically and in drug response) to the human cancer. Where the science permits we will use tumours under the skin, as those mice will have fewer health issues than KPC mice, reducing the suffering of individual mice used for therapeutic studies. Some of the mice that are tumour recipients will be genetically altered to express certain markers for detection of specific cell types. Others may be genetically altered to modify a gene suspected of being involved in the response (or lack of response) of the tumour to certain therapy. Mice that are to receive human tumour cells will need to be strains with defective immune systems, to avoid rejection of the tumours. Tumour-bearing mice are required to investigate the activity of anti-cancer agents. We need to test individual agents and also combinations of multiple drugs, possibly combined with radiotherapy and/or surgical removal of the initial tumour, to mirror the treatment that patients could receive. Where appropriate, non-invasive imaging methods are used to monitor tumour growth, and tissue sampling is normally post-mortem, but occasionally biopsies are required from tumours under the skin, to obtain samples from more than one time-point in the same animal (e.g. pre- and post-treatment). Animal suffering will be minimised by the use

	of anaesthesia and analgesia where appropriate, and environmental enrichment will be provided to promote the expression of species-appropriate behaviour. Most mice on studies will be killed before they show any signs of illness because the scientific endpoint of the study is reached before the cancer is too advanced, but mice will be killed if they do develop signs of cancer-related illness or drug-related toxicity, to reduce pain, suffering, distress and lasting harm.
Why can't you use animals that are less sentient?	Rodents are the species with the lowest neurological sensitivity likely to produce data predictive of the effect in man. Also, the majority of animal models of cancer have been developed in mice, and there is a longstanding literature on cancer biology and pharmacology based on mice, which provides the background to our studies. These cancer models require adult animals.
How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?	Via Mailing lists for initiatives from the NC3Rs, information circulated locally by Home Office Liaison Officer and Named Animal Care and Welfare Officers, and information from local annual 3Rs symposia. Changes to practice would be considered and pilot studies performed to ensure any changes do not compromise the ongoing science. **A retrospective assessment of refinement will be due by 28 May 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?
How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?	We have been using these tumour models and most of the procedures for years already and so the methods are already refined. However, we will scrutinise new guidance as it comes available and will look to adopt new best practice when advances are published. For procedures we have not used before, such as surgical removal of the primary tumour, we will use pilot studies to develop the protocol and evaluate the best surgical method, post-op care and pain management protocols. As our understanding increases of the timescale for metastasis growth in our models we will refine our endpoints for those studies to avoid mice suffering from the presence of metastases.
What published best practice guidance will you follow to ensure experiments are	We minimise suffering by adhering to best practice guidance, currently the "NCRI Guidelines for the welfare and use of animals in cancer research" by P.Workman, et al., Br. J. Cancer (2010) 102, 1555-1577 .

conducted in the most refined way?	For surgical procedures: LASA 2017 Guiding Principles for Preparing for and Undertaking Aseptic Surgery. A report by the LASA Education, Training and Ethics section. (E Lilley and M. Berdoy eds.). http://www.lasa.co.uk/publications/ For recording and reporting on experiments: Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG (2010) Improving Bioscience Research Reporting: The ARRIVE Guidelines for Reporting Animal Research. PLOS Biol 8(6): e1000412. doi:10.1371/journal.pbio.1000412

Project	**5. Nuclear receptor regulation of circadian rhythms, metabolism and inflammation**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	We aim to establish the interplay between the body-clock and both energy metabolism and inflammation. Increasing evidence supports the view that energy metabolism and inflammation are fundamentally linked with the same cells (e.g. macrophages), and networks (e.g. glucose metabolism), central to both processes. Our recent work suggests that the body clock plays essential roles in both how the body handles nutrition, and also how the body protects itself against damage, or infection. We can use these insights to develop new treatments for common diseases, by targeting various proteins that can	

	respond to drugs, or drug-like molecules. We think that these are the links between the body clock, inflammation and energy metabolism, and that they may prove useful targets for new medicines in the future. **A retrospective assessment of these aims will be due by 20 June 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?
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The benefits arising from this work range from new discoveries into how the fundamental processes of energy metabolism and inflammation are regulated, and how they affect one another. We expect to find new control circuits linking these two super-systems and anticipate that some of the proteins we find that can respond to drugs (receptors) and circadian regulated networks will lie at the centre of these links. Further we will find new targets for drug intervention which we will seek to test in follow on experimental studies in humans, eg asthma. We also expect to improve our understanding of how circadian control systems vary the responses of the commonly used drugs targeting the nuclear receptors across the circadian cycle. This will allow us to find new ways to use the power of our body clock to aid treatment of multiple human diseases eg diabetes. Our results will help design new drug trials in people. As an example, we now have a human clinical trial in asthma, which is testing the importance of the time of day at which a commonly used medicine is given. We are testing if optimising the time of day may result in greater benefits from a common and cheap medicine, which will bring rapid benefit to patients.

What species and approximate numbers of animals do you expect to use over what period of time?	The projected duration of the project is five years. We aim to minimise animal usage throughout, and constantly seek new refinements to reduce, replace, and refine the animal studies. We will use mouse as our subject species, as this is the lowest sentient form suitable to model processes relevant to human health and disease; and also because we are able to manipulate gene expression easily in the mouse. We anticipate a maximum number of mice used to be 16,500, but most of these numbers relate to simple breeding of mice for killing in order to collect cells and tissues. Such mice will be bred, and maintained in group-housing conditions, with cage enrichment, and ad-libitum food and water. Their well-being will be monitored, and signs of illness, or distress acted upon.
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?	Much of the animal use should result in a very low level of severity limited to breeding, tissue biopsy and humane killing. Many fewer mice will experience greater severity. Typical procedures would include non-invasive monitoring of home cage activity and metabolism following altered diet. Some mice will have altered diet, which will either cause them to gain of lose weight. They will then have injections to challenge their metabolism. Some will also have measurements of their body composition, or imaging of their body organs under general anaesthesia. Some mice will have small devices surgically implanted beneath the skin, or in the abdominal cavity, in order to record temperature, or to deliver a substance over a prolonged period to time. Some will also have a pellet implanted beneath the skin to cause prolonged release of a compound. In addition some animals will have blood testing for glucose concentrations after a glucose challenge, or an injection of insulin. Some animals will have drugs administered by injection, or by oral administration (adding to food, or water, or by gavage). We will make very limited use of low

	temperature challenges to the mice, which will result in compensatory changes including altered behaviour, nest building, huddling in a group, and the animals will experience being cold. In one protocol animals will have an inflammatory, or immune reaction started by administering a substance which will mimic the effect of an infection. In this way the animal will experience an incraese in body temperature, increased need for sleep, loss of appetite, andloss of social interaction. For some of the measurements the animals will be singly housed, which will cause some distress, as mice are social animals. At all times animals will be closely monitored, and will be humanely killed if any animal welfare issues emerge. Experimental animals will be humanely killed by an approved method at the end of the studies. **A retrospective assessment of these predicted harms will be due by 20 June 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?
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	As far as possible we will use isolated cell and tissues from human donors or humanely killed animals. As mice and humans share great similarity at the DNA level, and share many of the same responses to challenges relating to the immune system, or to excess, or insufficient food the mouse is a good model for aspects of human health and disease. Indeed, elaboration of an inflammatory response, or the adaptation to altered nutrient exposure, or temperature require live animals, and the live mouse is a useful, and accurate model. The systems we study are not well-conserved in lower vertebrates, but where possible we do make use of cell line models, human subjects, and genetic studies in large human cohorts. **A retrospective assessment of replacement will be due by 20 June 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?
2. Reduction Explain how you will assure the use of minimum numbers of animals	We carry out careful experimental design, using our full-time computational biologist and statistician, to ensure clear primary objectives, and consideration of the power of the study. We ensure sufficient animals to reliably test the hypothesis, and avoid studies with excessive variance, or noise, which makes obtaining clear data difficult. We monitor all our genetically altered animal colonies to minmise the numbers of animals generated, and to maximise the efficient use of any animal that is bred. We cryopreserve sperm from GA mice that are not in active use, to prevent breeding of mice that are not essential for the scientific purpose. We have a full-time statistician in the group to help with experimental design, and we adopt best practice by avoiding bias, cage effects, and observer effects by use of masking of the scientists as far as possible, eg the scientist performing the in-vivo part will code the animals, so the scientist analysing samples is not aware of group membership until the code is broken after analysis is complete. **A retrospective assessment of reduction will be due by by 20 June 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?
3. 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 use mouse as the lowest sentience mammal on which we can perform the studies. Mouse also offers us ease of transgenic manipulation, surgical intervention to target specific structures, and abundant previous data in the public domain which we can use to minimise new experimental data acquisition. We monitor all experimental studies carefully,

	and ensure rapid euthanasia is used to handle any animal in distress. We have developed new ways to test inflammation in the mouse, eg by creating an aerosol of an inflammatory stimulant we are able to induce a mild inflammation in the mouse lung without the need to directly inject the mouse. For those mice used in breeding protocols for tissue and cell harvest the animals will not be tested in-vivo, and will not be disturbed before humane killing. Some mice (circa 10%) will have changes to body function and behaviour eg inflammation, altered feeding, and altered ambient temperature. Severity limits will include weight loss, and signs of distress. Some specific examples are included here:- For some of the interventions we will need to use surgery, for example to implant telemetry devices under the skin, which will be used to track variables such as temperature without the need to disturb the animal repeatedly. In the case of surgery we will use general anaesthesia (so the animal does not feel the surgery), sterile technique (to reduce the risk of wound infection), and post operative pain relief (in a similar way to post operative care for people). We will try to minimise animal numbers by performing longitudinal studies where possible. For examplewe can use mice transgenic of a luciferase gene to report gene activity by using photon capture technology. In this way we can track gene expression through time, over many days, to get very high quality, and high resolution data from a far smaller number of animals, and the experience of the individual animal is restricted to the breeding, implantation of the slow-release compound delivery pump, and exposure within the recording cage. Dietary manipulations may cause a weight gain, or weight loss. For this reason we will monitor body weight, and have limits beyond which we will not take the animals. High fat diet can cause issues with coat grooming, and so we will monitor for coat condition during such treatment, and have limits in the event that the animals are seen to suffer. Changes in ambient temperature cause the animals

	to change their behaviour, and to use energy to maintain body temperature. We will use temperature probes, which transmit, so we can track the animal response. We have limits for body temperature change, which will cause us to return the animal to regular temperature for recovery. We will also use infrared temperature capture to non-invasively monitor animal body temperature. Some animals will have an inflammatory challenge. This will typically be the lowest exposure needed to see a response. Animals will be carefully monitored, and we have limits in place to stop the experiment and to humanely kill the animal in the event that the limit is exceeded. Where possible we use a minimally invasive route for administration, such as generating an aerosol, which the animal can breathe in. This approach results in localised lung inflammation, without further disruption to the animal's routine care. **A retrospective assessment of refinement will be due by by 20 June 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?

Project	**6. Pathology of chronic inflammatory disorders**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	tissues (this aberrant behaviour of the host's immune system is termed auto-immunity). These cells accumulate in the joints (in RA) or in the glands (in SS) causing severe inflammation. The role of the microbiome (the bacterial flora which colonise our guts) in the development of these auto-immune diseases will be scrutinised. In addition, we plan to investigate and develop new drugs capable of correcting the abnormal behaviour of the immune system cells in order to switch off inflammation and achieve long-term cure. **A retrospective assessment of these aims will be due by 02 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?
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	As a model of long-lasting inflammatory diseases that we focus our research on, RA is one of the most disabling joint inflammations worldwide. It affects approximately 1% of adults, reduces quality of life, increases mortality and results in large medical costs. National-Audit-Office figures indicate that 45% of RA patients are of working age and within 1 year of suffering the disease 30% are unemployed. RA costs the NHS/society £560 million annually and £4.8 billion in work-related disability. Several new drugs provide good results in treatment of 60-70% of RA patients, still 30-40% of patients do not benefit from these drugs. By understanding the underlying causes of the abnormal behaviour of the immune system in long term inflammatory disease, we seek to: (1) Better utilize current drugs by giving the right drug to the right patient. For example, by confirming that a specific target protein or cell is involved in causing the disease in our animal models, and finding that this target is abnormally elevated/decreased in the patient; then this patient can benefit from a category of drugs that specifically target this protein or cell. This would provide: i) better care as it would avoid delay starting a more effective drug; ii) prevent unnecessary exposure to potentially toxic drugs and iii) avoid wasting NHS money on drugs which are not going to work. This work has the potential to benefit patient in the near future as it uses approved drugs which have been modified to target diseased tissue and decrease systemic damage. (2) Develop new drugs that will target specific cells and mechanisms contributing to the development of long lasting inflammation. (3) Explore the interaction of the gut bacteria with the immune system and its role in the development of disease. Establish the benefits of patient stratification for personalised medicine. Determine if the

	gut bacteria can be manipulated by drugs/compounds or “good” bacteria.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use around 5000 mice within 5 years.
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?	Most methods used in this project are expected to involve mild to moderate distress for the animals. Some studies involve induction of joint inflammation which will be associated with mild to moderate joint pain, redness and swelling. Only where joint pain and swelling has been induced to mimic RA are these disease manifestations expected to be severe e.g. causing difficulty for the animals to get access to food and water as normally presented. In such cases, special care will be provided. If the condition of animals deteriorates they are killed to avoid further suffering. Drugs that induce reversible loss of sensation (Anaesthetics) will be used where possible and relevant during the studies e.g. when examining animals with painful joints. Drugs that relieve pain (Analgesics) will be used whenever it’s possible. At the end of the protocols animals are killed and their joints, glands, and tissues of the defence (immune) system (lymph nodes, spleen, blood) are analysed under the microscope and biochemically to assess disease manifestations and confirm whether the treatment had any effects. **A retrospective assessment of these predicted harms will be due by 02 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?
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We utilise several in vitro models that in many cases replace in vivo experiments or in some cases reduce their size. For instance, we have recently developed an organ culture in order to tests compounds penetrance and efficacy in modulating complex structures which are found in tissue and would be otherwise lost in traditional cell culture. However, diseases are

	complex and involve a great number of interactions between cells leading to the disease manifestations that cannot be assembled in the test tube. We therefore use animals to look at how cells, tissues and gut bacteria work together, interact with each other in the whole organism to produce disease, and how they may be corrected to stop the progression of the disease. **A retrospective assessment of replacement will be due by 02 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?
2. Reduction Explain how you will assure the use of minimum numbers of animals	A range of in vitro testing using cells and tissues obtained from humans or animals will be set up first to define targets that may play a role in inflammation. This strategy makes a vital contribution towards minimizing animal usage. Careful optimization of models allows us to reduce variability and consequently allow smaller treatment groups. Statistical advice will be sought to ensure that the experiments are powered up in order to reduce chances of having to repeat them. Longitudinal monitoring (collecting sequential samples from the same animals) will reduce the number of animals necessary for a statistically meaningful cohort size. **A retrospective assessment of reduction will be due by by 02 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?
3. 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	Experiments on induction, progression, and management of inflammatory disorders require in vivo (in animal) studies where the immune responses can be assessed over a period of time. Mice are animals of choice because reagents and genetic variations needed to

minimise welfare costs (harms) to the animals.	analyse mechanisms of inflammation are available in mice. Every invasive procedure will be performed with the animal unconscious (under general anaesthesia) and painkillers (analgesics) will be given pre-emptively and afterwards to relieve pain and ensure minimal distress and discomfort is caused to the animal. New methodologies assessing disease development have been implemented, such as in vivo imaging for accurate measure of mechanisms involved in the disease. This reduces experiment size as animal are followed through the disease. We have expertise with multiple different models that vary in severity; we will be able to match the appropriate model to the question asked so to avoid excessive suffering of a severe model. In models where limbs are inflamed special care will be taken to minimise suffering such as soft litter and soft food and water gel. Pain relief will be provided whenever possible. **A retrospective assessment of refinement will be due by by 02 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?

Project	**7. Perivascular clearance in dementia and neurodegenerative diseases**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Neurodegenerative diseases such as dementia and Alzheimer's disease (AD) affects more than 800,000 people in Britain and 35 million people worldwide. Old age, genetic factors and having high levels of cholesterol are risk factors for the development of dementia, but it is still not understood why. One of the pathological characteristics of dementia is the build-up of toxic proteins (such as amyloid beta (Aβ)) that kill brain cells. This build-up occurs as a result of the failure of the brain's capacity to remove these proteins. Aβ and other proteins are normally removed from the brain by drainage along membranes that are present in the walls of blood	

	vessels, called basement membranes. Therefore, increased deposition of toxic proteins may result from changes in the health and/or structure of the basement membrane. The objectives of this project are to understand how Aβ and other proteins drain from the brain normally, how risk factors for neurodegenerative disease affect the basement membrane and Aβ removal, and to identify possible routes of improving the drainage of toxic waste as well as vaccination against toxic proteins such as Aβ accumulating in the brain. **A retrospective assessment of these aims will be due by 24 September 2024** 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?
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Old age is the strongest risk factor for the development of dementia. As the proportion of people over 60 years old is growing faster than any other age group, it is predicted that over 131 million people will be affected by dementia by 2050. Current therapies do not stop, reverse or even slow the disease progression. Understanding how Aβ and other peptides are removed from the brain under normal and pathological conditions is essential to understanding how dementia develops. The findings from this project will a) give a better understanding of how factors such as age and cholesterol affect the efficiency of solute clearance from the brain and b) identify possible routes of vaccination against toxic peptides such as Aβ accumulating in the brain. This will provide a new direction for effective preventative and therapeutic treatments for dementia to be developed.
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 3800 mice will be used over the 5 year period.

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 proposed research involves breeding mice under mild, moderate and severe categories that have been genetically modified to develop adverse effects of neurodegenerative disease. We will also test wildtype mice for the role of a modified diet in the development of neurodegenerative disease. Mice will be used to either investigate how the brain deals with removing waste, undergo vaccination regimes against the toxic proteins that accumulate in the brain in neurodegenerative disease or be assessed for behavioural abnormalities. We do not expect to have adverse effects in the mild breeding protocol. However, mice bred under moderate and
	severe breeding protocols have the potential to show adverse effects. These will be inspected carefully for and closely monitored daily. Animals exhibiting abnormal features that cause signs of suffering that is greater than minor and transient or any unexpected harmful features will be killed (Schedule 1). We have chosen to use some strains that are bred under a severe protocol, as the advantages related to their pathological features that replicate the disease outweigh the disadvantages related to their phenotype. In the experimental protocols the level of severity is not expected to exceed moderate. Mice being vaccinated will receive injections of substances via different well characterised routes, in the tail vein or intramuscular. These mice may display chronic low levels of discomfort. Mice receiving a stereotaxic injection will require surgery in which a small burr hole is drilled through the skull to expose the brain. The majority of mice will undergo this protocol under non-recovery so will feel no discomfort or pain. Some mice will be allowed to recover and are expected to show a transient higher level of discomfort. These mice will be administered pain control before and after surgery and will be monitored closely. Some behavioural tests may cause low levels of stress and anxiety. We will monitor these closely. After exposure to the water maze test, animals will be dried off before returning them to their respective cages. Mice will be closely monitored and will be removed immediately from the tank if they appear panicked or distressed. We have designed our experimental endpoints to ensure that animals will be used before developing any phenotype that would cause long lasting pain or suffering. At the end of the experiments, animals will be killed humanely. **A retrospective assessment of these predicted**

	harms will be due by 24 September 2024 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?
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The complex nature of the brain makes it difficult to study using non-living models. The rodent brain functions in many similar ways to that of the human brain. Many aspects of neurodegenerative disease can be accurately modelled using genetically altered rodents and can be used to test potential new treatments. **A retrospective assessment of replacement will be due by 24 September 2024** 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?
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals to be used in the project has been calculated by power analysis to provide the minimum number of mice sufficient to support robust statistical analysis by standard methods such as Analysis of Variance, Students t-test and linear regression. **A retrospective assessment of reduction will be due by by 24 September 2024** 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?
3. 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)	Mice are the most refined model organism of choice to study neurodegenerative diseases such as AD, because the structure and function of the rodent brain is similar to that of the human. Further, the mouse genome can be easily used to make genetic alterations that replicate features of human neurodegenerative disease. Unfortunately, the causes of neurodegenerative disease are complex and multifactorial and it is highly unlikely to find one

to the animals.	animal model that will represent the condition as a whole. We have therefore refined our use of animal models by carefully identifying models that display specific characteristics comparable to that seen in humans that we feel will enable us to generate clinically relevant data and achieve our objectives successfully. Rodents also breed easily, with a short generation time, facilitating multigenerational and ageing studies. Finally, protocols for rodent husbandry and health management are well established. To minimise suffering, all animals will be assessed daily for signs of distress or ill health. Vigilant monitoring will be done in animals following surgical procedures. Any animals showing signs of distress and/or pain will be killed by a Schedule 1 method. Handling will be minimised to routine husbandry and procedures required for the project. **A retrospective assessment of refinement will be due by by 24 September 2024** 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?

Project	**8. Preclinical testing of snakebite therapies**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	To protect the health of human patients, this project will use a mouse model to preclinically test the effectiveness and safety of antibody based anti-toxins and other toxin-neutralising therapies. This will help ensure that ineffective or toxic therapeutics are identified before their use in human patients **A retrospective assessment of these aims will be due by 01 May 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?
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The benefits of this project will be the approval for human use of treatments and other toxin-neutralising therapies demonstrated to be effective and safe in a proven animal model. The project will also establish the initial dose of therapies for human trials and assist product manufacturers meet regulatory requirements. The relevant pharmacopoeias stipulate that the effectiveness of each batch of manufactured product should be preclinically tested in mice before it is used in human patients. Thus, in line with regulatory and public health policy, outputs from our previous project licenses resulted in: 1) Provision 37,000 vials of treatments to help save the lives in West Africa. 2) Cessation of manufacture of a poorly efficacious product destined for sSA - before it reached market. 3) Validation of efficacy and stability of products to be used by the NHS and European countries for human treatment. It is important that human victims of toxins receive appropriate treatment: the inappropriate distribution in Ghana, Chad and Central African Republic of untested products resulted in an increase in mortality of treated patients from 0.5% to 12%. This example demonstrates that while the cost to mice of these preclinical tests are high, that benefit to humans is greater. The cost/benefit ratio of preclinical testing is therefore greatly skewed in favour of human health benefit.
What species and approximate numbers of animals do you expect to use over what period of time?	We anticipate the need for 1245 mice for 35 assays to test the toxicities of these natural toxins (7/year), and 1945 mice for 55 assays to test the toxin-neutralising efficacies of antibody-based treatments or inhibitory molecules (11/year) over 5 years. We anticipate requiring 120 mice for dose range-finding studies and 180 mice to determine the potential toxicity of the antibodies or inhibitory molecules.
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?	Toxins cause severe cardiovascular and neurological effects in mice. Humane end points will be used throughout for rapid implementation of schedule 1 killing. At the end of the experiment all animals will be killed using a schedule 1 method

	A retrospective assessment of these predicted harms will be due by 01 May 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?
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The mouse model of toxicity has proved a satisfactorily accurate representation of the multiple effects of toxin-induced pathology in humans. There is no *in vitro* alternative assay yet devised that can accurately predict the (i) toxicity of a venom and (ii) the efficacy of an antivenom or small molecule inhibitor. We continue to actively investigate methods which may provide accurate assessment of therapeutic efficacy *in vitro*. **A retrospective assessment of replacement will be due by 01 May 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?
2. Reduction Explain how you will assure the use of minimum numbers of animals	The literature and results of previous experiments is closely examined to reduce the range of toxin doses, and therefore the numbers of mice, needed to establish the statistical validity of the assays. To further minimise the numbers of mice required to achieve the objective, we use (i) preliminary range finding studies and (i) dose-staging methods to significantly reduce the numbers of mice required to accurately determine the lethality of the toxin/s and/or potency of the antibody or inhibitor therapy. Statistical analysis is performed on all the results, and the minimum number of mice required for statistical validity is used throughout. During this project we will continue our vigilance to identify methods that show promise in reducing the numbers of mice required for these

	assays. **A retrospective assessment of reduction will be due by by 01 May 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?
3. 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.	Mice are the physiologically least advanced rodent species that could be used for the preclinical assays. All the previous preclinical tests on antibody potency have been performed on mice. It would therefore be illogical to change the animal model species. The physiology of mice has been well characterised and the effects of toxin/s can therefore be accurately determined. The consistent use of mouse genetic strains (eg CD1) reduces independent variability and therefore (i) reduces the number of animals required for statistical validity and (ii) increases the validity of comparing results from different experiments. To refine protocols, we will: ● Undertake *in vitro* tests to reduce the number of candidate therapies requiring *in vivo* testing ● Undertake *in vitro* tests to reduce the number of candidate therapies requiring *in vivo* testing ● Undertake *in vitro* tests to reduce the number of candidate therapies requiring *in vivo* testing ● Undertake *in vitro* tests to reduce the number of candidate therapies requiring *in vivo* testing ● Undertake *in vitro* tests to reduce the number of candidate therapies requiring *in vivo* testing ● Use tests of the shortest possible duration ● Employ human endpoint observations at

	frequent intervals throughout the experiments • Maximally implement analgesia • Use range-finding and dose-staging protocols to reduce the numbers of use required • Use existing and develop new less-severe humane end points to reduce pain, harm and distress **A retrospective assessment of refinement will be due by by 01 May 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?

Project	**9. Production of Biological Samples**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
		Translational and applied research
	X	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The success of any regulatory or non-regulatory study is dependant on the use of equipment that can generate datasets accurately; And this is very much reliant on the equipment used being validated and calibrated appropriately. Indeed, this is a fundamental requirement of working to Good Laboratory Practice. The work undertaken within this project licence will allow us to validate the suitability of equipment before it is used on important, scientific studies, calibrate it before use thereby, ensuring it is capable of generating data that is accurate and can be trusted. Another aspect of work that will be served by	

	the performance of studies under this licence is the development of methodologies that can be utilised on in-vitro studies e.g. in-vitro assays. The primary objective of this licence is therefore, to obtain blood and/or urine samples from laboratory animals for the purpose of establishing and/or validating methodologies for use on *in-vitro* studies, or to validate/calibrate laboratory equipment which in turn will allow future regulatory studies to be performed under Good Laboratoy Practice standards. The animals used on these studies will have been acquired from other regulatory safety or efficacy studies, which for a variety of reasons, including studies being terminated early and animals were not used or, the animals were used, but euthanasia was not part of the objectives. On these occasions, providing that the animals are in good clinical health, have been checked by a Named Veterinary Surgeon and the project licence has adequate authority for re-use, these animals may be considered for transfer to this licence. Transferring these animals to this licence has the benefit of reducing the number of animals used. Indeed, it would not be ethical to euthanise animals unnecessarily, only to obtain more animals at a later date for use on studies performed on this licence.
Retrospective assessment	**Retrospective assessment** Published: 06 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 programme of work as defined by the objective of the licence were fully met. A number of *in-vitro* and validation type studies using very few animals were performed during the lifetime of this licence enabling valuable data to be generated that was subsequently

	used to support regulatory studies involved with safety assessments of pharmaceutical materials conducted to Good Laboratory Practice (GLP) standards. Regulatory studies are typically run to GLP standards and a prerequisite of this is any equipment used that generates data should be appropriately validated in order to provide assurances that it is capable of generating data accurately, reliably and consistently and of course, is suitable for purpose. Similarly, such equipment often needs to be calibrated before use. Where equipment is used to analyse biological products such as blood and urine, control samples of blood and urine will be needed. This licence allowed us to obtain blood samples for these purposes. Similarly, once equipment had been successfully validated, they would often have to be calibrated before use. Again, blood samples were required in order to calibrate. Some of the blood samples taken were used as control matrices to support pharmacokinetic studies (the analysis of chemical metabolism from the point of administration to the time it is completely eliminated from the body) and toxicokinetic analysis (the analysis of systemic exposure). Whilst this work may appear relatively simple, the benefits were extremely valuable; by taking a small volume of blood from a small number of animals during its 4 years lifespan (3 dogs and 46 rats), we were able to successfully perform hundreds of regulatory safety studies that would otherwise not have been possible.

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The potential benefits associated with the attainment of the objectives are as follows: 1. The biological samples collected will allow in-vitro studies to be performed and the data generated used to make an assessment of the bio-availability of a drug and an indication of its pharmacological effects before going into animals. 2. The validation and calibration of equipment and the establishment of methodology prior to use on regulatory and non-regulatory studies will allow data to be collected in a Good Laboratory Practice (GLP) compliant manner which is acceptable to international regulators.
What species and approximate numbers of animals do you expect to use over what period of time?	In the majority of cases biological samples will be taken from rats and mice. However, there will be occasions when samples will be taken from rabbits and the beagle dog. Numbers will be relatively low and will not exceed 400 mice, 800 rats, 50 rabbits and 100 dogs in a five year period.
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?	Taking blood from animals using established methods causes trainsient pain or discomfort only. No adverse effects are expected and the severity is Mild. The volumes taken will be within Internationally accepted Good Practice guidelines for each species and should not interfere with the welfare of the animals. Effectively, no adverse effects should be observed and the expected severity is Mild. On the occasions where large volumes are required i.e. may exceed Good Practice Guidelines then samples will be taken under General Anaesthesia and the animal will not be allowed to recover. Urine samples will be collected from rats and mice by putting them into a purpose built cage (metabolism cage) which allows urine to collect in a glass vessel. Urine samples will be collected from dogs by catheterisation. Both methods will be of Mild severity and will cause trainsient discomfort only. Once samples have been collected, the animals may be considered for future sampling, or for re-use on future regulatory studies. If however, there is no justification to re-use them or further sampling is not required then they will be humanely euthanised.
Retrospective assessment	**Retrospective assessment**

	Published: 06 November 2023 **What harms were caused to the animals, how severe were those harms and how many animals were affected?** The procedures performed on this project were limited to venous blood sampling on one occasion only. In total, 3 beagle dogs and 46 rats were used in the four years of the project. The blood collected was used to support in-vitro studies and the validation and calibration of laboratory equipment which was subsequently used on regulatory safety studies. The methods used were well established and caused no more than mild, transient discomfort during their performance with no lasting effects.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The primary purpose of this licence is to obtain biological products i.e. blood and urine to support *in-vitro* studies which require these products as part of validation, calibration or method development. At this time there are no suitable substitutes for blood or urine that are internationally accepted for these purposes and it is therefore, still a necessity to acquire biological fluids directly from animals.
Retrospective assessment	**Retrospective assessment** Published: 06 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?** Due to the objective of this project being solely to acquire biological products (blood or urine) from animals for the validation and/or calibration of laboratory equipment that would subsequently be used to analyse biological samples on regulatory safety studies, or to establish background data on a new strain of animals for example, it was not possible to consider the use of alternative methods.
2. Reduction	In the majority of cases, the quantity of biological product necessary to achieve the objectives of the study will be known. By using

Explain how you will assure the use of minimum numbers of animals	readily available literature on recommended blood sampling volumes and circulatory blood volumes for the selected species it will be a relatively straightforward process in determining how many animals will be required in order to acquire the volumes required. Where necessary, animals will be considered for re-use, thus reducing the numbers of animals used on this licence even further.
Retrospective assessment	**Retrospective assessment** Published: 06 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 number of animals used was kept as low as practicable by acquiring samples from euthanised animals wherever this did not interfere with the quality of the sample; indeed only 49 live animals were used in total in a 4 year period.
3. 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.	Regulatory and non-regulatory studies performed at the establishment typically involve the use of rats, mice, rabbits and where justified, beagle dogs. Where *in-vitro* studies are needed to support in-vivo studies then it is necessary to use samples from the same species. Samples will be taken using established techniques that have been refined to the extent of causing the animals transient discomfort only. Where objectives allow, samples will be taken under terminal anaesthesia thereby, minimising pain, suffering and distress. The procedures required on this licence are of Mild severity only and will cause only transient levels of pain, suffering and distress to the animals.
Retrospective assessment	**Retrospective assessment** Published: 06 November 2023 **With the knowledge you have now, could the choice of animals or models used have been improved at all? How did you minimise harm to animals during the**

	project? The species selected were based on the regulatory studies the project would support. The majority of regulatory safety testing is performed on rodents; primarily rats and mice and for this reason, the majority of animals used on this project was rats (46 animals). It is acknowledged however, that in specific instances there is sufficient, scientific justification to use the beagle dog and for this reason, 3 beagle dogs were used to acquire blood in order to support future studies performed under separate authority that would include this species. The procedures performed, which were limited to venous blood sampling on one occasion only were well established and have been refined over many years. Indeed, under separate authority we have the resources to invest time into method development of new techniques and the refinement of existing ones, including the refinement of blood and urine collection.

Project	**10. Promoting tissue regeneration by targeting endogenous stem and progenitor cells**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The overall aim of this project is to elucidate the key events in tissue healing to identify novel clinical therapeutics for improving regeneration of skeletal, cardiac, bone and liver by targeting stem and progenitor cells already present in the body. Adult stem cells are an essential component of tissue homeostasis and have been shown to play indispensable tissue renewal and repair following injury. The greatest success in harnessing the regenerative potential of stem cells thus far has been for haematological disorders, although further improvements in engraftment would lead significant clinical benefit. A similar approach	

	based on administration of exogenous stem and progenitor cells has not yielded meaningful improvements for solid organs. Even if strategies that rely on ex vivo expansion of the own individual patient stem cells were to be shown to be effective, this approach would be prohibitively expensive and unlikely to become widely available in the near future. Targeting the body's own stem cells overcomes the many hurdles associated with therapies reliant on the administration of cells grown up outside the body and should be more cost effective. **A retrospective assessment of these aims will be due by 09 March 2025** The PPL holder will be required to disclose: • Is there a plan for this work to continue under another licence? • Did the project achieve its aims and if not, why not?
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	By better understand how the body's own stem cells repair damaged tissues and organs, we will alleviate the suffering of patients affected by a myriad of conditions, including trauma, skeletal muscle disorders, liver disorders such as cirrhosis and myocardial infarction. At the moment, there is no approved therapeutics to promote regeneration of any of these.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mice, no more than 26000 over a 5 year period.

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?	The protocols in this PPL cover tissue regeneration studies in general but the purpose of each protocol is different since each protocol covers one organ (bone, muscle, cardiac, liver) and the likely/expected severity is different depending on the protocol. The majority of our protocols are of moderate severity. The liver and cardiac protocols are severe and we will ensure the welfare of all animals by monitoring them closely and addressing any issues promptly. Following cardiac surgery, we will monitor regularly in the 2-3 hours post-surgery, then at least three times daily for the first week and as twice daily thereafter. For the liver models the animals are expected to show weight loss but not any other visible adverse effects, as confirmed by experience in other centres that are using these models. The mice will be monitored closely and we will use a scoring system to record potential adverse effects. We minimise the suffering by adhering to LASA guidelines throughout, surgery is performed under aseptic conditions, analgesia is administered before painful procedures and they are checked regularly afterwards, with further analgesia administered as necessary. We liaise closely with the NAWCO/vets when necessary, and work with our collaborators, who are experts in the field, to obtain training in procedures that are not yet full-established in our lab. Welfare costs are minimised by using power calculations (statistical methodology) to limit the number of animals in each group. Animals will be killed at the end of each experiment by appropriate methods and further analysis will be done to achieve the highest level of information possible from each animal. **A retrospective assessment of these predicted harms will be due by 09 March 2025** The PPL holder will be required to disclose: • What harms were caused to the animals, how severe were those harms and how many animals were affected?
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	*In vitro* work will be used to test hypothesis prior to *in vivo* and therefore reduce the number of animals required in subsequent *in vivo* experiments. *Ex vivo* assays will further analyse the *in vivo* findings. We will rely on extensive *in silico* modelling as well as *in vitro* assays using murine and human cell lines before proceeding to *in vivo* mouse models. Bone repair, haematopoiesis, skeletal muscle, cardiac and liver regeneration can only be fully followed in animal models. The healing of these tissues involves complex interactions between numerous cell types and cytokines, many of which have not been fully identified yet, so studying the regeneration of these tissues is not reproducible *in vitro*. The mouse genome is well-studied and more reagents e.g. antibodies and genetic knockouts are available for biological investigation than for any other laboratory animal, including rats. **A retrospective assessment of replacement will be due by 09 March 2025** The PPL holder will be required to disclose: • What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?
2. Reduction Explain how you will assure the use of minimum numbers of animals	Efficient colony management ensures that only colonies that are actively being used are allowed to mate and produce offspring. Those that are no longer required are cryopreserved and breeding and maintenance discontinued at the earliest opportunity. We manage our active colonies efficiently. Before commencing a new study, we draw up an experimental protocol, covering our objectives/hypothesis, an experimental outline, detailing experimental treatments, group sizes,

	the experimental material to be collected and a detailed method of analysis. We routinely use good laboratory practice aimed at reducing bias, such as randomisation of treatment and blinded assessment of outcomes. The number of animals will be minimised by performing multiple assessments such as non-invasive *in vivo* scanning on the same animal whenever possible and by using the maximum relevant tissues from each animal. We derive maximal information from any experimental sample. All group members have received training in statistical methods and we have consulted statisticians on aspects of our experimental design. Where appropriate, power calculations are performed prior to the start of an experiment to establish the required group size. In most cases, a significance level of 5% and 80% power will be used. In a typical study, consisting of more than 2 experimental groups, multiple comparisons will be made using ANOVA and eventually multiple t-test comparison. **A retrospective assessment of reduction will be due by by 09 March 2025** The PPL holder will be required to disclose: • How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?
3. 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 mouse genome is well-studied and more reagents e.g. antibodies and genetic knockouts are available for biological investigation than for any other laboratory animal, including rats. The mouse is a relevant species for tissue regeneration/translational research. The main healing events are similar to those seen in humans. Our different regeneration models are well established, and they have been refined to the highest standard. We continuously look for further potential options of refinement. Furthermore, either our group is already proficient on them or we have established the necessary collaborations for the expertise knowledge and training required for the best refinement models. We refine the procedures by adhering to LASA guidelines throughout. Surgery will be performed

	under aseptic conditions, analgesia administered before painful procedures and animals will be checked regularly afterwards, with further analgesia administered as required. We liaise closely with the NAWCO/vets when necessary, and work with our collaborators, who are experts in the field, to obtain training in procedures that are not yet full-established in our lab. Animal numbers are minimised by using power calculations (statistical methodology) to limit the number of animals in each group. Animals will be killed at the end of each experiment by appropriate methods and further analysis will be done to obtain the highest level of information possible from each animal. We have already established a fracture model using a plastic bridging bar that allows serial CT scanning of each animal over time, thereby dramatically reducing animal numbers. The animals are able to ambulate freely after surgery. Our skeletal muscle injury model selectively causes the death of the muscle fibres, with minimal associated inflammation. The animals are able to weight bear immediately following recovery from anaesthesia and do not exhibit any signs of pain after 12 hours. **A retrospective assessment of refinement will be due by by 09 March 2025** The PPL holder will be required to disclose: • With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?

Project	**11. Provision of Biological Materials**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
	X	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The aim of this project is to provide blood and biological products (including organs, brains, lungs and kidneys) from a range of animal species (mice, rats, hamsters, rabbits, chickens, turkeys and dogs) to support scientific research and both diagnostic and regulatory work. This can include ensuring new medicines are safe before release for use and checking the calibration of diagnostic devices used in treatment of both humans and animals. To do this we provide a service to customers, which includes academia, Contract Research Organisations (CROs) and pharmaceutical companies. For whom we produce fresh bloods, plasmas and serums, after assessing	

	individual customer requests looking at the purpose of the work to be carried out by the customer and the benefits it may provide. By storing frozen plasma, serum and organs we can then ship them internationally to customers, giving a consistent timely service across different end users working on similar work. This allows researchers to purchase the specific product required as opposed to animals having to travel. **A retrospective assessment of these aims will be due by 04 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?
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The benefits from conducting this work are dependent on the research projects of the customers ordering: a large proportion of products produced under the previous licences supported regulatory work, which is required under government guidelines and ensures the safety of drugs. Other products go to support calibration of assays and equipment to ensure results from work conducted are validated and that drugs produced are free of viral contamination. Regulations which guide the choice of species selected by customers to perform this testing include: Food and Drug Administration, World Health Organisation and the ICH (International Council of Harmonisation). The data from the assays performed will be used in regulatory submissions to the appropriate regulatory authorities or is used to help form a picture of the potential of putative new drugs to be more efficacious with a better side effect profile than existing therapies in a wide variety of human and animal health indications. These data may not always be positive, and hence, some of these tests may prevent the further development of such entities, preventing the un-necessary use of animals in efficacy and regulatory testing prior to testing in human or animal clinical trials. The scientific benefits directly linked to this licence are dependent on the research projects of our customers; but under previous licences the tissues have contributed to the knowledge of disease

	processes in man, animals and food crops, understanding of the development of the immune system and its regulation, and extension of the knowledge of neurobiology and associated neurological disease. By offering the different products and species from one location we can give consistency across the samples, allowing direct comparisons in the end work performed, even if this is at different locations by different customers. We are able to reduce the movement of animals by shipping blood products to end users across Europe who would otherwise have to transport animals increased distances to produce products themselves. We also can take organs after the death of the animal (for example brains and lungs) and store these until needed. The customers we supply have a preference to outsource this work so they can benefit from the high levels of specific experience and knowledge we provide.
What species and approximate numbers of animals do you expect to use over what period of time?	Over the 5 year term of the licence we expect to use up to 160,000 mice, 60,000 rats, 6,250 rabbits, 1250 Hamsters, 170 dogs and 1,060 birds (chickens and turkeys). The majority of animals used will undergo non-recovery procedures (i.e. collection of blood or organs and tissues); carried out under terminal general anaesthesia. However, approximately 250 rabbits, 70 dogs and 160 birds will be used for the repetitive collection of small blood samples. Dogs, birds and a small proportion of the rabbits (5%) would have blood withdrawn from a superficial vein at approximately fortnightly intervals resulting in each animal having approximately 24 samples taken per year. Most rabbits would only have one blood sample taken.
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?	The majority of the animals under this licence will only undergo procedures under non-recovery anaesthetic; these animals will only experience mild discomfort from the administration of anaesthetic such as experienced by human patients undergoing surgery and from being held still as the anaesthetic is introduced. The only difference is that they will not awaken from the anaesthetic and will have death confirmed or be humanely killed at the end of the procedure. Anaesthetic will be introduced either by injection into the veins or by inhalation of gas.

	For rabbits and dogs sedation may be used beforehand which will reduce anxiety and reduce the need for longer periods of restraint. Chickens, turkeys, dogs and rabbits will be kept for repeat blood sampling, and will have approximately 2 blood samples taken a month. These are small volumes that are under 10% of blood circulating volume and will be collected from superficial veins, similar to human blood donations. These animals will only experience minimal restraint during the period of sampling and it is not expected to cause any adverse effects. Where appropriate topical local anaesthetic will be applied to the area before sampling. **A retrospective assessment of these predicted harms will be due by 04 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?
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The work performed, that uses products produced under this licence, is required under safety and regulatory guidelines; these include testing of drugs (both medical and veterinary) prior to their release to market, as well as ongoing calibration and quality checks of equipment and processes to ensure accuracy of the results that are published. Currently there are no methods to generate animal specific blood products (cells, plasma, serum) without the use of animals. **A retrospective assessment of replacement will be due by 04 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?
2. Reduction Explain how you will assure the use of minimum numbers of animals	By keeping dedicated colonies of dogs, rabbits and birds we are able to take small blood samples across a period of time from the same

	animals. This reduces the number of animals needed overall and provides a consistent product decreasing the need for retesting. By collecting blood under non-recovery anaesthetic we are able to collect a higher volume of blood per animal compared to collection after the death of the animal. This reduces the numbers of animals used overall. Our customer services department provides a central point to order blood and other biological products from, for a range of customers from small university groups to large contract research companies. This means we can collect different products (blood and organs, including brains, heart, liver and lungs) from the same animal and provide to multiple end users. This is frequently done with blood products from birds. All tissues are collected after death from all species. **A retrospective assessment of reduction will be due by by 04 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?
3. 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 choice of animal is determined by the customers' and regulatory requirements, with species such as dogs only used where non-rodent species are required and it is the best model, due to either the research being related to dogs, or due to the similarities in systems that they share with humans. The methods used for the collection of blood samples are based on guidelines of volume and frequency that will cause the least harm to the animals. The processing after collection is designed to get the highest quality and quantity of product so sample sizes can be kept as small as possible; we consider storage methods from across multiple fields including human transfusion services to ensure that we can maintain the quality of stored product. Dogs and birds kept for repeat blood sampling are held in group living conditions, with dogs having access to both inside and outside areas as part of their housing; all are assessed individually and both their behavioural and physiological condition is monitored throughout

	the time they are held for use in procedures. Rabbits are only kept as repeat donors if the end user requires it, for example we work with a customer who uses fresh rabbit blood cells in human medical diagnostic work and before using the cells from any rabbit they have to validate it in line with ISO 15189 (International standards for medical laboratories). By keeping a donor rabbit they can complete the validation once and then only take small volumes thereafter. For dogs, chickens and turkeys a peripheral vein such as the jugular vein in the neck is used for collection of blood samples, this is a superficial, easily accessible, larger vein which means the time the animal is held for the procedure can be kept to a minimum and adverse effects, even for larger samples are rarely seen. For rabbits the marginal ear vein or artery will be used, with the vein mostly used as the samples taken are small and the vein has less chance of bruising. This is an accessible blood vessel that means the rabbit can be held in a natural position for the duration of the sample. For all the animals used in repeat blood sampling procedures the sample time and experience of feeling is similar to a human blood donation or blood test performed medically. Dogs will only be used when the product is required for work that cannot be done without using dog specific materials, currently there is a requirement under EU legislation that drugs are tested in a non-rodent species before release into the medical and veterinary markets. Dogs are used in cases where they have similarities with humans in how they deal with the drugs at a cellular level. Worldwide legislation also requires the use of the same type of species specific plasma or serum product as the test species to support regulatory toxicology work, hence dogs must be used to supply blood products to support toxicology studies in these species. **A retrospective assessment of refinement will be due by by 04 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?

Project	**12. Provision of Biological Materials**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
	X	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	To supply blood and tissue from rhesus macaques for use in *ex-vivo* studies. In *ex-vivo* studies the experiments are carried out on the blood and tissue rather than the living animal. Rhesus macaques are primates and primate blood and tissue are required for three main purposes. These are firstly to investigate primate specific features, secondly to meet regulatory requirements and thirdly to use in health and welfare studies of the primates themselves. **A retrospective assessment of these aims will be due by 12 June 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?
What are the potential benefits likely to derive from this project (how	The blood and tissue taken under this licence is used for a variety of purposes. The main
science could be advanced or humans or animals could benefit from the project)?	use of the blood is for regulatory screening of products destined for human use such as the influenza vaccine. The blood is used to ensure that there is no contamination of the vaccine by other viruses and that the vaccine is safe for use. Other blood may be used to investigate signs of good and poor welfare in the macaques with the potential benefits of improved health and welfare of the macaques. Anatomical studies on brain tissue are used to advance our scientific knowledge of the primate brain.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use up to 120 rhesus macaques for blood and tissue collection under non-recovery anaesthesia over the five year period. These are animals that are being killed for health or colony management reasons. Approximately 40 male rhesus macaques will be used for collection of small volumes of blood under sedation or short-term anaesthesia.
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?	The risk of adverse effects to the animals used in this study is very low. The centre has collected blood from recovery animals over a number of years and has had few adverse effects The volume of blood collected is kept low to minimise the risk of anaemia and animals are checked over by a veterinary surgeon between blood samples. The expected severity level is mild for recovery animals. At the end of the procedure the non-recovery animals will be humanely killed and the recovery animals will be returned to their social groups. Some of the recovery animals will be reused on the same protocol but no more than six times per year with a minimum of one month between procedures. **A retrospective assessment of these predicted harms will be due by 12 June 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?
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Animal tissue and in this case primate tissue is required for a wide range of experiments that cannot be replaced with non-animal alternatives. These include toxicology and studies of brain function. **A retrospective assessment of replacement will be due by 12 June 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?

2. Reduction Explain how you will assure the use of minimum numbers of animals	As a breeding colony we have animals that come to the end of their natural breeding life or are unsuitable for breeding and need to be humanely killed for health, welfare or colony management reasons. By taking blood and tissue under non-recovery anaesthesia we minimise the need for production of additional animals to supply these products and we can also provide tissues from a single animal to multiple users. **A retrospective assessment of reduction will be due by by 12 June 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?
3. 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 unit's expertise is with rhesus macaques. The products provided by this licence are intended to support and assist the development of methods and experiments that require primate tissue. The animals remain in their breeding groups and are kept in large enclosures with high levels of enrichment. This gives them the opportunity to perform a wide range of natural behaviours including mating and foraging for food, They are cared for by highly experienced animal technicians. As the blood and tissues can be collected at the unit there is no need to transport the animals removing possibility of stress due to transportation. **A retrospective assessment of refinement will be due by by 12 June 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?

Project	**13. Regeneration in the nervous system**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	To identify how ageing and disease impair myelin regeneration in the central nervous system (CNS, which comprises the brain and the spinal cord), by adult stem cells and to develop new therapies to promote myelin regeneration. The fibres of nerve cells (axons) are wrapped in layers of fatty membrane (myelin) which protect them and allow them to transmit electrical impulses very rapidly. Myelin in the central nervous system (CNS – which comprises the brain and the spinal cord) is produced by cells called oligodendrocytes. Oligodendrocytes and myelin are lost in diseases such as multiple sclerosis (MS). If myelin is damaged (demyelination) and not restored, the nerve	

	fibres will not work properly and will eventually die. The loss of nerve fibres is irreversible, and its accumulation due to myelin regeneration failure accounts for the currently untreatable progressive phase of MS. Although stem cells in the brain are capable of making new oligodendrocytes that restore myelin, this regenerative process declines with age. Currently, there is no therapy promoting myelin regeneration. To meet this urgent unmet clinical need, it is necessary to know exactly what causes this failure and how to overcome it. **A retrospective assessment of these aims will be due by 14 July 2024** 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?
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The project will advance the understanding of 1) myelin regeneration in the nervous system, 2) the factors and conditions that influence this process, and 3) why myelin regeneration fails in diseases and ageing. Our discoveries may be harnessed to develop new regenerative therapies in the treatment of MS.
What species and approximate numbers of animals do you expect to use over what period of time?	This project will use rats and mice. We expect to use up to 13,600 mice (including breeding genetically altered mice) and 2,700 rats over a five-year period. The actual number of mice is expected to be lower than the above figure because some of the mice bred under breeding protocols will be used in the experimental protocols on this licence.
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?	We will predominantly use animal models that involve creating a very small area of injury in the CNS. Most of these models require surgical procedures, which usually take 30-60 minutes under anaesthesia. More than 90% of the animals will recover spontaneously without showing apparent deficits in normal functions. A small proportion (<10%) of rats undergoing brain injury may exhibit a significant loss in movement control and balance, which will be killed humanely. Some animals will be subjected to

	experimental interventions such as delivering drugs by injection or implantation of slow releasing minipumps, and imaging under a relatively long period of anaesthesia. These procedures likely lead to considerable discomfort, stress and even pain to the animals, indicated by signs such as reduced activity, subdued behaviour. Animals receiving certainsubstances (e.g. compounds to be tested) may suffer considerable weight loss due to toxicity and affected food and water intake. We will make sure the animals undergoing procedures to have adequate pain relief as standard, e.g. during and after surgery. If animals are identified suffering considerably from experimental procedures (reaching the established end points) indicated by e.g. loss of mobility, sharp reduction of body weight, signs of distress or pain not readily alleviated, they will be humanely killed. Some animals in this project will be subjected to calorie restriction or exercise or behavioural tests, which are expected to cause no more than temporary stress until the animals have acclimatised. **Certain strain related defects may develop in genetically modified animals such as malocclusion, the overgrown of misaligned front teeth in rodents which affects normal eating.** **Although these animals may be maintained healthy by trimming the overgrown teeth, we will only carry out the procedure if we cannot obtain healthy alternatives to minimise stress and incidence related to the procedure.** All animals used in the project will be killed under anaesthesia or humanely using another method and at end of studies, tissues are harvested for further analysis. **A retrospective assessment of these predicted harms will be due by 14 July 2024** 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?
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	CNS (brain and spinal cord) myelin regeneration is a complex biological process involving many interacting cell types locally and also affected by factors of whole body (e.g. from blood). At present, there is no non-animal substitute that can accurately replicate CNS regeneration. Although non-protected animal alternatives (non-vertebrates) may be useful for studying fundamental cellular processes, they do not exhibit the same level of complexity in the CNS, i.e. their nerve fibres do not have myelin sheaths. For this reason, mammals are the only option from which we can gather enough information to meet the objectives of the project. We therefore use mainly mice for our work and a lesser number of rats. There are some alternative techniques that can be considered as a partial substitute: using cultured cells isolated from animals; using human induced pluripotent stem cells (cells capable of generating any cell type in the body) and using synthetic fibres to replace axons. However, these techniques are still in their infancy and cannot completely substitute the use of animals. We will however, conscientiously explore the opportunity to develop and maximise the use of these techniques wherever possible. **A retrospective assessment of replacement will be due by 14 July 2024** 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?
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will ensure our experimental design is sound, and we aim to use the minimal number of animals that will give statistically meaningful data. Experimental groups will be randomly selected to consist of animals with same age, sex and strain. The number of animals in each group will be determined by a statistic principle based on existing data from similar studies or early phase so called 'pilot' experiments. In addition to randomisation, proper controls and sample

	sizes, we will avoid the introduction of bias that may otherwise influence interpretation of results by “a blind” approach, so that the researchers are hidden from the information and identities of the animals (e.g. control or treated) during the experiments until all the data have been obtained. Where possible, repeated non-invasive tests (e.g. imaging, behavioural tests) will be performed on the same animal to maximize the efficiency and robustness of data acquisition, reducing overall animal usage whilst simultaneously ensuring there is no increased harms to animals used. We will continue to use cell and tissue culture in our studies to obtain preliminary data before using animal models. This ensures only the most promising experiments are progressed and performed on animal models. Where possible, we will use human tissue and cells with appropriate ethical permissions in our work which will reduce the use of animals. We will ensure our materials, e.g. control tissues and data are shared with other researchers to eliminate the unnecessary repetition elsewhere of the same experiment using animals. **A retrospective assessment of reduction will be due by by 14 July 2024** 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?
3. 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 animal models proposed were chosen because of the minimal functional loss that they induce. Among the models available, we will always choose the one which is least harmful to the animals, gives the clearest results and is the most consistent. In conducting animal work, we will always ensure most appropriate handling and housing, minimising the impact of single housing of animals (which is at times scientifically

	necessary) and other sources of stress. We will take appropriate measures (pain relief and anaesthesia) to minimise pain in the animals undergoing procedures. We will limit the numbers of procedures on individual animals to prevent cumulative and unnecessary harm. Whenever feasible and scientifically appropriate, we will choose the least harmful procedures for our tests. **Non-specific developmental defects or adverse signs may occur in genetically altered mouse lines, such as retarded growth, or 'runt', and malocclusion, overgrowth of misaligned front teeth. We will always ensure proper selection of breeding animals to avoid the presence of such traits in the background based on their recorded health status in the 'family tree', so that the occurrence of the defects may be eliminated or minimised.** Before testing a new compound which not previously been used in animals we will perform small scale 'pilot' experiments with low numbers of animals to determine effective dosage and toxicity to minimise potential harm on larger scale studies. We will continue to improve the method of detecting the impact of adverse effects of procedures on animals, using a consistent, objective measurable way to record pain, stress and functional deficits e.g. utilising 'scoring' systems where appropriate. We will ensure adequate training of all researchers who carry out animal work and continue to develop effective measures to reduce pain and discomfort for animals under procedures such as incorporating pain relief agents into food supplements after surgeries as an alternative to giving by injection, and work with animal technicians to strengthen our monitoring system. **A retrospective assessment of refinement will be due by by 14 July 2024** 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?

Project	**14. Regulation of membrane transport in cardiac health and disease**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The heart is a pump that contracts and relaxes rhythmically from birth to grave. Contraction and relaxation is achieved simultaneously in all the cells of the heart as a result of the movement of ions across the surface membrane of a cell. Contraction expels blood and relaxation allows the heart to refill with blood ready for the next beat, so understanding how ions are moved and how this can go wrong is central to understanding how the heart works and diseases arise. We have discovered a new way in which this process of ion transport is controlled in cells of the heart. This is a	

	chemical change made to certain proteins that changes the way they work and where they go in a cell. All of the main routes for ions to enter and leave the cell undergo this new regulatory mechanism that we have found. This programme of work will investigate the role of this chemical change in controlling a protein highly relevant to cardiac function. The project will investigate what the chemical change does to this protein in the heart, and how this goes wrong in disease. It aims to determine the cellular control mechanisms and find out how the chemical changes control how proteins work in both healthy and diseased hearts.
Retrospective assessment	**Retrospective assessment** Published: 20 June 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?** This project achieved all its primary aims. The project was focussed on a transporter protein called NCX1 which moves tiny, charged particles called ions from inside to outside a cell. This controls the force of contraction in muscles such as the heart, making NCX1 an important protein that controls how the heart works. It has been known for a long time that drugs that stop NCX1 from working would be an effective way to treat the symptoms of heart failure, but none of the drugs that do this that have been identified to date can be used in the clinic because of unwanted side-effects. This investigation offered important new insight into the way that cells control NCX1 that are currently being leveraged to identify clinically useful NCX1 inhibitors. Consequently, an important outcome of this study is that we can realise the potential of many decades of NCX1 discovery science by leveraging our understanding of this protein to generate new drugs that stop it working. Ultimately this could

	enable the discovery of drugs that could lead to new treatments for chronically debilitating cardiac diseases that significantly reduce quality of life and represent an enormous burden on health services worldwide. In Key Objective 1, we investigated how an integral element of NCX1 called the XIP domain switches off NCX1. We found that whether XIP can switch NCX1 off or not depends on a small chemical change that the cell makes to NCX1 called palmitoylation. When NCX1 is not palmitoylated XIP cannot switch it off. If XIP can't turn NCX1 off then NCX1 moves more ions into a cell, which changes muscle contraction. We worked out exactly how XIP turns off NCX1. This knowledge enabled us to leverage further funding to identify new drugs that mimic the effect of XIP, which we predict will stop NCX1 from working and have clinical applications for treatment of heart failure, arrhythmias and heart attack. In Key Objective 2 we identified how cells control whether NCX1 gets palmitoylated. We found that the hormone insulin, which was linked to regulation of NCX1 many years ago, controls NCX1 activity through palmitoylation. The important implication of this finding is the link between cell metabolism (controlled by insulin) and palmitoylation of many proteins, which we are currently following up in other settings. In Key Objective 3 we investigated how NCX1 palmitoylation regulates force of contraction in the heart using a genetically modified mouse model in which the normal copy of the NCX1 gene was replaced by a modified form of NCX1 that cannot be palmitoylated. Surprisingly despite the clear effects of palmitoylation on NCX1 in cells, blocking NCX1 palmitoylation in this animal model did not alter force of contraction in the heart. Based on these results we concluded that in cardiac muscle from mice NCX1 palmitoylation makes a small contribution to regulating force of contraction. In Key Objective 4 we evaluated the role of NCX1 palmitoylation in the behaviour of the sino atrial node which controls how fast the heart beats. We implanted animals with

	telemetery devices to measure heart rate. We could find no difference in heart rate when NCX1 could not be palmitoylated in these animals, which led us to conclude that NCX1 palmitoylation does not control sino atrial node activity. In Key Objective 5 we investigated whether changes in NCX1 palmitoylation make a difference to the damage to the heart during a myocardial infarction (heart attack). We found that the amount of tissue that dies during an experimentally-induced heart attack was not altered in hearts in which NCX1 was not palmitoylated. We therefore rule out targeting NCX1 palmitoylation to treat the injury associated with heart attack. In Key Objective 6 we investigated the contribution of NCX1 palmitoylation to the development of heart failure. We found onset and development of heart failure was indistinguishable between hearts expressing 'normal' NCX1 and NCX1 that can't be palmitoylated. This led us to conclude that NCX1 palmitoylation does not make a difference to the early stages of disease progression. We also evaluated several other experimental models of heart failure, and most importantly assessed how much NCX1 is palmitoylated in cardiac biopsies from organ donors and heart failure patients. The overall picture of how much NCX1 is palmitoylated in heart failure is inconsistent: there is less palmitoylated NCX1 in the early stages of heart failure in animal models but significantly more palmitoylated NCX1 in the late 'severe' stage of the disease in humans. Our experiments do not identify a direct role for NCX1 palmitoylation in the evolution of heart failure. The important consequence of increased NCX1 palmitoylation in human heart failure is that NCX1 will retain its sensitivity to XIP, which we hope to exploit in a recently-funded drug discovery project.

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Movement of ions across cell membranes is essential for all sorts of processes in the cardiovascular system, and is known to go wrong in certain diseases. The research will make a fundamental difference to our understanding of how ion transport works, and may uncover new treatment targets in heart disease. Such treatments would reduce the socioeconomic burden of heart disease in the UK and reduce the morbidity/mortality of patients suffering from heart disease.
What species and approximate numbers of animals do you expect to use over what period of time?	Over a period of 3 years, it is expected that 600-1000 mice (WT and transgenic mice expressing 'targeted' genes encoding ion transporters) will be subject to regulated procedures (one or more of the regulated procedures detailed within this license). The research project requires a colony of mice with both copies of the ion transporter gene targeted to change its chemical properties. It is therefore estimated that up to 1500 mice may be required in order to produce/maintain the homozygous transgenic mouse colony throughout the duration of the project. The total number of mice to be used within this project is estimated at 1500, meanwhile it is estimated that 20 rats will also be used to complete the body of work (i.e. cell-related experiments). Rat hearts contain 10x more tissue than the hearts of mice therefore using 20 rats (instead of 200 hundred mice) within the project will keep animal numbers to an absolute minimum and comply with the N3CRs guidelines.
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?	Mice will be kept in a tightly controlled environment with excellent welfare conditions. The expected levels of severity vary throughout the project depending on the procedures the mice are subject to and vary from mild to moderate to severe. Mice may have telemetry devices implanted to monitor their vital signs e.g. heart rate and blood pressure throughout the course of the project which is deemed to be of a moderate severity. Additionally, these mice may undergo ligation of the aorta to induce hypertrophy of the left ventricle which may result in heart failure (although heart failure is unlikely to occur during the short time we are investigating these mice for post-ligation/banding (9 weeks) as heart failure

	usually occurs over a longer period of time). This procedure requires complex microsurgery and would be classed as a 'severe' in terms of the level of severity. Our expertise and care ensures that pain, suffering and unexpected deaths are minimised. The duration that the animals will be exposed to cardiac disease will be the minimum required to obtain sufficient data about the acute and chronic changes in cardiac structure and function. These mice will also undergo minimally/non-invasive imaging and/or pressure-volume loop analysis under general anaesthesia to determine the level of damage to and cardiac function of the heart and are expected to experience little adverse effects in response to either. The blood vessels of the mice may also be cannulated for the administration of substances or the withdrawal of blood, however, this would likely be at the very end of the protocol and would be done under terminal anaesthesia. Post-mortem, organ/tissue/cell assessment will be performed in order to establish changes to gene/protein expression that occur after disease. It is at this time that detailed structural and biochemical analysis will be conducted. At the end of the study, animals will be killed humanely by anaesthetic overdose or by cervical dislocation where death will be confirmed by severing of the femoral artery. Any animals which fall ill unexpectedly will be humanely killed immediately.
Retrospective assessment	**Retrospective assessment** Published: 20 June 2023 **What harms were caused to the animals, how severe were those harms and how many animals were affected?** The procedures used in this licence were described in four protocols: Protocol 1 - breeding and maintenance (severity category mild), Protocol 2 - heart failure with preserved ejection fraction (severity category severe), Protocol 3 - cardiac phenotyping (severity category moderate), Protocol 4 - organ / tissue / blood removal (severity category mild). Animals used under Protocol 1 experienced

	no harm. They were maintained in social housing with free access to food and water. Animals used under Protocol 2 were housed in single cages during the period on diet so that we could monitor their food and water intake. They experienced moderate impairment of their well-being associated with being kept in cages by themselves (but were always housed in close proximity to other animals). No animal was singly housed for more than 7 weeks. Animals were fed a high fat diet and blood pressure elevating agent (in the drinking water) to induce symptoms of heart failure. Animals gained weight and their blood pressure increased. Animals' wellbeing was monitored daily, and their blood pressure was monitored weekly. No animals displayed any signs of distress and no additional health complications arose as a result of the increase in body weight or increase in blood pressure. The classification of the severity limit of this protocol as severe was due to the danger that the high blood pressure induced by administering a drug in the drinking water can sometimes cause sudden death. This did not happen and no animals experienced severe pain, suffering or distress. These animals experienced up to 7 weeks of moderate impairment of their well-being associated with the obesity and high blood pressure they developed. Animals used under Protocol 3 experienced moderate discomfort, with one exception in which an animal was found dead the day after being dosed with a drug. This exceeded the severity limit for this protocol and was reported to the Home Office. We immediately stopped administering this drug and did not resume these experiments. Some animals were implanted with telemetry devices to monitor their blood pressure and heart rate, which meant they had a small transponder implanted under their skin. All animals were monitored daily after surgery, and none exhibited signs of discomfort or weight loss. Total: 904 animals experienced no harm under Protocols 1 & 4 (mild). 24 animals experienced moderate impairment of well-being under Protocol 2. 42 animals experienced short-term moderate pain under

	Protocol 3. 1 animal experienced severe discomfort under Protocol 3.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It is difficult to obtain viable human heart muscle including suitable non-diseased heart muscle for comparison purposes. There is considerable variation in age, medication and underlying pathology of any obtainable human tissue and there is the likelihood of progressive disease being present. It is also not possible to investigate the processes at well-defined time points after a single incidence of damage. Heart muscle cells do not divide, so cannot be grown in cell culture like other tissue types. It is simply scientifically invalid to use other types of cells grown in culture as models of cardiovascular disease. Cultured cells are usually unable to contract, don't resemble cells from the heart, and do not make connections with each other, so lack all of the structural features of a living beating heart. Substantial prior and continuing cell experiments will inform and limit the number of animal experiments required and where possible as much information from one animal will be obtained. Despite this, experiments involving cells present limitations in terms of the physiological information that can be derived. It is therefore necessary to appropriately acquire tissues from animals at times in order to gain a comprehensive understanding of the role of ion transporters in the heart and any other organs/tissue which may be physiologically relevant.
Retrospective assessment	**Retrospective assessment** Published: 20 June 2023 **What, if any, non-animal alternatives were used or explored after the project started, how effective were they and are there any lessons worth sharing with others?** The heart is unique in that it lacks the ability to

	regenerate itself by growing new cells, which also means that heart cells cannot be grown in cell culture. Therefore in order to have access to a source of contracting heart tissue to understand how heart muscle cells change during the onset of disease researchers must either obtain human tissue or work with animal models of cardiac disease. During this project we made contact with collaborators in the USA who provided us with diseased human tissue from a biobank which allowed us to 'validate' some of the changes we saw in our animal models of heart failure. However, some of the important scientific questions about how cardiac muscle works during the disease process that we aimed to answer needed us to work with living, beating hearts that are being exposed to changes in 'haemodynamic load' (in other words, experiencing changes in blood pressure during the onset of disease, just as happens in people). These cannot be obtained from a biobank, which contains frozen tissue that cannot be revived. Therefore we were unable to entirely replace animal models in this project.
2. Reduction Explain how you will assure the use of minimum numbers of animals	This lab has considerable expertise in minimising the number of animals required for experiments whilst ensuring the generation of robust data as evidenced by our publication track record. We will use advice from statisticians in our Institute where required. Sample sizes will be set from our knowledge of the literature, previously performed experiments and statistical analysis. Before we start any experiments we determine how many animals that experiment might need based on a ‘power analysis’. This takes into account the likely magnitude of the effect that will be seen in the experiment, and the likely variability within the experiment. Based on this, we can calculate the minimum number of animals that should be investigated.
Retrospective assessment	**Retrospective assessment** Published: 20 June 2023 **How did you minimise the number of animals used on your project and is there anything others can learn from your experience?**

	Our reduction measures proceeded as planned through the project. All studies were discussed with Named staff. The introduction of an 'Experimental Request Form' midway through the project for all animal studies in our institution enhanced oversight of the planned work. We constantly scrutinised the scientific literature to ensure we avoided duplication. We continually monitored experimental numbers to ensure we used the minimum number of animals to give statistically robust results. We completely redesigned the experimental model of heart failure that we used in the investigation following a publication in 2019. This refined model of heart failure did not rely on surgery (see 'refinement' section) and instead used substances delivered in the diet and drinking water to cause heart failure. Introducing this refinement meant that the healthy control animals in our heart failure study no longer needed to go through a 'sham' surgical procedure (as had been planned in the original experimental design). They could simply continue to receive normal diet and normal drinking water. This reduced the number of animals that experienced regulated procedures.
3. 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.	Less sentient species i.e. fish are not appropriate for the current body of research as it is not possible replicate the disease model we wish to investigate in such species nor is it possible to determine the cardiac phenotype of these animals with sufficient precision using the current technologies available i.e. PVL analysis. The mouse is therefore the least sentient species in which it is possible to replicate the disease model we seek to investigate while allowing us to conduct in depth cardiac phenotyping of this model. The mouse and rat are the lowest mammalian vertebrate group with which the scientific community have been able to fully characterise the alterations in cardiac structure (including ventricular remodelling) and function in disease models such as left ventricular hypertrophy. The models chosen closely resemble the pathological changes in human heart disease. There is no alternative to using these models, however, we will continue to utilise our current

	laboratory animal and organ/tissue/cell data to inform whether particular aspects of the project's severe procedures are required or whether the information we seek can be best obtained using protocols of lower severity. Wherever possible, experimental design will be 'longitudinal' (making several observations as disease develops in a single animal – for example using telemetry or echocardiography), rather than focussing on experimental endpoints that involve a terminal procedure (for example, the insertion of a pressure catheter into the left ventricle to measure cardiac function). We will constantly review the literature for ways to refine the severe disease models. Distress and suffering of any animal will be minimalised by conducting regular visits to the animals which have recently undergone surgery, completing post-operative monitoring forms, administering analgesia as and when appropriate and liaising with the vets (when necessary) to raise any concerns and to discuss the welfare of any animal which is demonstrating signs of distress and suffering.
Retrospective assessment	**Retrospective assessment** Published: 20 June 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?** Harm was minimised throughout the project by monitoring the health and wellbeing status of animals daily. The choice of the mouse as the animal used in this project could not have been improved because it is the least sentient species in which it is possible to undertake detailed cardiac phenotyping during the onset of heart failure while remaining as equivalent as possible to the human. A new experimental model of heart failure in mice caused by eating a high fat diet and administration of agent that increased blood pressure in drinking water was described in the scientific literature early in the project. We amended the project licence to eliminate the

	planned MTAB model of heart failure (original protocol 2, which is associated with a high risk of death during or just after surgery), and used this diet induced model instead. The elimination of the need for surgery for our model of heart failure was a significant refinement in this project.

Project	**15. Regulatory Testing of Biological Toxins & Antitoxins**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
		Translational and applied research
	X	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	To undertake testing procedures to ensure the safety, efficacy, stability and overall quality of toxins and associated proteins used for medicinal products in accordance to registered marketing authorisations held with national and international regulators and in accordance with Good Manufacturing Practice. To provide testing services to assist with product development, improvement and clinical trials associated with toxins and associated proteins. **A retrospective assessment of these aims will be due by 14 October 2024**	

	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?
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The work detailed in this project will allow the continued safe development, production and use of botulinum toxins and their derived products for the treatment of a wide range of medical conditions.
What species and approximate numbers of animals do you expect to use over what period of time?	Potency Assay for Biological Toxin: 500,000 mice Biological Toxin Antibody Assays: 10,000 mice Neutralisation Assay for Biological Toxins: 10,000 mice
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?	Occasionally mis-injection into the lumen of the intestine could potentially cause peritonitis and mis-injecting into the subcutaneous tissue can cause abscesses although the latter has never been seen in practice. Occasional injection into the bladder can occur. Careful injection by experienced technicians reduces these risks and any animal suspected of being mis-injected will be killed by a schedule 1 method. All animals except possibly those in the very low dose groups will show typical signs of the toxins to some degree; this includes difficulty with breathing (wasp waisting, deep gasping or abdominal breathing), cyanosis, ataxia, lethargy, ruffled coats, an inability to move and some limb paralysis. Some animals will recover from these signs over the course of the test so all need to be kept alive until they are showing severe clinical signs of toxicity at which point they are killed by a schedule 1 method. Despite frequent observations (hourly) some animals may die due to the potential rapid onset of the symptoms. **A retrospective assessment of these predicted harms will be due by 14 October 2024** 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?

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The mode of action of toxins is complex; this cannot be fully replicated in cell culture or other in-vitro techniques and currently requires an animal model. For example the neurotoxins are proteins that have similar molecular structure and molecular weights. They are usually associated with non-toxic protein both naturally and in-vitro. They have a di-chain structure consisting of a light chain which is the toxic portion of the molecule and the heavy chain which is responsible for targeting the cholinergic neurones. These neurotoxins act presynaptically by blocking the release of the neurotransmitter, acetylcholine, at the neuromuscular junction. Even with the development of non-animal assays there is still an ongoing need for the mouse potency assay. This is true for manufacturers who have yet to successfully developed an alternative as a replacement, for high potency products (where alternatives may not be sensitive enough) and as a "back up" to ensure product availability due to non-animal assay failure. There may also be a requirement to use the mouse assay for the qualification of reference standards and the comparability of both assays. Some manufacturers have developed toxin specific cell culture assays but these methods are not always available to other manufacturers and it may not be possible to validate other toxins, even those of the same serotype. **A retrospective assessment of replacement will be due by 14 October 2024** 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?
2. Reduction Explain how you will assure the use of minimum numbers of animals	The animal numbers required for the potency assay has been reduced as experience has been gained at closely targeting the expected values. Careful design of the assays using a geometric progression of dilutions that results in a symmetric design about the known estimated potency ensures a robust assay with

	maximum precision from the number of mice used in the assay designed to be appropriate to meet the regulatory requirements to safely control the production and release of the product. The number of animals currently required per annum is based on the test history of these assays at this facility. The majority of samples received for potency assay are determined by the Routine Quality Control (QC) Assay. **A retrospective assessment of reduction will be due by by 14 October 2024** 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?
3. 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.	Historically the majority of the work undertaken on biological toxins has been with mice as the animal model being the lowest neurophysiological model considered appropriate. The mouse lethality assay for the biological toxin test requires death as an end point; however, suffering can be reduced by killing, using a Schedule 1 method, any animals that it is predicted will die during the course of the test. Mice are observed at regular and frequent intervals, those showing severe symptoms will be killed. Approximate proportions of animals experiencing mild, moderate and severe severity are 7%, 32% and 61% respectively. However regular observations ensure that approximately 90% of mice are humanely killed before death from the effects of the toxin. The assay has also be refined for new clients in that wet mash is supplied to all animals. This allows for easier access to food and water and provides a more palatable system for animals that are affected by the toxin. It is not possible for all current tests as the benefit is outweighed by the number of animals required for comparative studies to introduce into validated assays. **A retrospective assessment of refinement will be due by by 14 October 2024** 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?

Project	**16. Remodelling and reverse remodelling in heart failure**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Heart failure is a massive health issue in the UK and the world. It is characterised by a degeneration of the heart known as myocardial remodelling. This project aims at investigating myocardial remodelling and studying new approaches that can lead to reversal of this phenomenon and ultimately to treatment. New therapies for heart failure that include manipulation of mechanical forces applied to the heart muscle., gene and cell therapy have given promising early results in reversing remodelling, but are still insufficient to induce significant changes. Left ventricular assist devices (LVAD) are mechanical pumps that are implanted in	

	patients to unload the heart from the excessive work load due to heart failure. This therapy induces improvement in the structure and function of the heart muscle in heart failure, yet the rate of clinical recovery in patients remains low. With this project, we aim to define additional combination therapies, including drugs, gene and cell therapies that may lead to an improvement in the rate of clinical recovery obtained with LVADs. The project utilises animal models of disease to induce heart failure (by obstructing blood vessels of the heart in rats and mice) and animal models of mechanical unloading to simulate the LVAD action (by transplanting the failing heart in the abdomen of another animal). By unloading the failing hearts, we recreate the clinical scenario of heart failure patients treated with LVADs. The animals are then treated with pharmacological, gene or cell therapies, or the animals carry genetic mutations, so that we are able to identify therapeutic targets or strategies that may be beneficial in patients. Given the complexity of the clinical condition and the interactions occurring in the patient, living animals are necessary and in vitro approaches are not suitable. Extreme care is taken to minimise suffering and to use the minimum number of animals. This is achieved by maximising the use of each single animal to produce samples from several experiments. In addition, the use of animal with the same genes abolishes the risk that the body rejects the transplanted heart avoiding the use of toxic drugs employed to avoid this. The reason why rats will be used in this project is that no other species with identical genes are available. The protocols involved are designed to produce models of disease and this would involve some degree of discomfort for the animals. However, we take extreme care to minimise this discomfort using painkillers and, in several years of experience with these procedures, we have noticed that the vast majority of treated animals only show signs of mild discomfort during the post-operative period (24 hours), with minimal problems thereafter. Performing the

	studies described in this project will achieve a number of important goals: 1) to determine the mechanisms underlying heart failure and its progression, 2) to assess a number of therapeutic approaches, including mechanical and pharmacological therapy, 3) to explore novel hypothesis and targets using gene manipulation technology, which is essential to open unexplored alternatives for treatment. Overall, this project will provide not only a profound advancement in the understanding of the mechanisms of heart failure and its treatment, but also indicate some useful strategies to manage thousands of patients suffering from heart failure and with poor prognosis. **A retrospective assessment of these aims will be due by 09 December 2024** 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?
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This project directly aims at understanding and testing new therapies for heart failure will potential to find a cure or alleviate symptoms for millions of people.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice (4900 in 5 years) Rats (3000 in 5 years) Rabbits (250 in 5 years
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?	Adverse effects are limited to normal complications occurring during surgery, such as bleeding and infections. Complications due to heart failure are possible and these include breathlessness, weight loss, and general deterioration. Given the large experience accumulated over several years of research, these effects occur at a low rate and are detected and dealt with very promptly. The expected level of severity is moderate because the animals will be culled humanely before the onset of significant clinical signs. The animals will be humanely killed at the end.

	A retrospective assessment of these predicted harms will be due by 09 December 2024 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?
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The syndrome of heart failure is a complex disease and no alternative, non-animal platforms exist. Our laboratory is pioneering the use of human in vitro models using stem cells, but these systems are still inadequate. **A retrospective assessment of replacement will be due by 09 December 2024** 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?
2. Reduction Explain how you will assure the use of minimum numbers of animals	Animals will be used in numbers sufficient to obtain meaningful results and calculated using well-established statistical techniques. Particular care will be taken to collect the maximum number of measurements from each animal and optimise the use of tissue obtained, in order to perform several series of experiments using the same group. **A retrospective assessment of reduction will be due by by 09 December 2024** 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?

3. 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 use rodents which provide solid and well characterised models of disease. The animals are housed in modern facilities, they are looked after by highly qualified and caring staff and are supported with professional veterinary advice at all times. Animals will be continuously monitored for pain and distress and, if these exceed acceptable, objectively-determined limits, they will be humanely sacrificed. We will use animals with the same genes to avoid the use of toxic drugs to prevent transplant rejection. This will also reduce the variability of the data helping reduce the number of animals. **A retrospective assessment of refinement will be due by by 09 December 2024** 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?

Project	**17. Repair and regeneration of cardiac and skeletal muscle**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project aims to understand the effects of stem/progenitor cells, exercise and ageing in the repair and regeneration of skeletal and cardiac muscle. As the majority of patients that will require reparative/regenerative therapies will be elderly, we are interested in ways to prevent or treat the effects of ageing to improve muscle repair and regeneration **A retrospective assessment of these aims will be due by 23 April 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?
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	My research is at the forefront of current molecular and cellular biology of stem/progenitor cells and muscle regeneration, and the findings are directly transferrable to the treatment of cardiovascular disease/failure, muscular dystrophy and age-related muscular diseases. The work has major societal, medical, technological and economic impact amenable to widespread use at a reasonable cost. My labs work has contributed towards the advancement of regenerative therapeutic cell approaches where the repair/regenerative mechanisms are activated in situ REDACTED. An effective therapy that could prevent a moderate proportion of patients from progressing to a chronic condition would lead to significant improvements in the social impact of the disease, and large reduction in healthcare costs. My research aims to address this unmet medical need.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice and rats will be used. Approx. 6500 will be used over 5 years. 62% of this number is for breeding and maintenance of genetically modified animals.
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?	Some animals will be given ligation of an artery in the heart, to simulate a heart attack. This is considered severe. All animals will be anaesthetised so the level of pain and suffering should be zero during this time. Animals will receive stem/progenitor cells and/or perform exercise training to repair their heart so they should show improved cardiac function. The exercise training which is expected to be of enjoyment to the animals, who prefer an active lifestyle rather than a sedentary one. Some animals will receive drugs which target aged and damaged cells. These drugs have been shown to prevent or delay tissue dysfunction, physical dysfunction and extend health- and lifespan. At the end of the protocol the animals will be killed in a humane way and their tissues will be collected for us to analyse and determine the efficiency and effectiveness of the repair and

	regeneration process. **A retrospective assessment of these predicted harms will be due by 23 April 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?
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Due to the types of procedure involved (i.e. damage to cardiac muscle due to ligation of a major artery) there is no alternative other than to use animals. Together with ethical constraints for removal of human heart tissue, this amount and sort of analysis cannot be obtained with human samples. We obtain human myocardial and skeletal muscle samples but these are small (~200mg) and limited to the atria and individual skeletal muscle. We use human cells to model ex vivo 3D culture systems and screen for relevant agents (i.e. senolytics) involved in cell survival and proliferation. However, due to the complexity of the interactions between different cell types present in each tissue and the body, we are unable to completely model what happens using cell culture or ex vivo systems. **A retrospective assessment of replacement will be due by 23 April 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?
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have undertaken statistical tests to estimate the number of animals needed to obtain significant results. We archive tissue samples. **A retrospective assessment of reduction will be due by by 23 April 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?
3. 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.	My lab strives for and is committed to a constant refinement in our protocols to improve the welfare of the animals. For example, we do not use electric shock grids to enforce exercise on animals. We encourage the animals to exercise by prodding with a paintbrush or tapping the side of the treadmill panel. We have invested in training in the microsurgical techniques and refinement of protocols and procedures. All personnel are trained and signed off as competent in each procedure. Animals are monitored closely during recovery from surgery to assure adequate analgesia. Our experiments are conducted under the supervision and advice of veterinary surgeons and named animal care and welfare officers. **A retrospective assessment of refinement will be due by 23 April 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?

Project	**18. Research into infectious fish disease**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	3 years and 3 months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals

Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	To better understand, diagnose, control and prevent fish diseases, thus improving production and welfare of farmed fish and protecting wild aquatic life. There are two main sub-objectives: a) To improve understanding of aquatic animal disease (host susceptibility, infectivity, and pathogenicity); b) To develop and apply methods examining the efficacy of substances for therapeutic and/or diagnostic use
Retrospective assessment	**Retrospective assessment** Published: 06 December 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?** This licence was a continuation of an extensive programme of work established in this laboratory in the 1970's and progressed through a series of similar licences. Through this the laboratory has gained an international reputation as a leading organisation in the diagnosis, treatment, and control of aquatic animal diseases. This expertise forms the basis of the UK governments response, both scientific and statutory, to control of aquatic animal disease in the farmed and wild environments. Aquaculture now accounts for more than half of total global food fish supply. Losses to infectious diseases are a major economic constraint and welfare problem and the contiguous nature of the aquatic environment means infections transfer easily between farmed and wild stocks. The two main elements underpinning the research need addressed by this licence are: the improvement of production and welfare of farmed fish and the protection of wild fish from disease spread, ultimately protecting biodiversity. There were two main sub-objectives: to improve understanding of fish disease (host susceptibility, infectivity, and pathogenicity); and to develop and apply methods examining the efficacy of substances for therapeutic and/or diagnostic use. Under PPL62762172 we have undertaken: two studies to assess the susceptibility of UK native species to

	spring viremia of carp virus (informing import policy for a disease controlled under national measures); five studies to assess the pathogenicity of bacteria associated with disease in cleaner fish (leading to the development and deployment of vaccines to improve welfare in these species increasingly used as biological controls for sea lice impacting cultured salmon); a study on amoebic infections in salmon (supporting the development of chemical control for amoebic gill disease); three studies characterising novel viruses (supporting risk assessment for their potential impact and informing policy decisions on control requirements); three studies investigating latency and developing new blood biochemical and molecular surveillance tools for koi herpesvirus (informing policy and potential control options as discussions develop on potential de-listing of this disease); four studies on ectoparasites of trout and ornamental fish (advancing characterisation of their diversity and identifying potential targets for novel control); a study on shedding rates of a significant, listed, exotic virus of rainbow trout (providing key data for parameterising quantitative risk assessment models informing control policy); two proof-of-concept studies assessing the efficacy of vaccines produced by novel methods (plant and algal grown vaccines for a salmon viral disease, supporting control of endemic diseases in aquaculture); two studies investigating infection in early life stages of trout including fundamental studies on environmental manipulation (heat shock) in early development, (informing host response and uncovering potential new disease control methods as well as potential for reduction/refinement using less sentient life stages in disease characterisation studies); and one study developing a challenge method for a significant listed disease of salmon (enabling future planned studies to identify key disease resistance genes in salmon using genome editing and selective breeding). As such the work conducted under this licence made significant progress on the broad aims and objectives. The data has been used to inform risk assessment and form policy and regulation at national and international level, output often in the form of reports to the Department for Food and Rural affairs (Defra) as well as peer reviewed papers. Fundamental work undertaken, as well as characterising the pathogenicity of disease-causing agents, also enabled the development of new diagnostic tools and methods, such as developing baseline information on blood biochemistry in healthy and diseased animals. A large part of the work directly informed and impacted control of aquatic animal disease in aquaculture settings. Nineteen peer reviewed papers

	have been published directly from the studies undertaken, with a further nine currently in preparation. As indicated above, this is a broad remit and new diseases are continually being discovered in cultured and wild aquatic species, often associated with the domestication of new species. These require characterisation and control. The work program was significantly impacted by COVID-19 restrictions and several planned studies did not proceed. Thus, despite the advances made, future work remains essential and further licence applications will be made along similar broad lines. Project amendments he project licence was amended three times. Firstly, in June 2018 to enable repeated close examination, non-invasive sampling and or blood sampling of fish under anaesthesia or, in circumstances where non-invasive procedures can be undertaken rapidly, without anaesthesia. The second, in September 2020, to allow more significant environmental manipulation, in the form of heat shock, beyond the range normally experienced under natural conditions. This was to enable the studies investigating the impact of environment on heritable mechanisms affecting response to disease. The third amendment, made in July 2021, was largely typographical to provide clarity and consistency across all areas of the licence referring to the option to take large volume blood samples at point of humane killing of fish under various relevant approved non Schedule 1 methods.
What are the potential benefits likely	With the ongoing depletion of wild fish stocks,
to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	fish farming is increasingly a critical sector for aquatic food security. Despite major advances over the last 40 years, infectious diseases continue to be a major constraint, reducing productivity, fish welfare and resource use efficiency. Fish farms are usually in contact with the surrounding river/sea environment, meaning infections can move easily between farmed and wild stocks. Research to understand diseases and develop control methods (e.g. prophylactic treatments, vaccines, disease resistant strains) is integral to assuring the future sustainability of aquaculture. The UK has a high aquatic health status, which is under constant threat from emerging and introduced diseases. Government policy is to eradicate any notifiable diseases disease by slaughter and disinfection if possible. This policy requires reliable validated diagnostic methods and an understanding of disease

	risks to wild fish populations. Maintenance of the UK's aquatic biosecurity and compliance with national and EU legislation on aquatic disease requires knowledge of aquatic disease supported by long-term programmes of diagnostic tool development, disease monitoring, disease control and prophylaxis. These form the statutory and scientific basis of the aquatic animal disease research programmes for wild and farmed fish covered by this licence.
What species and approximate numbers of animals do you expect to use over what period of time?	We seek authority to work with any fish species because fish from different environments and continents contribute to food security. In terms of wild fish disease research, we are likely to use endemic species. We seek authority to use a maximum number of 99,000 fish over a 5-year period; however, this number is expected to be much lower as it includes a large contingency in case a fish disease outbreak occurs requiring additional investigations
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?	One main procedure is used in four of the six protocols in this licence; this involves pathogen challenge, i.e. controlled exposure to pathogens. By nature of the serious pathogens of interest, the potential adverse effects are generally severe. The actual adverse effects are managed by defined humane endpoints implemented by intensive monitoring which involves both direct visual checks and desktop observations of live videos from in-tank underwater cameras. To our knowledge, we are the only research facility employing such remote monitoring to manage fish welfare.
Retrospective assessment	**Retrospective assessment** Published: 06 December 2022 **What harms were caused to the animals, how severe were those harms and how many animals were affected?** Twenty-four studies were performed over the five years of this licence using a total of 10,017 fish. This is considerably less than the proposed number authorised (see table 1) although this was in part expected as a significant contingency of numbers was included in case a large disease outbreak occurred requiring additional immediate unforeseen investigations as part of our national response. The number used continues a trend of decline in numbers observed in the two previous similar licences. This is likely due to a combination of reasons including studies being delayed or not performed because of the impact of COVID-19 and the fact that the laboratory officially

withdrew from membership of the UK's Good Laboratory Practice and Good Manufacturing Practice compliance programmes for veterinary medicines and safety studies so there was less requirement for preliminary studies supporting that type of work. This reduced number also in part reflects our attempts to actively reduce numbers of fish used as part of 3Rs responsibilities by exploring suitability of embryonic or pre-first feed life stages to provide the required scientific evidence. A total of 4121 pre-first feeding fish were used within 3 studies investigating disease in early life stages.

Table 1 -Studies performed and fish use by protocol.

Protocol	No. of studies	No. of fish used	Max no. of fish authorised *	Severity
1 Infectivity and pathogenicity	17#	7,649	55,000 5,000	severe
2 Development of methods for therapeutics	3	1,203	10,000 5,000	severe
3 Production of cohabitees	2#	840	2,000	severe
4 Maintenance of pathogens	2	225	1,000	moderate
5 Maintenance of diseased fish	2	100	1,000	moderate
6 Maintenance of genetically altered fish	0	0	10,000 10,000	mild
		10,017	99,000	

#Two studies encompassed use of fish under two protocols (protocols 1 and 3). *Where two figures are given, the first number in a cell refers to category "fish-all other fish" and the second number refers to "fish-zebrafish".

Two further studies were approved but did not proceed. In one case this was because the stock of fish brought in for the study were deemed not fit for purpose and terminated during holding. In the second case, diseased fish brought in for holding and isolation were terminated and sampled on receipt due to arrival in poor condition (see refinement).

Most of the studies undertaken were under protocol 1 "Infectivity and pathogenicity of fish pathogens" with the rest spread evenly across protocols 2-5. No studies were performed on genetically altered fish that had reached protected status, although genetically altered

	fish were used at the pre-first feeding stage. No fish were reused. Challenge with an infectious agent is a common procedure in all these studies and therefore the majority were authorised as severe. Under these protocols we prior estimated the maximum proportion of test animals likely to experience each level of severity of adverse effects was 20% severe, 40% moderate, 20% mild and 20% sub threshold. Increased frequency of observation, attention to disease specific clinical signs and appropriate setting of humane endpoints resulted in reported actual severities of only 3.6% (359 fish) severe, 40.4% (4,045 fish) moderate, 39.1% (3,914 fish) mild and 17.0% (1,699 fish) sub threshold. Harms experienced by individual fish varied according to pathogen under investigation but typically involved one or more of (in increasing apparent severity) lethargy, loss of appetite, lesions, atypical respiration, oedema and swelling, haemorrhages, irregular swimming patterns and intermittent loss of balance. Observation of three of more of these signs indicates near moribundity (death inevitable) and triggered euthanasia of individual fish and removal from the study. More severe clinical signs were on occasion observed including exopthalmia, loss of buoyancy or complete lack of response to stimuli, indicating a moribund state (death imminent), observation of any one of which triggered euthanasia. For the first time in this laboratory, one of the studies employed heat shock as a significant environmental stressor in early developmental stages to induce heritable phenotypic changes independent in changes of genome. This raised the potential for future developmental abnormalities, but no associated developmental abnormalities were observed in these fish via focussed monitoring.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The development of disease or resistance is difficult to study without a whole animal model as it involves multiple tissues and organs. The early parts of our investigations are conducted in nonanimal models; however, the infection, pathogenesis, host immune response, treatment and vaccination responses require complex metabolic, anatomical and immunological mechanisms that cannot yet be modelled in vitro or in surrogate invertebrate species.
Retrospective assessment	**Retrospective assessment**

	Published: 06 December 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?** Two of the studies undertaken under this licence relate to novel viral pathogens targeting the gills of carp that cannot be cultured in existing established cell lines in vitro. Currently very few fish gill cell lines exist and none from carp. A significant and ongoing amount of work attempting to develop either new primary gill cell lines or to manipulate existing non gill cell lines from common carp by gene editing has been undertaken during the lifetime of this licence and remains ongoing. Such in vitro tools though not completely replacing in vivo work would enable characterisation of these fastidious gill tropic viruses. Work has also been undertaken to develop an in vitro model of the gill epithelium in salmon. The asymmetric, three-dimensional model we developed has allowed the short-term study of host response of salmonid gill cells to exposure to pathogenic amoebae in seawater. One of the studies involved examination of disease development and host response across several life stages of the test species, from pre first feeding to juvenile. A sub objective of the work sought to determine if the early life stages (pre first feeding alevins) offered a less sentient, non-protected alternative model for studying a bacterial infection in Rainbow trout. Early data indicate in this case the early life stages did not succumb to disease or replicate the outcome observed in more traditional later stages. In another piece of work, we investigated use of pre protected stages of Atlantic salmon to characterise resistance to disease. Several groups of recently hatched genome edited salmon alevins were exposed to a viral pathogen and did support infection, but variance in hatch date, both within and between groups, and the short window between hatch and protected status meant data on response to disease challenge was compounded by normal hatch mortalities at this early stage.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Every effort is made to minimise the numbers of animals used in studies: statisticians advise on the numbers required to achieve meaningful results, animal husbandry experts advise on fish social needs and the ethics committee (AWERB) scrutinises each study plan. Members of the AWERB include scientists, veterinary surgeons, animal husbandry experts and lay people; they all have the power to veto study plans.

Retrospective assessment	**Retrospective assessment** Published: 06 December 2022 **How did you minimise the number of animals used on your project and is there anything others can learn from your experience?** Where the appropriate data and previous experience existed demonstrating effective consistent tank replication for host species with which we were very familiar, negative controls were reduced to a single tank rather than replicates. On one occasion a study was not progressed because suitable samples were available from naturally infected field sites visited by colleagues during the performance of their statutory duties. On two occasions samples taken at termination of study were utilised for a second purpose – namely development of baseline blood biochemistry values for relevant fish species both healthy and experiencing disease. Modification of tank infrastructure to enable variable and reduced tank volumes to be set allowed for further reduction in fish numbers for those species for which a relatively high stocking density is required to maintain maximum welfare (typically salmonids).
3. 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 want to have the ability to work with any fish species, of interest to both conservation and food production. The main tool we will employ to refine prospective severe procedures and minimise suffering is **close monitoring**. Fish are typically sourced from our own breeding establishment to ensure disease- free, high quality animals acclimated to experimental tank conditions. Externally sourced fish are health screened on arrival and quarantined to ensure a good health status and acclimation before use. We have a dedicated, high-tech aquarium facility, with monitoring and call-out alarms (water temperature, flow, depth). Named persons oversee staff training and performance, care of fish, and dissemination of information. Close links with the international fish research community ensures we are aware of any developments in fish care and biosecurity. Consideration is given to all aspects of the environment (including enrichment) e.g. space, water quality and current, conspecific density, lighting, shading, refuges and diet. We have a dedicated team of specialist

	aquarists complemented by long-standing experience in fish husbandry. Stock and experimental fish are closely monitored and interventions (including veterinary treatments) are implemented wherever possible. We believe we have a strong institutional culture of care and have review processes to identify where improvements in care can be made.
Retrospective assessment	**Retrospective assessment** Published: 06 December 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?** The main tool used to refine prospective severe procedures and minimise suffering was close monitoring including increased frequency of observations and remote observation via low aspect in water cameras as well as overhead observation in person. On one occasion diseased fish transported to the laboratory and due to be held and sampled over time were received in poor condition and sampled immediately on receipt. Despite prior discussion with company biologists and the involvement of their external veterinarian in fitness to travel assessment this is a situation that will require closer scrutiny if required in future. We refined the clinical signs database to work with species new to us. A notable example being the observation of natural lethargy in healthy wrasse and the absence of fast escape response to tail touch representing a key clinical sign. Tank enrichment was also improved during the term of this licence with artificial weed introduced for wrasse and now being used with carp and other cyprinids. The anatomy of wrasse also required the identification of new sites to locate visible implant elastomer tags for marking compared to the mark sites used in salmonids. Several new procedures with associated standard operating protocols were created. Key examples being prolonged detailed examination and health scoring of gills under light anaesthesia in shallow troughs; blood sampling with recovery (rather than euthanasia) using maintenance strength anaesthesia via oral intubation; rapid skin mucus sampling by swabbing without anaesthesia; marking by immersion in fluorescent dye.

	The last example required considerable adaption and refinement on first use as the duration and strength of osmotic shock and fluorescent dye immersion we first followed from published protocols resulted in adverse effects too significant to comply with our desired welfare parameters for marking. We believe this combination of close and frequent monitoring and continued infrastructure and procedural improvement in the facility led to the effective implementation of humane endpoints and the observed lower levels of actual severity experienced by fish under our care than was prior estimated.

Project	**19. Research to improve the treatment of envenoming**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals

Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	To protect the health of human patients, this project will use mouse models to preclinically test the effectiveness and safety of antibody-based anti-toxins and other toxin-neutralising therapies. **A retrospective assessment of these aims will be due by 02 March 2025** The PPL holder will be required to disclose: • Is there a plan for this work to continue under another licence? • Did the project achieve its aims and if not, why not?
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The benefits of this project will be the demonstration of the preclinical efficacy and safety of new therapies designed to treat various aspects of toxin-induced pathologies, that affect >500,000 people each year across the world. These new therapies are designed explicitly to provide superior efficacy and safety than existing conventional anti-toxin therapies, which have many limitations that result in poor patient outcomes in victims. Our findings will therefore underpin the selection of highly promising therapies for future use in early clinical trials in humans, following the demonstration of prerequisite safe and effective use in a proven animal model. This data will also be used to establish the initial dose of new therapies for those human trials, and thus be utilised for regulatory requirements during product translation. The relevant UK/EU Pharmacopoeias and WHO guidelines stipulate that the effectiveness of anti-toxin therapies should be preclinically tested in mice before use in human patients. Thus, in line with regulatory and public health policy, outputs from our previous project licenses relating to this work resulted in: 1) Provision of 37,000 vials of effective treatments to help save lives in Africa 2) Cessation of manufacture of a poorly efficacious anti-toxin product destined for Africa before it reached the market. 3) Validation of the efficacy and stability of products to be used by the NHS and in European countries for treating intoxications. It is important that human victims of intoxification receive appropriate treatment: the inappropriate distribution of untested products in numerous African countries

	resulted in an increase in mortality of treated patients from 0.5% to 12%. This example demonstrates that while the cost to mice of these preclinical tests are high, that benefit to humans is greater. The cost/benefit ratio of preclinical testing is greatly skewed in favour of human health benefit.
What species and approximate numbers of animals do you expect to use over what period of time?	We anticipate requiring a total of 3,950 mice and 18 rabbits over a five year period.
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?	Many of the proposed experiments are classified as severe due to the likely adverse effects the natural toxins being used to challenge the mice with are likely to cause. Such toxins cause a variety of severe pathologies, summarised broadly as cardiovascular, neurological and/or local disturbances. Thus, specific adverse reactions relating to this toxin research could present as: neuromuscular paralysis, laboured respiration, seizure, anaemia, haemorrhage, oedema, weight loss, and/or local inflammatory, haemorrhagic and/or dermonecrotic lesions. Previously defined humane endpoints (see refinement) will be implemented as indicators for euthanasia, in order to minimise animal suffering. We do not anticipate a high incidence of adverse effects for experiments relating to animal immunisation and the toxicity testing of new anti-toxin therapeutics. These experiments do not use high doses of natural toxins, and thus experiments relating to the safety testing of new therapeutics have a moderate severity designation, with potential adverse events relating predominately to the longer-term maintenance of mice (e.g. weight loss, hypothermia, immobility). Immunisation experiments have a higher risk (although still relatively low) of observing adverse events, including the potential for ulceration at the injection site, along with longer term health issues as the result of mice ageing (e.g. tumour development, inner ear infections, skin lesions and weight loss). At the end of the experiments all animals will be euthanised using a schedule 1 method.

	A retrospective assessment of these predicted harms will be due by 02 March 2025 The PPL holder will be required to disclose: • What harms were caused to the animals, how severe were those harms and how many animals were affected?
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Currently there is no *in vitro* alternative method for raising polyclonal antibodies - animals must be used. The mouse model of toxicity has proved a satisfactorily accurate representation of the effects caused by these natural toxins in humans. There is no *in vitro* alternative assay yet devised to supplant these animal tests (see below) because natural toxins can cause a variety of different physiological effects simultaneously, and intoxications are the result of complex mixtures of these toxins at the same time. Although we have investigated if *in vitro* techniques could be used in lieu of *in vivo* testing, we have found that they are insufficiently accurate to accurately predict the outcomes of *in vivo* tests. We continue to actively investigate methods that may provide accurate assessments of therapeutic efficacy *in vitro*. **A retrospective assessment of replacement will be due by 02 March 2025** The PPL holder will be required to disclose: • What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?
2. Reduction Explain how you will assure the use of minimum numbers of animals	The literature and results of previous experiments are closely examined to reduce the range of toxin doses, and therefore the numbers of mice, needed to establish the statistical validity of the assays. To further substantially reduce the numbers of mice required to achieve the objectives, we use (i) preliminary range finding studies and (ii) dose-staging methods to accurately determine the lethality of the toxin/s

	and/or potency of the antibody or inhibitor therapies. Statistical analysis is performed on all the results, and the minimum number of mice required for statistical validity is used throughout. During this project we will continue our vigilance to identify methods that show promise in reducing the numbers of mice required for these assays. **A retrospective assessment of reduction will be due by by 02 March 2025** The PPL holder will be required to disclose: • How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?
3. 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.	Mice are the physiologically least advanced rodent species that could be used for the preclinical assays. For the immunisation experiments we will also use a low number of rabbits following optimisation experiments conducted in mice. Rabbits are the most appropriate model for these experiments as their blood volume will permit collection of sufficient antibodies for later preclinical testing of new anti-toxin therapies. All of the previous preclinical tests on the efficacy of antibody-based therapies have been performed on mice. It would therefore be illogical to change the animal model species. The physiology of mice has been well characterised and the effects of toxin/s can therefore be accurately determined. The consistent use of mouse genetic strains (e.g. CD1) reduces independent variability and therefore (i) reduces the number of animals required for statistical validity and (ii) increases the validity of comparing results from different experiments. To refine protocols, we will: ● Undertake *in vitro* tests to reduce the number of candidate therapies requiring *in vivo* testing ● Undertake *in vitro* tests to reduce the

	number of candidate therapies requiring *in vivo* testing ● Undertake *in vitro* tests to reduce the number of candidate therapies requiring *in vivo* testing ● Undertake *in vitro* tests to reduce the number of candidate therapies requiring *in vivo* testing ● Undertake *in vitro* tests to reduce the number of candidate therapies requiring *in vivo* testing ● Use tests of the shortest possible duration ● Maximally implement analgesia ● Use terminal anaesthesia for certain procedures to reduce pain and suffering ● Use range-finding and dose-staging protocols to reduce the animal numbers required for the experimental outcomes ● Use existing and develop new less-severe humane end points to reduce pain, harm, and suffering and distress to the experimental animals ● Facilitate the implementation of these endpoints, and in turn further reduce animal suffering, by implementing rigorous and frequent animal observations. **A retrospective assessment of refinement will be due by by 02 March 2025** The PPL holder will be required to disclose: • With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?

Project	**20. Studying Gene expression in development and Disease**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	(1) Understand the processes that maintain normal healthy growth, development and function in the body (2) Identify changes that can contribute to the development of diseases that are relevant in humans (3) Test for potentially new and more effective approaches for preventing or treating common human diseases These studies are necessary to understand how normal function is maintained in healthy individuals and changes the can cause commonhuman diseases. The human body, like	

	many higher organisms that develop from a single fertilised egg, must undergo very tightly controlled changes that give rise to the highly specialised and complex organs (e.g. brain, heart, liver, kidney) and maintain function under different conditions. However, because of the complexity of such organisms, identifying the changes that lead to abnormal functions or diseases can be very challenging. Our research is specifically aimed at understanding how normal specialised cells and tissues are produced and maintained from the common blueprint DNA code, which is found in every cell of an organism. Such unique functions are linked to specific regions of DNA, called genes, which carry the code that is used to produce different cellular proteins. However, the DNA must first be copied (transcribed) to produce an RNA template, which is in turn, used as the instructions to produce specific cellular proteins that carry out specialised function in different cells. One of the most important levels of control in the cell is the production of RNA from DNA (called gene transcription) and is largely driven by specialised proteins called transcription factors, which govern the assembly of the machinery that increase some genes while blocking others from being made. In this regard, transcription factors can be considered as master regulators that control cell fate and when altered can cause abnormal gene production that contribute to disease. Therefore, it is important to study how such master regulators act in different cells in the whole animal but also to identify disease-causing changes. Such knowledge can be harnessed for early detection and prevention or treatment of specific diseases. Different organs in the body must work very closely together to maintain normal function in healthy organisms, so it is important that we identify and study disease-causing changes in a whole animal rather than in isolation, to understand what happens during the transition from health to disease. The master regulators that we are studying are evolutionarily conserved e.g. 90- 95% similar between mouse and humans. This means that by studying animal models we can gain essential information on the changes that underlie common diseases in humans but also identify

	more effective ways of preventing / halting the progress of such diseases or looking for new ways of treating many diseases. **A retrospective assessment of these aims will be due by 03 July 2024** 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?
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Increase of knowledge: One of the main benefits of these studies will be to increase our knowledge and understanding of how complex and highly specialised organs arise and function normally but also identify changes that lead to abnormal function associated with common human diseases such as cancer, diabetes and heart disease. Such increased knowledge can lead to more effective ways of preventing or treating such diseases. This is very important because many common human diseases develop over a long time, but in general, these are not detected until later stages when damage has already occurred in cells and tissues. Potential application for patients: The benefit for patient can arise by increasing our understanding what controls mportant cellular events such as cell survival, division and death, which when uncontrolled can lead to human diseases. Mouse models are particularly important because mutant strains with changes in specific master regulators have already shown relevance to human diseases including abnormal heart formation in babies (congenital heart disease), death of specialised cells such as heart muscle after stress and stiffening or abnormal function in blood vessels. Other relevant diseases such as cancer could also benefit from better understanding using some of the proposed studies. Results from using such models could help in early detection of disease-causing changes by identifying potential biomarkers but could also highlight new approaches to treatment at early stages and thereby prevent long-term damage. The zebrafish provides some unique advantages for studying heart development at very early stages that are not possible in mouse and humans.

	This can be particularly beneficial for understanding heart defects, which can account a large proportion of miscarriages or heart defects in newborn babies. Secondly, the zebrafish has a unique capacity to repair specialised tissues e.g. heart muscle after damage, which not seen in higher organisms such as human and mouse. Such damage, which commonly occur after a heart attack, can lead to heart failure and death in humans so studying the factors that help to prevent cell death and promote regeneration in the heart will provide significant benefits in treatment of patients with heart diseases.
What species and approximate numbers of animals do you expect to use over what period of time?	This project will mainly use mouse models because existing models have already provided preliminary data that is relevant to human diseases. We will use existing mouse mutants but also produce targeted mutants using available and tested technologies and we anticipate that approximately 4000 mice will be used over the duration project. Rat and zebrafish models will also be used for specific studies where these are experimentally warranted with up to 2000 of each species used during this project.
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?	In general, we do not expect adverse effects during pre-treatment phases of the experiment (e.g. drug administration) but will be carefully observed at all stages during any treatment. Pain relief will be given to animals deemed to be suffering (using defined scoring sheets). Similarly, pain relief will be given to animals undergoing any procedures that are judged to result in pain and discomfort and especially in animals recovering from operations. To ensure that no adverse effects arise from procedures undertaken under anaesthetic, all animals will be monitored closely during and after surgery, until the conditions are stable. Potential risks of infection or other complications during procedures can be minimised by using clean surgical equipment and aseptic techniques. All procedures will be undertaken by qualified and experienced staff who can observe experimental animals closely and identify and treat any pain and discomfort at early stages. Any animal that is deemed to be suffering unduly, will be

	promptly culled. By keeping monitoring records, we can identify any procedures with adverse effects and seek advice from the named veterinary surgeon. Additional steps will be taken tosure comfort of animals after any procedure e.g. extra clean bedding and provision of mashed food and water. Majority of the experiments to be undertaken will be carried out under the mild or moderate severity and experiments involving higher severity level will be limited as much as possible. **A retrospective assessment of these predicted harms will be due by 03 July 2024** 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?
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	When trying to understand the changes associated with human diseases, it is necessary to consider the complex interaction between different organs of the body under normal conditions and identify changes that may contribute to abnormal responses and disease formation. Such complex crosstalk between organs and tissues are essential for normal health and function and can only be studied in intact animals. Similarly, to identify changes that cause common human diseases, it is important to mimic the whole-body conditions as closely as possible. For these reasons, experiments using animal will only be undertaken when it is necessary to recapitulate the complex internal environment or mimic the interaction and crosstalk between different tissues or other physiological changes seen in an intact organism that closely mimic the human systems. **A retrospective assessment of replacement will be due by 03 July 2024** 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?

2. Reduction Explain how you will assure the use of minimum numbers of animals	Careful experimental design will be carried out to minimise the number of animals used in all experiments. For all studies, animals will be matched in terms of age, gender and weight to minimise variation and therefore reduce the number of animals used. In addition, whenever possible control studies will be performed on the same day to maintain environmental conditions (e.g. temperature) as closely as possible. Whenever possible, in-vitro models such as cell lines and three-dimensional cell cultures will be used to confirm findings and undertake in-depth cellular and molecular analyses that can then be progressed to primary cell culture, which uses fewer animals to derive many experimental points and test the number of interventions from specialised cells (e.g. heart or nerve cells). In addition, wherever possible, we will use carry out serial measurements and paired analysis in order to reduce animal numbers at a different time points/duration of treatment. **A retrospective assessment of reduction will be due by by 03 July 2024** 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?
3. 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.	For all experiments, relevant models will be selected based on the unique properties in relation to the human diseases being researched. For example, mouse models will be the primary systems used for the studies because these models are extremely well characterised but also because many processes that regulate development and/or function of different tissues are highly conserved between rodents and humans. On the other hand, the zebrafish will provide a better model for experiments aimed at understanding the factors that control heart regeneration. For the different studies, we have used information from existing literature and previous studies to refine the scoring system that will be used to monitor experimental animals. Such guidance, when combined with regular monitoring for clinically relevant measurements,

	will ensure that changes can be detected early and treatment given before adverse effects can occur. For all experimental animals, steps will be taken to maximise animal welfare with provision of additional bedding, pain relief, easy access to food and water, if necessary. Most of the experimental plans are designed to be carried out in a staged manner so to avoid any undue pain and suffering. In all cases, experimental studies will only be undertaken by experienced researchers with appropriate training trained for any specialised techniques. **A retrospective assessment of refinement will be due by by 03 July 2024** 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?

Project		**21. Survival of commercially caught and released marine fish**
Key Words (max. 5 words)		
Expected duration of the project (yrs)		5 years 0 months
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
		Translational and applied research
		Regulatory use and routine production
	X	Protection of the natural environment in the interests of the health or welfare of humans or animals
	X	Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)		To increase our understanding of the survival of marine fish after they have been caught by fishing vessels and released back to the sea. This knowledge will be used to advise UK and international government, stakeholders and NGOs and support sustainable fisheries management. **A retrospective assessment of these aims will be due by 11 December 2024**

	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?
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	brought ashore and will die, which could reduce the sustainability of fishing; ii) in setting the permitted amount of fish catches, it is important to know how many fish are killed by commercial fishing. Currently, it is assumed that unwanted fish catches that are returned to the sea do not survive. Information on the amounts of fish that do survive, will improve the data used in setting sustainable fishing levels.
What species and approximate numbers of animals do you expect to use over what period of time?	Adult, sub-adult and juvenile marine fish. Up to 4000 animals would be used over the 5-year period of the work.
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?	There are two procedures proposed, i) attaching tags to fish and releasing them back to the sea; ii) using holding facilities to observe and monitor fish that would have been released back to the sea. i) The tagging is assessed as 'Mild' severity. Possible adverse effects are pain during tagging, scarring and/or infection of the tagging location. Where needed, pain relief will be used on the tagging location. Adverse effects will be minimised by using practiced tag attachment methods. Aseptic precautions will minimise the risk of infection. After the tag has been attached, tagged fish will be discharged from the Act and returned to the sea. ii) The observation method is assessed as 'Severe'. The aim of the work is to estimate how many fish survive after release, and it is possible that some fish will die during the monitoring period. We will introduce humane end points at the earliest point of the monitoring period. Fish will be killed if they display a health condition that is linked with a low chance of survival or they stop feeding. Therefore, the procedures will evolve as we learn more about how the health of fish relates to its chance of survival. At the end of the monitoring period, the fish will be killed by an approved method. **A retrospective assessment of these predicted harms will be due by 11 December 2024** 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?
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The project will provide knowledge on the survival levels of commercially caught and released fish. This information does not exist, and the survival levels are likely to be different depending on the species of the fish, and on how the fish are caught. It is currently not possible to find a non-protected animal alternative. The project will seek, review and incorporate alternatives throughout the project duration. **A retrospective assessment of replacement will be due by 11 December 2024** 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?
2. Reduction Explain how you will assure the use of minimum numbers of animals	The methods and numbers of animals used will be based on experience and previous research. There will be input from a statistical team and the Animal Welfare and Ethical Review Body, so that the minimum number of animals are used. We will find out how the health of fish, at the time they would be released from the fishing vessel, relates to their survival chances. If a relationship can be found, it will be possible to assess the fish at the time they are released, to predict their survival levels, and there will be no need to tag or observe fish. This method will also be used within studies. The tagged and observed fish will show how health condition relates to chances of survival. A larger number of fish will have their health assessed at the point they would be returned to the sea. The health-survival relationship will be applied to all of these assessed fish to give an overall survival estimate. This reduces the number of fish needed for tagging and observation. Where possible, data and knowledge from other research will be used instead of using animals. For example, sharing of knowledge and data will continue at the International Council for the

	Exploration of the Seas Working Group on Methods for Estimating Discard Survival. Opportunities to reduce the number of animals used will be assessed throughout the project. **A retrospective assessment of reduction will be due by by 11 December 2024** 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?
3. 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 project will provide new knowledge on the survival levels of commercially caught and released fish. This information does not exist, and the survival levels are likely to be different depending on the species of the fish, and on how the fish are caught. It is not possible to find a non-protected animal alternative. A range of commercial fish species will be studied. Where survival chances are shown to be high, this work will support requests to allow fish to be released back to the sea. Therefore, the species and fisheries selected for study will be those where the potential for survival is highest. The methods proposed are based on direct experience of fish tagging and husbandry techniques developed over 20 years. The tag attachment method will be one that minimises pain and suffering. Tagged fish will be checked that they are fit enough to be released to the wild, and those assessed not to be fit, will be killed by an approved method. For the observation method, the holding facilities and husbandry techniques will be chosen to minimise suffering to ensure reliable results. Humane end-points will be used at the earliest point in the monitoring time for fish that are unlikely to survive. This will be continuously evaluated so that any suffering is minimised and the results are useful. The monitoring periods will be as short as possible while still producing robust results. Refinements will be reviewed throughout the project; information will be sought from other relevant research and from NVS/NACWO.

	A retrospective assessment of refinement will be due by by 11 December 2024 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?	
Project	**22. Targeted mouse models for pre-clinical studies in cancer**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
	X	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals

Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Cancer is the leading cause of death of children aged 1-15 in the UK. There have been improvements in survival rates for children with leukaemia and lymphomas, however, there is still a great need for progress in treatment of solid tumours. Neuroblastoma is the most common childhood “non-brain” solid tumour and medulloblastoma the most common malignant brain tumour in children. A significant proportion of children with these tumours are considered high risk, that is, despite intensive treatment they have a very poor prognosis. In addition, many of those children that do survive suffer a number of late effects that are severe or life limiting. There is a definite need to improve treatment options for these children. The current treatment for medulloblastoma involves tumour removal, chemotherapy and or radiotherapy. When this fails there is currently no further treatment option available. By mimicking this treatment plan we wish to understand how these tumours respond to current treatments and determine how new treatment approaches could be implemented to improve outcomes **A retrospective assessment of these aims will be due by 09 July 2024** 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?
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Developments in molecular screening technologies have meant that it has been possible to identify more of the defects associated with these high-risk tumours. Using this information, we can develop animal models of high risk disease and use these to develop improved and more targeted treatments. We will use preventative experiments using animals prior to tumour onset to assess whether therapy can delay or prevent tumour development. We will carry out intervention trials using animals bearing tumours of a specified size dependent upon the tumour type and location. These are

	performed to assess the effect upon tumour growth and/or survival of the animal. This includes assessing when tumours become resistant to treatment drugs. Combination experiments will be designed based on current clinical practise whereby up to three drugs are administered together cyclically and may be followed by a fourth treatment with a different drug. We are proposing these experiments in order to identify treatments that are more effective and less toxic to children with these tumours. We anticipate that our work will lead to the development of treatments that will be more specific for an individual's particular cancer type and therefore giving the doctors treating them a better choice of more effective medicines.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use genetically engineered mice that are pre-disposed to develop tumours that represent the high-risk disease seen in children. We anticipate breeding approximately 35,000 – 40,000 mice over the
	next 5 years. Of this number there will be a number of animals that are not the correct genetic make-up to produce tumours and these will be used for future breeding or killed by humane methods.

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?	Neuroblastoma tumours normally arise in the abdominal cavity and occasionally in the thoracic cavity, the primary adverse effect is weight loss, if the animals lose 15-20% body weight that doesn't respond to diet supplements they are humanely killed. The primary symptoms of medulloblastoma, a tumour in the back of the brain, are either circling behaviour or domed heads, when they show these signs they are humanely killed, where possible we use non-invasive imaging to detect tumours before the onset of these symptoms. Using our existing models, we have a great deal of knowledge as to the expected symptoms and time of onset and thus are able to prevent progression of tumours to the stage that they cause ill health. With our new models we will monitor the health of the animals carefully and where possible use non-invasive imaging techniques prior to onset of symptoms. We will test methods of treatments such as surgery and drugs. This could cause pain and weight loss. Pain relief and diet supplement will be given and if they don't respond they will be humanely killed. **A retrospective assessment of these predicted harms will be due by 09 July 2024** 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?
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The development of tumours depends upon a number of interactions between the tumour cell and the local tissue environment. Therefore, we are currently not able to replicate this environment in a laboratory setting. There are a number of factors vital for drug testing that are not possible to address in the laboratory, such as does the drug get into the tumour? Does it require modification by some other process in the body? Are there any toxic side effects that can only be assessed in a live animal? The mouse is the species of choice due to the amount of genetic mapping information available and the ease in which they can be genetically modelled.

	A retrospective assessment of replacement will be due by 09 July 2024 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?
2. Reduction Explain how you will assure the use of minimum numbers of animals	When breeding our genetically engineered mouse models we will design good breeding programmes to prevent over breeding and to keep numbers to a minimum. We have a workflow process whereby we test the validity and relevance of our models and use this to make decisions as to how to proceed with models. When testing drugs, we follow a workflow process to determine the effectiveness and use this to determine how far to proceed with a particular drug. Experiments are designed to use the minimum number of mice whilst providing statistically significant results. **A retrospective assessment of reduction will be due by by 09 July 2024** 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?

3. 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.	Mice are widely used for *in vivo* drug development and there is a lot of information available for comparison. Genetically engineered mouse modelling technology is well established, and we have previously demonstrated that we are able to produce and utilise our models to advance clinical trials. Using information from our current models as to potential symptoms we will closely monitor all animals that are expected to develop tumours. Any new drug regimens will be discussed with Named Persons, i.e. the vet and animal care and welfare officers, in advance. We will use refined techniques such as clinical score sheets, regular observations, combining drugs to reduce the number of injections and good handling techniques. We will use analgesia and anaesthesia as recommended to minimise any pain from surgery. **A retrospective assessment of refinement will be due by by 09 July 2024** 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?

Project	**23. The effect of peripheral homeostatic disturbance upon the brain's defences**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The aim of this project is to investigate how the defensive mechanisms of the brain are affected by changes in the rest of the body, and to study the links between chronic ill health and development of neurological conditions such as Alzheimer's disease. This project has two arms, firstly we will compare the effects of chronic inflammatory disease, modelled using the very common dental condition periodontitis, with acute inflammation upon the two main defences of the brain, the blood-brain barrier and the immune cells called microglia. Periodontitis has been associated with an increased risk of	

	developing Alzheimer's disease in humans, but little is known about how these links occur; this project will directly investigate whether periodontitis increases the vulnerability of the brain to damage. The second main arm of this study is to establish how changes in the microbial communities of the body can influence the brain's defences. There is substantial evidence linking a low quality diet and poor brain health, including an increased risk of Alzheimer's disease, but again the linking mechanisms are unclear. The microbial communities of the gastrointestinal tract respond to changes in diet, producing many chemical mediators that affect the rest of the body. This project will directly investigate how diet-induced changes in the gut microbes affect the brain's defences, and their importance in linking a poor diet with the risk of neurological disease. **A retrospective assessment of these aims will be due by 17 September 2024** 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?
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	As the average age of the population increases, the most significant risks to health come from age-related neurological disorders, of which Alzheimer's disease is the most common. While the cause of Alzheimer's disease is still unknown, it is increasingly clear that a significant factor is the failure of the normal defensive mechanisms of the brain. In this project we aim to investigate how two common, chronic disruptions to normal physiology can affect these defences and increase the risk of developing Alzheimer's disease. The experiments to be performed in this project will allow us to assess the state of brain health after chronic physiological disruption, providing valuable insights into the mechanisms underlying the increased risk of Alzheimer's disease and other neurological disorders associated with either chronic inflammatory disease or poor diet. This will serve as an essential platform for future studies

	investigating ways to counteract these changes and protect the brain from disruption, hopefully reducing the risk of developing age-related disease.
What species and approximate numbers of animals do you expect to use over what period of time?	The majority of the animals to be used over this 5 year project will be wild-type mice and we expect to use a maximum of 1800 animals in this period. We will also use selected genetically modified animals to test specific hypotheses, and we anticipate using a total of 400 such animals over the 5 year period.
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?	The majority of procedures to be performed in this project are of mild severity, and we expect few adverse effects, primarily acute and self-resolving sickness from the administration of inflammatory agents. A number of mice will receive injections of inflammatory agents directly into the brain, a procedure of moderate severity given the route of administration and the central position of the brain in health. The inflammatory agents we will use however, are deliberately chosen to be relatively mild, and are not expected to cause significant defects in behaviour or health. All animals will be killed by a humane method at the end of the procedures or if they show unexpectedly severe adverse effects. **A retrospective assessment of these predicted harms will be due by 17 September 2024** 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?
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We are investigating the impact of changes to normal physiology upon the brain's defences and the risk of developing neurological disease. As such, we will be examining interacting changes in several highly complex systems, namely the immune response, the microbial communities of the body, and the brain. Currently, there are no computer models able to effectively replicate these complexities. Similarly, the immune response and nervous systems of non-protected animals are too simple to model the interactions that occur in mammals, and we would not be able to satisfactorily address our research questions using these organisms. We will investigate our research questions as far as possible using *in vitro* techniques or through analysis of human clinical samples, such that we will only move towards animal experiments when all other approaches have been exhausted. **A retrospective assessment of replacement will be due by 17 September 2024** 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?

2. Reduction Explain how you will assure the use of minimum numbers of animals	All the experiments in this project have been carefully designed to use the absolute minimum number of animals, through rigorous statistical analysis of the numbers of animals required to efficiently detect biologically meaningful differences in our experiments, informed by our previous experience in the field and by the scientific literature. Where possible, we will make use of non-invasive imaging techniques to permit repeated analysis over time of individual animals, significantly reducing the number of animals needed. **A retrospective assessment of reduction will be due by by 17 September 2024** 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?
3. 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 mouse has been exceptionally well characterised in terms of its physiology, genetics and microbial communities, and represents the best available model system to ensure results are applicable to human health. Moreover, there are a large number of genetically modified mouse strains available, which will allow us to examine the role of specific components of the immune, nervous and vascular systems in maintaining brain health. The use of such strains will significantly enhancing the precision of our experiments, ensuring that the data we obtain has greater clarity than can be gained from studies of wild-type animals or those of other species. In this project, we will use minimally invasive techniques wherever possible, including for example modification of gut microbes by changing diet or including antibiotics in drinking water, both to reduce welfare costs to animals and to avoid triggering a stress reaction, which we know from previous work to modify the response of animals to inflammatory stimuli. **A retrospective assessment of refinement will be due by by 17 September 2024**

	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. Treatment and Prevention of Auto-immune and Immune-mediated Diseases

Project duration

5 years 0 months

Project purpose

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

Key words

Uveitis, Psoriasis, Sjogren's, Multiple Sclerosis, Thrombocytopenia

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?

This project aims to develop novel therapies to treat auto-immune diseases and immune-mediated diseases.

A retrospective assessment of these aims will be due by 16 December 2024

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.

What are the potential benefits that will derive from this project?

Auto-immune diseases affect millions of people worldwide causing pain, impaired function and diminished quality of life. We will be facilitating the development of new therapies for diseases which cause significant ill health and for which current treatments are inadequate. By contributing to the development of new immune-modulatory drugs, our project will benefit the patients, improving their quality of life and reducing suffering.

By providing high quality services and scientific expertise, we can make the testing of such drugs more informative.

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 estimated number of animals to be used over the duration of the project (five years) is 12200. Mice will be used in more than 90 % of studies with rats being used in the remainder. No other species are to be used.

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?

We expect animals to develop some clinical signs relating to the diseases induced under this project licence. Inducing auto-immune encephalomyelitis under our treatment and prevention of experimental autoimmune encephalomyelitis project is likely to cause adverse effects such as bodyweight loss, paresis and paralysis of the tail and hind limbs. The expected level of severity for this model is severe. Specific measures are taken to limit harms such as frequent monitoring and scoring of disease, specific clinical signs and non-specific clinical signs. Moderate signs are not tolerated for more than 24 hours and severe signs will not be tolerated. At the end of an experiment, all animals will be humanely killed to enable further in vitro testing of samples.

For our treatment and prevention of systemic lupus erythematosus (SLE) projects the adverse effects we expect to see are impaired kidney function, increased pro-tein in urine, swelling to the paws, changes to breathing patterns and abdominal distention.
On our treatment and prevention of psoriasis we expect to see changes to the skin such as skin thickening, flaking, crusting, redness and itching. Treatment and prevention of dry eye syndrome (Sjogren’s) can cause reduced tear and saliva production and onset of diabetes.

For mice and rats used on our treatment and prevention of experimental autoimmune uveitis (EAU) we can expect the animals to develop inflammation to the front and back of the eye.

Under our antigenic challenge protocol expected adverse effects are changes to body and coat condition, decreased activity, changes to breathing patterns and irritation or break in the skin around adjuvant administration site.

The adverse effects expected under the Treatment and Prevention of Amyloidosis include bodyweight loss, changes to coat condition and posture, changes to breathing patterns and decreased activity levels.

The expected level of severity for all the above models is moderate. Measures are taken to limit harms such as frequent monitoring of disease-specific clinical signs and non-specific clinical signs for early identification of adverse events. Moderate signs are not tolerated for more than 24 hours and severe signs will not be tolerated. At the end of an experiment, all animals will be humanely killed to enable further in vitro testing of samples.

Expected adverse events: Animals showing signs of several major human diseases will be given new treatments by injection or slow release implants and the effects on the diseases measured. Diseases modelled include mul-tiple sclerosis, in which animals may show weak-ness, difficulty moving and bodyweight loss.

In other models, animals may develop increased protein in urine, crusting, skin flaking, skin thickening, reduced saliva and tear function; or eye inflammation; changes to body condition or weight loss.

Most protocols show moderate signs of disease, other than EAE which displays severe disease.

Most animals will develop only mild disease, a small number of animals may develop more severe disease,

Measures taken to limit harms: frequent monitoring of animals for early identification of adverse events, Animals showing moderate signs for more than 24 hours will normally be humanely killed. Models of multiple sclerosis can be more severe, and animals will be monitored very closely and given extra care. At the end of an experiment, all animals will be humanely killed to enable further in vitro testing of samples.

A retrospective assessment of these predicted harms will be due by 16 December 2024

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.

Immunology involves multiple systems, multiple organs and multiple cell types. The complexity of the immune response cannot be reproduced in laboratory tests.
In addition, the symptoms of auto-immune diseases – paralysis, eye inflammation, reduced salivary/lachrymal function, skin flaking and thickening - cannot be modelled in a laboratory. Experiments on cell lines and on cell cultures will be performed. However, the limitations of these methods do not allow them to replace the use of experimental animals: there is no alternative to the use of a living animal that would allow the objectives to be met.

A retrospective assessment of replacement will be due by 16 December 2024

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.

Statistical tests will be performed at the planning stage to determine the necessary number of animals to be used to obtain scientifically sound data. Where suitable, previous experimental data from our establishment will be used to allow for comparison. In addition, if possible, we encourage the use of a shared control or untreated group among different studies using the same model.

A retrospective assessment of reduction will be due by by 16 December 2024

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.

Most of our studies will be done in mice, which are the lowest species that develop these diseases in the same way as humans. Rats are occasionally used when the disease cannot be modelled in mice, if the test compound does not work in mice, or if a larger animal is needed.

Animal suffering will be limited by ensuring that the models used cause the least amount of harm to the animals. The mildest disease inducing agent or dose will be used, and studies will be kept as short as possible. Animals are monitored frequently for signs of discomfort, and appropriate action taken promptly. We will monitor animals closely throughout the studies, and they will be treated or humanely killed if they develop signs of excessive suffering.

Animals are housed in groups and kept in an appropriate environment with plentiful bedding and nesting material and suitable object that allow them to express normal behaviour. All staff are trained in good animal handling procedures. Animals are always handled gently and humanely, especially animals which may be in pain. Animals may be acclimatised to being handled prior to the experiment starting so that they are less stressed once the study begins.

Animals are provided with a bowl of mashed food on the cage floor if moving may be uncomfortable. When substances need to be administered, we will give the smallest volume possible and administer it in the way that causes the least distress.

A retrospective assessment of refinement will be due by by 16 December 2024

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?

Project	**25. The role of inflammation in neurodegeneration**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The number of people with dementia or vision loss is increasing in our ageing population and novel treatments are desperately needed. The typical memory loss, mood changes and loss of vision, are a result of dying nerves in our brain or the loss of rod and cones in our eyes, a process collectively known as ‘neurodegeneration’. For many years, it was believed that neurodegeneration is a disease of the brain, but we now know it is much more complex. Research has shown that inflammation can damage nerves. The inflammation occurs in the brain as a result of ageing, but is worsened as a result of other diseases, which are commonly seen in elderly, such as infections, arthritis, gum disease or diabetes. Some of these so-called ‘co-morbidities’	

	are a result of our life style and our genes. The animal models proposed in this work are designed to study the inflammatory triggers that lead to the onset and/or progression of neurodegeneration and allow us to investigate how manipulation of the immune system may prevent, halt or treat age-related dementia or vision loss. **A retrospective assessment of these aims will be due by 08 October 2024** 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?
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Worldwide, millions of people are affected by dementia or vision loss, for example caused by Alzheimer's Disease, Parkinson's Disease or macular degeneration. Currently there is no treatment to halt or cure these diseases. Better understanding of the biological process(es) that results in memory loss or vision, may result in novel ways to treat these devastating diseases of the brain. When we age, certain proteins form clumps in our brain or eyes, these are known as plaques. These can cause damage to the brain, but we don't know how. Inflammation has been recognised as a factor that can also cause damage to nerves. This may occur via the activation of specialised immune cells in the brain, called microglia, or, alternatively as a result of chronic infections, or lifestyle choices. Identifying the cells and proteins/molecules involved in nerve damage may result in a delay of memory loss, mood changes or vision loss. This will improve the quality of life of patients and their carer with dementia.
What species and approximate numbers of animals do you expect to use over what period of time?	To provide a better understanding of the processes of how local and systemic inflammation affect neurological disease this project will over a five year period use about 11,000 mice. We will use knowledge from our own previous research and from other scientists to calculate the number of mice required for a reliable scientific experiment.

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?	In our programme of work we propose to model Alzheimer's and Parkinson's Disease via injection of misfolded proteins directly into the brain, which is expected to cause disease similar as seen in humans. We do not expect any adverse effects of these injection by themselves, but mice may develop dementia or motor symptoms, similar to humans. We are interested in the early stages of disease to identify novel ways to prevent or treat age-related neurodegenerative diseases and we will use behavioural tasks that can detect changes in the brain before overt clinical symptoms. This will reduce potential suffering to moderate levels. Some animals will be exposed to inflammation, which will activate, or inhibit, their immune system and some animals will be aged to study how normal aging contributes to brain dysfunction. This may induce sickness symptoms and reduced activity, reduced vision, and/or reduced motor strength. To model age related vision loss as a result of macular degeneration, we will use a laser to injure the retina; this procedure is painless in human and we do not expect this to be different in mice. We will carefully monitor their behaviour and any mouse in distress will be culled. Surgical procedures may induce infections and this may make the mice ill, or delay their recovery. Animals will be carefully monitored for any signs of infection and sterile equipment is used to prevent them. When the experiment is finished we will take brain or eye tissue, blood, and organs and analysis them for signs of nerve damage and inflammation. We will also look at the gut and look in poo samples, to get information about bacteria that can cause inflammation as a result of lifestyle and diet. We typically use a range of techniques, and state-of-the-art microscope to zoom into the brain and stain cells for disease markers, or measure genes following treatment of the animals. Similar techniques are used to analyse human tissues or cells grown in dishes, so we can compare the results. **A retrospective assessment of these predicted harms will be due by 08 October 2024** 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?
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot	We intend to use cells grown in dishes, where possible, for example nerves or microglia. We also intent to use brain and eye tissue from people that have died with late stage dementia or vision loss.

use non-animal alternatives	However, this work is aiming to understand the role of inflammation before clinical symptom occur and it is unethical to conduct experimental on humans where removal of parts of the brain or eye is required for investigating the role of inflammation at the early stages of disease. Due to the complex interaction between the immune system and the nervous system there is no alternative that would entirely replace the use of living animals. **A retrospective assessment of replacement will be due by 08 October 2024** 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?
2. Reduction Explain how you will assure the use of minimum numbers of animals	The design of the individual experiments will be optimized to ensure that the maximum amount of data is obtained from the minimum amount of resource. We have already optimized many of our methods which means we can use small amounts of tissue for analysis. We will use the ARRIVE guideline to inform our experimental design. **A retrospective assessment of reduction will be due by by 08 October 2024** 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?
3. 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 mouse models which are proposed in our programme have many features in common with human neurodegenerative diseases, including loss of nerves, the activation of the immune system and typical behaviours such as memory loss and mood changes. We already showed that real bacterial infections in mice induce symptoms seen in humans and that a high fat diet results in subtle inflammation in the blood, similar as seen in humans. Previous studies using mice have provided a wealth of information on the role of inflammation in disease and these have resulted in novel treatments. A good example is a novel anti-inflammatory drug for people with arthritis that blocks inflammation and delays the progression of disease. This anti-inflammatory drug is now one of the most

	successful drug to date. The knowledge obtained from these mouse studies can be used in our models of dementia and vision loss. The immune system of the mouse has been studies for decades and the parallels with the immune system of humans are well known. Importantly, mice are also the only species where genetic manipulation has been carried out to permit further comparison and this allows us to investigate how genetics risk factors of dementia and vision loss are related to inflammation, life style choice or infection. To reduce lasting harm we will restrict our methods to induce mild systemic inflammation and regularly monitor for pain, distress and sickness behaviours using a well-being score and a pain score.If this score is exceeded, we will terminate the experiment. **A retrospective assessment of refinement will be due by by 08 October 2024** 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?

Project	**26. The Role of Pattern Recognition Receptors in Immunity and Homeostasis**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Our objective is to understand how molecules found on our immune cells, called pathogen recognition receptors (PRRs), enable our immune system to combat disease causing organisms and how these PRRs are involved in the development of autoimmune diseases of the joints (arthritis), lung (asthma) and eye (uveitis). Experimentally, to understand how particular PRRs are involved, we compare immune responses of normal mice to those of mice lacking that PRR and/or other components of the immune system. We may also need to use drugs to alter the immune system or types of	

	immune response so as to understand the underlying mechanisms of resistance or susceptibility to disease. Using these approaches we can determine which PRRs are important, and the mechanisms that they use to control the development of disease. **A retrospective assessment of these aims will be due by 18 September 2024** 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?
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The direct benefit of our research is the furthering of scientific knowledge of the underlying mechanisms of our immune system. These advances allow us to understand the disease process, and lay the foundation for new and better treatments for humans in the future. Our work has already led to substantial advances, as evidenced by our publication in scientific journals and the impact these have had on other scientists, measured by the number of times our work is cited by them. Our discoveries have already led directly to a greater understanding of what can cause disease in people, such as genetic alterations that cause predisposition to infections, and also a novel therapy for a fungal skin infection.
What species and approximate numbers of animals do you expect to use over what period of time?	29700 mice and 20 rats will be used in experimental procedures during the 5 year PPL. These numbers represent the theoretical maximum, and in practice will likely be less. Estimates are based on the required group sizes for experiments, experience on how many experiments are required to complete the studies, and on the number of researchers that will be working under this PPL

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?	Mice are given substances that can induce diseases which we need to study, including inflammation, eye disease, arthritis, and asthma. We also retain a small number of mice and allow them to age, to see what diseases develop, like in older humans. While the majority (approximately 70%) of mice used in our experiments will have no significant adverse effects, in some studies the mice can become unwell for a few days. These animals may show weight loss, become less active, have ruffled fur and appear hunched. During models of arthritis, their limb joints may become slightly red and swollen, and the mice may limp when walking on solid surfaces. We also use models of infectious disease, where mice are infected with microbes that cause illness. In most studies the mice can become very unwell and therefore this protocol is listed as severe. Animals will show up to 30% weight loss, become less active and isolated, have ruffled fur and appear hunched. In all our experiments, mice which become very ill are closely monitored and killed as soon as possible, once we have the scientific data we need. We anticipate that no more than 20% of the animals will show severe severity and for less than 24hrs. The time when we kill the animals has to have strong scientific justification and be agreed with the senior animal technicians and the veterinary surgeon. We have already established a very thorough system for monitoring these animals, which we use to minimise pain and suffering as much as possible. All animals are killed at the end of the study and their cells, organs or tissues are used for scientific analyses. **A retrospective assessment of these predicted harms will be due by 18 September 2024** 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?
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Our mouse models are for used for studying diseases found in people for which there are no other laboratory or computer based alternatives. However, we make use of non-animal models (such as human cells or laboratory grown cells)

	whenever possible and constantly look for new methods that would enable us to replace animals in research. **A retrospective assessment of replacement will be due by 18 September 2024** 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?
2. Reduction Explain how you will assure the use of minimum numbers of animals	We reduce the numbers of animal we use for experiments, by maximising the number of scientific measurements we make for each animal and identifying and using the correct numbers of animals to give us the most robust scientific results. We also use highly inbred strains of mice of the same sex and age to increase the robustness of our results and cease breeding of mouse strains that we do not use. We also carefully monitor the breeding of all our mouse lines, to minimise the production of excess animals. In our experience, these approaches help to reduce our animal usage by at least 25%. **A retrospective assessment of reduction will be due by by 18 September 2024** 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?
3. 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.	Mice are a worldwide used model to study human disease and the working of the immune system. Most of the tools we need to use for our investigations have been developed for working in mice, including genetically altered animals. We also use rats to generate new tools that we can use in our mouse models, such as monoclonal antibodies. The use of other animal species will not provide the key insights we need to understand human disease. We make sure that our experimental approaches are the most appropriate and performed as best as they can be. We are constantly scanning the scientific literature and talking to other scientists to make

	sure we are using the best possible approaches. Where we start a new line of experimentations, we first make use of a small number of animals (a pilot study) to learn about the impact of these new approaches on the animals and how best to minimise any suffering. Where there is potential suffering for the animals, particularly during our infection protocols, this is minimised by ensuring that all the people performing the experiments are appropriately trained, and through the use of techniques for the alleviation of this suffering (e.g. such as pain relief medication). In all our experiments, we are constantly looking for new ways to improve the well-being of our animals. **A retrospective assessment of refinement will be due by by 18 September 2024** 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?

Project	**27. Toxicology testing of chemicals**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
		Translational and applied research
		Regulatory use and routine production
	X	Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	To produce good quality information on the toxicity of chemicals, largely those that will be used in the oil and gas industry. This information will then be used by the regulator to decide if the chemical should be used, and what controls will have to be placed on the chemical's use. **A retrospective assessment of these aims will be due by 04 January 2025** The PPL holder will be required to disclose: • Is there a plan for this work to continue under another licence? • Did the project achieve its aims and if not, why not?	

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This will protect the environment by letting the regulator know how harmful a chemical will be, and preventing or restricting the chemical's use.
What species and	6000 sheepshead minnows 100 zebrafish 100 turbot
approximate numbers of animals do you expect to use over what period of time?	
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?	The fish could suffer toxic effects from exposure to the chemicals, up to and including death. The procedures are Severe. The fish will be euthanized after testing. **A retrospective assessment of these predicted harms will be due by 04 January 2025** The PPL holder will be required to disclose: • What harms were caused to the animals, how severe were those harms and how many animals were affected?
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The Offshore Chemical Regulation Guidelines (2005/1) state that it is mandatory to carry out a fish toxicity test on all oilfield chemicals or their components. While we also conduct toxicity studies on algae and crustaceans, these are in addition to the fish testing and cannot replace it. **A retrospective assessment of replacement will be due by 04 January 2025** The PPL holder will be required to disclose: • What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

2. Reduction Explain how you will assure the use of minimum numbers of animals	Our standard test is done at a concentration determined by the algae and crustacean tests. By using these preliminary tests we significantly reduce the number of fish that we use. We only run a set of tests when we have 3 or more chemicals to test. This reduces the number of fish that we use as controls. **A retrospective assessment of reduction will be due by by 04 January 2025** The PPL holder will be required to disclose: • How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?
3. 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 strictly follow the guidelines to ensure that our results will be accepted by the regulator. Fish that are not going to survive the test are euthanised immediately to relieve suffering. If a chemical is having a significant effect on a group of test fish, the test is immediately terminated and the fish euthanised to prevent any further suffering. **A retrospective assessment of refinement will be due by by 04 January 2025** The PPL holder will be required to disclose: • With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?

28. Training in complex surgical procedures

Project duration

5 years 0 months

Project purpose

- Higher education or training for the acquisition, maintenance or improvement of vocational skills.

Key words

Training, Minimally invasive, Surgery, New Procedures

Retrospective assessment

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

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Required at inspector’s discretion

Objectives and benefits

Description of the 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: 02 November 2022

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

No answer provided

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.

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?

300 pigs and 100 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, at the end of the procedures, animals are given an anaesthetic overdose and then all possible organs are harvested for use in other studies being conducted by a range of scientists at the institution as well as for use in other training courses.

Retrospective assessment

Published: 02 November 2022

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.

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: 02 November 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?

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: 02 November 2022

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

No answer provided

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: 02 November 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?

No answer provided

29. Training in Complex Surgical Procedures

Project duration

5 years 0 months

Project purpose

- Higher education and training

Key words

Training, Minimally Invasive Surgery, endoscopy, Robotic, Laparoscopic, Trauma, neurosurgery

Animal types	Life stages
Rats	adult
Pigs	adult, juvenile
Sheep	adult

Retrospective assessment

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

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Education and training licence
- Required at inspector’s discretion

Objectives and benefits

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

What's the aim of this project?

This project aims to continue delivering training to surgeons on advanced surgical techniques including open and minimally invasive, surgical procedures including neurosurgery, endoscopy, laparoscopy, robotic and vascular surgery.

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

Surgical procedures continue to improve and ongoing training is required to ensure patient safety. Surgical techniques and technology are also evolving and minimally invasive surgical (MIS) procedures are gaining popularity worldwide nowadays. These procedures include keyhole or natural orifice surgery that can be carried out using endoscopy (camera test through natural orifice), laparoscopy (keyhole through the abdominal wall) and thoracoscopic (keyhole through the chest wall). More recently, all these techniques could be facilitates with the robotic techniques. It is proven that MIS procedures are significantly better for patients compared to open procedures as they are associated with less trauma resulting in faster recovery, less pain, less post-operative adhesions, easier postoperative care and much faster return to active life. Consequently, many new minimally invasive procedures are being developed to replace larger, open procedures across all specialities such colorectal, urology, gynaecology and other digestive disease and vascular surgery. Optimal training on these advanced skills is required as insufficiently trained use of these new procedures results in unacceptable complication rate including higher death rates. As non-animal alternative has yet to be devised that satisfies the requirements of the training of surgeons in these advanced techniques, training on live animals is required to ensure competency before practising on patients. There is also a need to improve the efficiency of the current training pathways due to the increasing demand to train more surgeons to cope with the increasing pressure on the National Health Service (NHS). With the adoption of certain national screening programme in a number of specialities such as colorectal cancer and vascular surgery, these are contributing to identifying 30-40% more patients who requiring surgical intervention for their conditions and the number of surgeons qualified in the new procedures is very limited, resulting in long waiting lists across the NHS. Through this licence, we aim to continue delivering teaching to surgeons on these complex procedures, in terminally anaesthetised animals, to ensure an adequate supply of appropriately trained and competent surgeons. Additionally, management of trauma and unexpected serious events during surgery have not been taught properly due to the lack of simulation (physiological bleeding model), resulting in decreased level of confidence on how to deal with these events on patients. Traditionally, this type of advanced training is run in certain centres in Europe, but give the COVID restrictions on travel and the implications of BREXIT as well as the pressure on the NHS staff to cope with the growing backlog, s local British solution is required to provide this important training in the UK for the safety of our patients. there are courses that are provided in Europe

How will course attendees use their knowledge or skills in their future careers?

These are hands on training of various aspects of advanced surgical techniques. These courses are part of training curricula which often involves mentorship programme following attending the course. This involves an expert travel to the learner's centre to supervise them undertaking these skills during the first few cases to ensure that the learned skills are practiced appropriately. We are also developing assessment tools (formative) that can guide and monitor training and competency.

What are the principal learning outcomes from the course?

Each course has its specific learning objectives.

Robotic Surgery across all surgical specialities: at the end of the course, participants will have knowledge of

· Technical description of commonly used robotic systems
· Coordination hands & pedals / navigation control / energies
· Principles of Robotic Surgery
· Basic concepts in robotic surgery (triangulation, priorities, exposure, tools)
· Injuries, prevention, strategies, troubleshooting
· Role of the team work (nurse, assistant, anaesthetist)
· Robotic operating Room set up
· Ergonomics and organization in a robotic program
· System boost and trouble shooting

Laparoscopic courses

To enhance delegates skill in advanced upper and lower gastrointestinal surgery including morbid obesity, reflux, gastric and oesophageal cancer as well as colorectal cancer. Specific outcomes includes:

safe access techniques and ergonomics
safe identification and dissection of tissue planes
practicing haemostasis and safe ligation/ division of blood supply of organs
safe application of stapling devises, organ resection and restoration when required.

Endoscopic Courses

To enhances the delegates skills in both diagnostic and therapeutic application of endoscopy.

Specific outcomes include:

Safe dissection of a simulated pathology (polyp) in the stomach and or the rectum, including identification of tissue planes

-Safe haemostasis and application of energy sources to secure haemostasis
-Safe application of emerging endoscopic techniques to facilitate better diagnosis and or therapy of endoscopic surgery Vascular and microsurgery Endovascular:

a. Correct deployment of stent
b.Correct activation of coil/plug c, Correct anchorage of stent

Avoidance of endoleaks and procedure for closing if they occur
Avoidance of vessel rupture by recognition of pre-rupture conditions
Femoral nerve protection
Microsurgery

The purpose of this licence is to train and educate practising surgeons to attain microsurgical skills that can be utilised in the clinical practice of their particular speciality. Specific learning outcomes include safe microsurgical techniques to perform:

Dissection of small blood vessels and securing haemostasis
End to end anastomosis of small arteries and veins
End to side anastomosis of small arteries and veins
Graft vein into an artery
Repair of Sciatic Nerve
Emergency Trauma course

A two day course that has been recently introduced to NPIMR to support surgeons needs covering all aspects of emergency trauma of the abdomen and thorax (see a new protocol). Specific outcomes include:

Damage control during major trauma of both abdomen and thorax, including solid organ injuries (liver/ spleen/ kidneys)

Control bleeding from major vessels injury in abdomen and pelvis, including packing; intravascular shunt and vascular repair

Safe application of haemostatic techniques including adjuncts of haemostasis and principles of tissue sealing (see the table)

How are these learning outcomes important to the people on the course?

These are essential skills for surgeons attending these courses to practice and achieve competency prior to perform these advanced skills on patients. Currently, these skills are not taught anywhere else in the UK and given the COVID travel restrictions, there is a clinical need to provide this essential training locally here in the UK.

Who or what will benefit from the transfer of knowledge, or acquisition of skills that this course will deliver?

Patient safety is the ultimate benefit of these training courses. The overarching aim of this advanced training it to reduce surgical errors and near misses which can be achieved with the training at NPIMR.

The potential benefits are outlined here:

1-reduce operative and postoperative complications
2-enhances patient recovery and reduce hospital stay by the adoption of Minimally Invasive Surgery 3- reduce health care cost by providing training locally in the UK
4- improve the efficiency of health service by minimising staff leaves for travel to undertake training abroad

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

Planning to implement a quality assurance process of training to measure the impact of teaching on live animals and the outcome will be published in peer review journals. The outcome of this process will be used to enhance the teaching, improve the adherence to the 3R principles of reduction, replacement and refining the use of animals. We have already started this process and we conducted an independent review on one of our courses (microsurgery) by a group of students from UCL (see attached a consultancy report, evaluating the course and its impact on the students). We are planning to expand this work to other courses.

Species and numbers of animals expected to be used

- Pigs: 1350
- Rats: 3000
- Sheep: 150

Predicted harms

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

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

Pigs are the animals of choice for central vascular access and navigation as the central vascular system is very similar to that of humans. However, pig's legs are too short for peripheral long wire access and sheep would be the animal of choice for these manoeuvres. We will investigate modification of the equipment used for EVAR training to possibly allow simultaneous or sequential training of both central EVAR procedures and peripheral endovascular procedures in the same animal thus introducing reduction and refinement. For all laparoscopic, endoscopic and emergency trauma training, with the exception of head and neck, the animal of choice will be the pig due to its close comparison to man with respect to upper and lower GI tract in addition to organ size and blood volume. However for training involving head and neck, the sheep presents closer resemblance to human anatomy and would therefore, for these courses, be the animal of choice. We will investigate the potential for simultaneous or sequential training in endoscopic head and neck procedures and lower peripheral endovascular procedures in the same animal and, if possible, will adopt that process to reduce numbers of animals used.

For microvascular surgery, that there is currently no acceptable alternative to the use, ultimately, of live rats for gaining microsurgical skills. Simulated tissues can be used to gain basic technical skills but it is only in a live model complicated by the problems of thrombosis, haemorrhage and biochemical reactions to tissue manipulation that the true viability of an anastomosis can be tested.

Alternatives (simulated tissue – silicone membranes and 'mock 'vessels made from soft rubber) are used on the first morning to familiarise surgeons with the use of the microscope, correct handling of micro instruments and sutures, correct anastomotic technique and the placement and tying of micro sutures. Only when competence has been achieved in this exercise can the surgeons go on to complete anastomosis in anaesthetised animals.

Videos are used to demonstrate all exercises of the course and appropriate videos are shown continually during the week for student reference. This avoids the use of separate animals for demonstration purposes.

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

Animals will be anaesthetised and the steps vary according to each protocol.
In Endovascular course, an access to the blood vessel is obtained and stent is inserted

and placed in a blood vessels under X-ray. For microsurgery, blood vessels will be cut and rejoined and for robotic and key hole surgery; an access to the abdomen will be carried out prior to inflating the stomach and set up the robot to start removing certain organs.
All animals will be terminated at the end of each procedure.

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

The impact on the animals will be minimal since they will be under non-recovery anaesthesia for the entirety of the regulated procedures being applied

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)?

Non-recovery in all courses

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

- Killed

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

As yet, there are no simulators that truly represent the full physiological state necessary to teach these complex 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 however, to develop better simulators as these courses progress.

It is the nature of animal laboratories that progress in the knowledge required to perform their various tasks is constantly sought in the scientific press, meetings and conferences as well as personal communications between laboratories. A non-animal alternative has yet to be devised that satisfies the requirements of the training of surgeons in microsurgery.

It is important, however, to be able to develop a feel for live perfused tissue and learn how to deal with the problems of haemorrhage, blood clots and the way that real tissue and

blood vessels behave during handling. A living animal is therefore essential and the rat is chosen for microvascular course as it is the smallest animal in which this work can reasonably be performed. For minimally invasive surgery, the pig and sheep have been chosen 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.

Why can't your aim be met by observing or by participating in ongoing research or clinical procedures?

No answer provided

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

This is based on the current and planned number of courses during the next 5 years across all the four protocols

What in silico or ex vivo techniques will you use during training?

We have a large number of physical simulations (simulated synthetic organs) that surgeons practice on at the initial phase of their training. This applies to all specialities including silicon tubes for microsurgery; synthetic skin for laparoscopic and robotic skills and synthetic bowel for endoscopy manipulation. Additionally, we routinely use ex vivo for bowel surgery (both robotic and laparoscopic) training courses.

Will these techniques reduce animal numbers? If so, how?

Yes, especially with the new adoption of Kindheart model for robotic surgery (see below); as all the first robotic course across all specialities does not involve live animals, which are now only used in the later stage of training (second and third course).

What other measures will you use to minimise the number of animals you plan to use in your project?

In robotic surgery, we have already adopted the use of new semisynthetic models (Kindheart) which involve a combination of 3D printed synthetic materials and ex-vivo tissue to minimise the use of live pigs.

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

Rats for microvascular surgery
Pigs/sheep for endovascular, laparoscopic, endoscopic and robotic training

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

Adult animals are required to survive long surgery including anaesthesia and therefore less sentient animals such as fish or amphibia, or invertebrates are not suitable.

Also adults required due to the size of the tissues and organs used providing a more faithful model for surgeons to practise on..

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

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. Through running several courses, a number of refinement strategies have been in place.

Rats are easily anaesthetised and provide easily accessible vessels of a similar diameter to human finger’s vessels. When repaired, these vessels can be assessed for viability of the anastomosis up to a few hours post-operatively.

We have also taken note of the way other microsurgical courses are run, and their types of refinement, both in this country and abroad and where applicable have introduced new ideas. Through our experience we have been able to structure the courses such that the maximum number of exercises is carried out in each animal used. This has come in part from refinement of instructional technique and in part from refinement of exercises.
When required, we intend to collect blood samples from animals prior to termination for research purposes to minimising harming other animals for this purpose.

When applicable, we will use rats for microsurgery that have already been used in unrelated research project to reduce the overall number of animals that are used at

NPIMR.

Anaesthesia is induced and maintained by skilled assistants. The depth of anaesthesia is assessed frequently and body temperature is maintained by heat from bench lamps and by covering the rats with a small surgical drape. Local anaesthetic is applied topically throughout the exercises (on advice from the veterinarian). Fluids are applied topically via the wound site, to ensure hydration of the animal and to ensure care of the operative field. The named Veterinary Officer and the Named Animal Care and Welfare Officer may also periodically monitor animals throughout the course to ensure animal welfare compliance.

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

LASA Guidelines on Aseptic technique
Quality Improvement Guidelines for Endovascular Treatment of Iliac Artery Occlusive Disease
Dimitrios Tsetis Æ Raman Uberoi. Cardiovasc Intervent Radiol (2008) 31:238–2
Guidelines for the optimization of microsurgery in atherosclerotic patients. Chen HC1, Coskunfirat OK, Ozkan O, Mardini S, Cigna E, Salgado CJ, Spanio S. Microsurgery. 2006;26(5):356-62.

A CONSENSUS DOCUMENT ON ROBOTIC SURGERY This document was reviewed and approved by the Board of Governors of the Society of American Gastrointestinal and Endoscopic Surgeons (SAGES) in Nov 2007. Prepared by the SAGES-MIRA Robotic Surgery Consensus Group

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

Involved in research and publication of novel models that can replace or complement live animals

A retrospective assessment of refinement will be due by 19 May 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?

Project	**30. Understanding the fundamental biology of dangerous pathogens**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Infectious diseases REDACTED pose a serious threat to life. The aim of the work detailed in this project licence is to i) develop accurate models of disease to support our experiments; ii) determine which environmental conditions affect pathogen infectivity to allow us to understand the hazard posed by the disease; iii) determine the effectiveness of existing and novel therapeutics to advise physicians of the most appropriate response in emergency situations; and iv) use animal models to assist in the discovery of factors involved in disease which can be used as the targets in drug discovery programmes to generate the next generation of antimicrobial agents.	

	A retrospective assessment of these aims will be due by 09 October 2024 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?
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The most basic output of this work will be an increased understanding of these pathogens and their interactions with their hosts. This knowledge forms the basis for efforts to combat infections caused by these pathogens by indicating effective medical countermeasure strategies in the short term and providing potential diagnostic and therapeutic targets to be developed in the longer term.
What species and approximate numbers of animals do you expect to use over what period of time?	The expectation is that this licence will use no more than 10,000 adult mice, no more than 700 adult rats and no more than 450 adult hamsters over the 5 year course of the project
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?	One aspect of the work on this licence involves understanding the disease process for a range of serious pathogens. This is to establish accurate models of disease and identify targets for the development of medical treatments. Animals will be exposed to pathogen either by injection (e.g. intra-peritoneal, sub-cutaneous) or by intra-nasal instillation under general anaesthesia or by inhalation whilst physically restrained in an aerosol exposure tube. The procedures by themselves are expected to result in few adverse effects. However, animals exposed to pathogens are at risk of developing disease; they may become ill and without intervention could die. Clinical signs of disease may include piloerection, hunched posture, reduced activity and responsiveness. They might also experience laboured breathing or neurological signs such as circling behaviour, twitching or tilting of the head and progressive limb paralysis; such animals would be classed as experiencing severe suffering and would be killed. In parallel, another aspect of the work on this licence will examine the effects of potential therapies such as antibiotics or vaccines in non-infected animals. These animals will receive these treatments by the same routes as described above for the

	pathogens. The procedures by themselves are expected to result in few adverse effects and the expectation is that the therapies will have few adverse effects. These animals would be classed as undergoing mild to moderate suffering. Finally, the therapies will be tested to see if they work against the disease. The expectation based on information from previous licences is that the majority of medical treatments tested would be successful to an extent so that animals receiving these therapies would be protected from disease and its consequences, either fully or in large part. However, some of the medical treatments may not work well and these animals would be expected to develop disease with all of its consequences. All animals will be killed at the end of studies. **A retrospective assessment of these predicted harms will be due by 09 October 2024** 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?
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non- animal alternatives	The interactions between a disease-causing microorganism and its host are extremely complex and not well understood. This is especially true because the primary interface includes the host immune system, an incredibly interconnected network of host responses involving virtually every cell type in the body. Although some isolated aspects of these host-pathogen interactions can be recapitulated in non-animal systems in the laboratory, only an animal allows the complexity of the host-pathogen interactions to be fully expressed and studied. **A retrospective assessment of replacement will be due by 09 October 2024** 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?
2. Reduction Explain how you will assure the use of minimum numbers of animals	Studies will be designed with the advice of a team of statisticians to ensure each study has suitable levels of power without unnecessary use of animals. Pilot studies will be conducted where appropriate to provide information to inform these power calculations. Where possible, studies will be run in parallel to utilise the same control groups. To reduce inter-animal variation, animals within a study and within a series of studies will be matched in age and weight and studies typically use only male or female animals from a consistent supplier. Similarly, inbred strains of animals will be utilised in order to reduce the inter-animal variability. **A retrospective assessment of reduction will be due by by 09 October 2024** 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?
3. 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 animal models we use in place of humans to study disease are scientifically appropriate because they reproduce the disease seen in humans in terms of severity, eventual outcome and clinical features of disease. There are other animals which could be used in place of mice; these would also be suitable models because they also reproduce the disease in humans. However, these would be higher order animals such as non-humans primates which are perceived to have a higher neurophysiological response than mice and rats. Since disease would manifest with the same severity in these higher order animals as in mice and rats, the rodent models offer the more refined option. This also allows advantage to be taken of the numerous commercially available murine reagents and genetically modified mouse strains which will increase the amount of data that can be obtained from each animal. Animal suffering during the infection will be minimised by close monitoring of animals during study to allow the rigorous application of established humane endpoints. The success of the humane endpoints will be assessed after each study and will be continually refined during

	the course of this licence to alleviate suffering. Unfortunately, keeping the animals sedated for the duration of the studies, which is weeks at a minimum, is not practical, but brief periods of anaesthesia will be used for specific procedures which would otherwise be stressful. Environment enrichment will be provided appropriate to the species and will include nesting materials, plastic and cardboard dome houses, chew block and transfer of own scented material following routine cleaning of cages etc. Where appropriate animals will be familiarised to the procedure (e.g. handling, environment) to reduce stress during the actual procedure. **A retrospective assessment of refinement will be due by by 09 October 2024** 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?

Project	**31. Understanding the mechanistic origins and sequelae of epilepsy at all life stages**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
What's the aim of this project?	In a certain proportion of adults, especially those that are untreatable with current medications, there is a risk of sudden unexpected death in epilepsy (SUDEP) in adults. The aetiology of this condition is not fully understood, but there is the possibility that neurological dysfunction associated with the epileptic fits may lead to cardiorespiratory failure. Our aim is to understand the mechanisms by which epileptic fits could compromise the function of cardio respiratory neurons which in turn could lead to SUDEP, and to look at new treatments that could prevent it from happening. **A retrospective assessment of these aims will be due by 16 June 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?
Why is it important to undertake this work?	In the UK, around 600,000 people – almost 1% of the population – have epilepsy. Of these people with epilepsy approximately 600 die every year from SUDEP. Many more people have to live daily with the risk of SUDEP hanging over them. Whilst it is known that seizure-induced cardiorespiratory autonomic dysfunction is a potential cause of SUDEP, identification of effective therapies to prevent SUDEP is hampered by poor understanding of the underlying neural mechanisms that lead to seizure-induced cessation of breathing and/or circulation. Improved knowledge of microglial activation or inhibition in regulating cardiorespiratory function and seizure threshold/frequency will help to inform the future development of therapeutic interventions. Developments in therapeutic care will ultimately benefit the patients, clinicians and the health care system. It will reduce the duration of hospitalization and disabilities due to uncontrolled seizures in patients with epilepsy and reduce suffering of their family members, and improve the quality of life in lessening concerns over seizures and SUDEP. We hope that in the longer term, the UK health care system will benefit by saving money and resources spent on epilepsy patients.
What outputs do you think you will see at the end of this project?	**Sudden unexpected death in epilepsy (SUDEP)** • Identification of the key cardiovascular and/or respiratory autonomic neurons that are impacted by seizures. The knowledge of functional changes in neurons in specific cardiorespiratory nuclei will help us to understand how these changes may lead to SUDEP. • Potential for development of functional biomarker tests for people with epilepsy who are at higher risk of SUDEP. • Establishing whether inhibition or activation of microglia could be effective in preventing seizures and maintaining the activity of cardiorespiratory networks during acute or chronic seizures. • High impact publications and generation of new intellectual property.

Who or what will benefit from these outputs, and how?	Our project may lead to direct patient benefits in the **short to medium** term: Non-invasive testing of the responses of cardiorespiratory
	autonomic neurons to CO_2 (biomarker test) at different phases of epilepsy might identify people with epilepsy who are at higher risk of SUDEP. This would allow adjustment of lifestyles (e.g. supervision, nocturnal breathing support) to mitigate risk. Minocycline is a commonly prescribed antibiotic, that crosses the blood brain barrier. There is evidence to suggest this drug also blocks microglial activation, and if we can identify that microglial activation could lead to SUDEP then this drug might be a potential intervention to lower the risk of SUDEP. **Longer term** The identification of the mechanisms that underlie SUDEP - specifically the alterations to brainstem neural circuitry may suggest new strategies to lessen the risk of SUDEP e.g. use of stimulants that might act specifically on certain key circuits. Additionally these new therapeutic targets could lead to new drug development or repurposing of existing drugs that already act on these targets.
Will this work be offered as a service to others?	No
How will you look to maximise the outputs of this work?	In addition to disseminating our findings through high quality scientific papers and conference presentations, we shall have a systematic program to engage with a wider audience. This wider engagement will involve: • Dissemination through funders and relevant charities (Redacted) • Engagement with relevant patient groups • More general public engagement e.g. with communities local to the University, Science Fairs, Schools.

Explain why you are using these types of animals and your choice of life stages.	We use mice throughout the project as they are an excellent genetic model. This gives the advantage of a rich suite of genetic strategies to target specific cell populations in the brain either to specifically record their activity, manipulate their activity, or alter their gene expression. These strategies are essential to allow rigorous analysis of underlying mechanism. To study the potential pathways that lead to sudden unexpected death in epilepsy (SUDEP) in man we will use adult mice. Mice have proven to be an excellent model for the acute and chronic states of epilepsy which permit rigorous investigation of underlying mechanisms and are used by many labs around the world.
Typically, what will be done to an animal used in your project?	A small hole will be made in the skull to enable precise injection of virus particles to give expression of a protein that will report the activity of the neuron that it is expressed in by its intensity of fluorescence. The mouse will be allowed to recover and 2 weeks later another hole made into the skull and a special lens implanted into the brain so that the fluorescence can be measured. At this time electrodes to allow the recording of brain waves and heart signalling, and a cannula to allow delivery of a substance to induce seizures, will also be implanted. The mouse will be allowed to recover and a further week later a special baseplate will be glued to the head in final minor surgical procedure. This baseplate allows mounting of a mini-microscope that will allow recording of neural activity as the mouse behaves normally. Following this preparative work we will record activity of neurons in these mice for up to 4 months. Seizures will then be induced by brief infusion of kainic acid through the cannula. This will cause acute seizures, followed by the establishment of a chronic epileptic state where the mouse will exhibit occasional spontaneous seizures. We will also study neuronal activity in this chronic state.
What are the expected impacts and/or adverse effects for the animals during your project?	The mice recover well from the surgical procedures and show minimal adverse signs either during or between recording sessions. Following kainic acid treatment, the mice will experience acute seizures for up to an hour. This will produce longer term changes in animal behaviour such as increased anxiety, memory/learning deficits and chronic epilepsy.
What are the expected	For about 75% of animals the expected severity is severe.

severities and the proportion of animals in each category (per animal type)?	For the remaining animals on the protocol it will be moderate.
What will happen to animals at the end of this project?	Killed **A retrospective assessment of these predicted harms will be due by 16 June 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?
Why do you need to use animals to achieve the aim of your project?	This project seeks to understand phenomena that arise in the whole brain, and rely on interconnections within the brain such as: the mechanisms that underlie acute and chronic seizures; and how seizure activity can disrupt autonomic activity -specifically the neural circuits in the brainstem that control breathing and cardiovascular regulation. Catastrophic disruption of the activity in these neural circuits is likely to underlie SUDEP. These phenomena can only be studied in whole animals and tissues taken from animals, in which seizures have been previously induced. It would be unethical to use human subjects in these experiments. Brain organoids replicate to a very limited extent some of the circuitry of the brain. While these are unlikely any time soon to give detailed recapitulation of brainstem cardiorespiratory networks suitable for our aims, we shall monitor developments in this area, in case advances may make them suitable for investigating cellular and molecular mechanisms within the relevant neuronal subtypes and subcircuits.
Which non-animal alternatives did you consider for use in this project?	Induction of seizure like activity in isolated brain tissue

Why were they not suitable?	Seizure induction in isolated tissue: These models are acceptable for investigation of rather general phenomena related to the mechanisms of seizures. Brain slices are typically cut from one region. Our investigation of the mechanisms that could lead to SUDEP requires an interconnected brain whereby a seizure initiated in the cortex can invade, through many interposed steps, the key brainstem nuclei involved in cardiorespiratory control. It is impossible to achieve this type of interconnectivity in vitro. In vitro models are inadequate to progress the aims of the project. **A retrospective assessment of replacement will be due by 16 June 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?
Enter the estimated number of animals of each type used in this project.	mice: 300
How have you estimated the numbers of animals you will use?	We have performed power calculations based on expected effect sizes and consulted statisticians to optimize our experimental program. Power calculations from our previous experiments suggest that we will require around 100 mice to achieve our aims. However, if we find that particular subsets of neurons are affected by seizures we may need to perform further analysis with genetic targeting of these neurons. To allow for this we have anticipated that this may be the case for 2 additional populations and adjusted total numbers accordingly.
What steps did you take during the experimental design phase to reduce the number of animals being used in this project?	We took statistical advice on the design of the experiments. We examined the NC3Rs Experimental Design Assistant, but concluded that we did not need to use it for our studies at this stage. We have attended NC3Rs 2019 Symposium to keep abreast of new thinking in the field. We used the best estimate of effect size based on pilot data or published literature to inform our power calculations.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?	Planning and execution of our experiments will be according to best practice as exemplified by the PREPARE guidelines (Lab Anim. 2018 52:135-141. doi: 10.1177/0023677217724823). https://norecopa.no/PREPARE Pilot studies will be performed to get good estimates of effect size. Should our program demonstrate that our estimates of effect size are wrong (either too big or too small) we shall reperform the power calculations to ensure we arrive at a rigorous outcome. If it appears that the effect size is much smaller than anticipated for a particular experiment, we shall consider abandoning that part of the program. We shall routinely harvest tissue to enable biochemical, genetic, cellular and morphological analyses and share this tissue with our collaborators. The in vivo microscopy permits repeated imaging sessions from the same animal and this reduces the number of animals required in the study as the repeated measurements will give greater statistical certainty and will allow study of the acute and chronic stages of epilepsy in the same animal. **A retrospective assessment of reduction will be due by 16 June 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?
Which animal models and methods will you use during this project?	Rodents are a well-established model for studying the neural mechanisms underlying epilepsy. This gives a robust literature and wealth of potential data obviating the need to repeat past findings. Rodents in general are seen as a simplified and experimentally tractable model for far more complex mammals such as humans. Wherever possible, physiological testing of phenotypes will be achieved by using non-invasive methods such as: whole body plethysmography for detection of breathing movements. More invasive procedures will be used only when there is good reason (from the non-invasive experiments) to expect mechanistic insight.

Why can't you use animals that are less sentient?	Immature life stage: Mouse pups cannot be used because the cardiorespiratory control circuitry is still developing and will not model the adult circuits. As SUDEP occurs in adulthood, we need to study the effects of seizures on the mature brain stem circuitry. Sentience: Lower vertebrate species such as fish, lamprey, frog, while being able to generate seizures are not a good model for epilepsy and its effect on cardiorespiratory control networks as their cardiorespiratory control networks do not correspond to those of mammals. By choosing mice, we have selected the least sentient model to study the relevant underlying mechanisms that could lead to SUDEP Terminal anesthesia: Terminal anaesthesia alters the operation of the brainstem networks by for example, disinhibiting certain networks and generally depressing respiratory drive. Thus, use of
	terminal anaesthesia will not allow us to uncover the relevant mechanism of altered neuronal signalling that may lead to SUDEP.
How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?	We subscribe to the NC3Rs newsletter and we receive update Emails from this organisation. We make efforts to attend their symposia. We network with colleagues from other universities to ensure that we stay abreast of any developments. As an example of our openness to best practice, we have implemented improved mouse handling procedures designed to minimize stress as a result of coming from advice from NC3Rs.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?	We have considerable experience with the *in vivo* imaging methods and have considerably refined them already over the past 2-3 years. We have implemented standardised post-operative monitoring methods to ensure that outcomes and adverse effects from our surgical procedures are carefully assessed. We use this information to optimise post-operative care and pain management. We shall use rigorous aseptic techniques to protect against infection of the animals. We train the animals with a dummy camera to acclimate them to the imaging procedure and to the imaging cage to minimize stress. As imprecise targeting of viral injections can increase animal usage, we shall be very careful in this aspect of our procedure. We shall ensure we use calibrated stereotaxic frames, check the alignments thoroughly and regularly use the injection of fluorescent beads (in a non-recovery terminal procedure) to ensure that correct targeting is achieved prior to commencing the lengthy procedures required for the SUDEP part of the project. We continually look to refine and improve our experimental models and procedures. This is a regular topic of discussion in weekly lab meetings.
What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?	We shall take careful note of advice from collaborators and researchers highly experienced in these methodologies to ensure that we refine our experiments as much as possible. We shall follow the Laboratory Animal Science Association guidelines on performing aseptic surgery to ensure best practice for the recovery surgery involved in all surgical procedures. We shall follow the guidelines published in J Neurosci Methods. (2016) 260:2-25. doi: 10.1016/j.jneumeth.2015.09.007 which comprises a NC3Rs-sponsored metareview of models of epilepsy and has comprehensive advice on best choice of mode and refinements. Planning and execution of our experiments will be according to best practice as exemplified by the PREPARE guidelines (Lab Anim. 2018 52:135-141. doi: 10.1177/0023677217724823). https://norecopa.no/PREPARE To ensure effective and rigorous reporting of our results, we shall write papers according to the ARRIVE guidelines which are recognised as providing excellent transparent standards for reporting of research using animals and were developed by the NC3Rs.

Project	**32. Understanding the signals that regulate liver development, repair and cancer.**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The liver houses a network of tubes that moves bile from your liver into your intestines where it is needed to help digest food. There are lots of questions though around how these tubes are formed in the embryo as the liver grows and also how, following damage to these tubes in an adult, they regrow to ensure the liver continues to function normally. The first two aims of this license seek to address these questions. Cells do not just happen to form into ducts – it requires the cells to talk to each other and understand where they are and	

	importantly who their neighbours are – cells do this using special proteins. By understanding these proteins we can alter them to see if we can change the way a liver develops (which is important for patients who's liver doesn't develop in the right way, such as children who have a disease called Alagille Syndrome) or we can try and help the liver regrow in the adult following injury. Over the last five years, my lab has found that lots of the proteins that allow the liver to grow normally in the embryo or in the adult are used by liver cancers to help them grow. The final aim of this license is to see whether we can find out how these proteins help a liver cancer to grow and by working with other scientists and companies who develop medicines, figure out whether we can alter these proteins and what they do to slow down or stop liver cancers growing. **A retrospective assessment of these aims will be due by 18 January 2025** The PPL holder will be required to disclose: • Is there a plan for this work to continue under another licence? • Did the project achieve its aims and if not, why not?
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	There are an increasing number of people in the UK with liver disease and as a consequence of this there is an increasing number of people in the UK with liver cancer. The work we will do during this license will help us understand 1. How liver disease develops in adults 2. How liver disease can become cancer and finally, whether we can develop medicines with other universities and with companies that can help people with liver disease.
What species and approximate numbers of animals do you expect to use over what period of time?	In this study, we will use both mice (approximately 3000 over the five years) and rats (1000 over five years), some of which will be genetically modified. Most mice will be used for relatively short studies to understand how liver diseases progress and cancers form; however, some mice and the majority of rats will be used longer (for around 1 year) to understand how cancer grows.

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?	For 8 in 10 of the animals used in this study, most animals will experience moderate severity procedures and might lose 20% of their bodyweight; however for many of these animals they will not reach a 20% weight loss and will only experience modest loss of weight. Throughout, we will closely monitor animals and have developed a number of interventions that can support animals if they become unwell. For 2 in 10 animals During this license, 2.5% of animals (5 in every 200 animals) will be subject to a severe protocol (mainly rats) we will use a severe protocol, where animals will develop progressed and advanced liver cancer. These animals will experience weight loss and could progress to a point where their livers begin to fail, we will closely monitor animals for signs of this and mice will be provided with support. Following the study animals will be culled using a recognised procedure and we will study the livers and other tissues from these animals. **A retrospective assessment of these predicted harms will be due by 18 January 2025** The PPL holder will be required to disclose: • What harms were caused to the animals, how severe were those harms and how many animals were affected?
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Organs are made up of lots of different types of cells and we want to understand how these cells talk to each other in disease and cancer. We do use human tissue and human cells wherever possible, and we have also developed ways of growing these cells in the lab so that they behave more like adult tissue. But a limit of this is still growing lots of different cell types together which are coordinated into a tissue. For this, we require animals and even lower organisms, such as flies, do not have the same level of tissue complexity that we find in mice and human. **A retrospective assessment of replacement will be due by 18 January 2025** The PPL holder will be required to disclose: • What, if any, non-animal alternatives were used or explored after the project started,

	and is there anything others can learn from your experience?
2. Reduction Explain how you will assure the use of minimum numbers of animals	We use minimal animal numbers throughout all experiments by performing calculations (known as power calculations) which predict how many animals we need. We also use the very best models available that mean each animal gives us essential information, with little wastage. Tissues from the animals we use in research are stored and kept for future analysis, meaning that we do not need to repeat the experiments to generate further tissue. A major limitation of using genetically modified animals in cancer research is that we must generate large numbers of mice that do not have the right genetic changes in order to breed enough to use in our studies. We have developed a new model, where we can use wild-type mice and change cancer genes directly in the liver without having to breed lots of mice. This has saved us having to breed ~250 mice in the last year that would not have been usable in our studies. One of the changes that we are beginning to make now is to use new small animal approaches to watch diseases form and cancers grow. These approaches are similar to MRI or PET imaging that are used in hospitals. **A retrospective assessment of reduction will be due by by 18 January 2025** The PPL holder will be required to disclose: • How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?
3. 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.	Over the course of our previous license we have tried to make the liver disease and liver cancer models we use the very best we can. We have introduced a high protein diet to mice and rats that have established cancer as we have found that this prevents some of the weight loss seen in these animals. We have also established a score to look at how animals' appearances have changed over the time of disease and cancer. This way we have a record of any changes and know what to look out for in future experiments. **A retrospective assessment of refinement will be due by by 18 January 2025** The PPL holder will be required to disclose:

	• With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?

Project	**33. Zebrafish Models for Neuromuscular Diseases**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
	X	Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Inherited neuromuscular diseases such as muscular dystrophy are currently incurable and have a devastating impact on patients and their families as well as requiring substantial resources from the health service. Our overall aim is to examine in animal models how changes identified in the DNA of patients lead to the development of a neuromuscular disease and to evaluate existing and novel pharmacological treatment options. Muscle, brain and heart are the organs affected by the diseases we are studying; all of these are very complex organs made up of multiple cell types. This means that cell culture models, which generally consist of a single cell type, have limited	

	applicability to patients. Therefore, many pre-clinical studies can realistically only be achieved in whole animals. In these very basic studies into the underlying basis and mechanisms of disease we have elected to use the simplest vertebrate model available, the zebrafish. Zebrafish have a number of significant advantages for these studies including a short time to reach maturity, transparent embryos (which can be viewed under a microscope) and a muscle structure closely related to mammalian muscle (unlike invertebrate models). **A retrospective assessment of these aims will be due by 07 May 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?
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The major benefit of this research is to the scientific community by progressing our understanding of how deficiencies in specific genes lead to a neuromuscular disease. By providing this basic understanding we aim to enable the development of novel and better treatments for patients with neuromuscular disease, although much of this more directed research lies outside the scope of this project. We also aim to test drugs for their efficacy and mechanism of action to inform ongoing drug development programs. In the future, we hope that the continuation of our research will also benefit patients and their families.
What species and approximate numbers of animals do you expect to use over what period of time?	We estimate that we will need to use approximately 20000 zebrafish over the 5 year course of the project. The majority of adult fish will be used for breeding purposes only. A small number of zebrafish (250) between the age of 0-3 months will be used for pilot studies to assess dosage toxicity and effectiveness of pharmacological compounds. Approximately 4750 animals, mainly young embryos and larvae, will be used for testing pharmacological treatments. We minimise the number of zebrafish used by keeping breeding pairs to a minimum and using efficient genotyping strategies.

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?	The experiments that we undertake involve the use of animals as models for human neuromuscular diseases and so the effects on the animals in part reflect those diseases (muscle weakness and wasting). However, we will limit the effects on the animals to the embryonic period wherever possible and so the expected level of
	severity is moderate. The zebrafish embryos will be used for the creation of new strains and to test pharmacological compounds and so may exhibit muscle weakness and wasting as a consequence of the genetic alterations. A small number of zebrafish (250) will be exposed to therapeutic compounds as part of dosage pilot studies, however as the optimal dose of these compounds will be unknown, there is the potential for adverse side effects. Adverse effects of specific therapeutic compounds are difficult to predict in advance even when in-vitro laboratory testing has been undertaken as this is unlikely to be relevant to zebrafish. Therefore, we cannot rule out acute toxicity and lethality in the case of novel experimental therapies. Consequently, the adverse effects have the potential to be severe. All animals will be monitored closely during and after the pilot to look for any signs of distress and will be humanely killed if adverse effects present. Adult zebrafish will be used solely for the purpose of breeding and are not expected to exhibit adverse effects. Most animals will be humanely killed at the end of the experiments except those required for breeding who will be expected to be only mildly affected, if at all. **A retrospective assessment of these predicted harms will be due by 07 May 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?
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Muscle and brain are complex organs consisting of multiple cell types which interact both during development and as a mature tissue. Unfortunately, currently it is not possible to model this structure in cell culture (which generally consists of single cell type) with sufficient fidelity to make the conclusions drawn applicable to patients. Where possible, we use primary cells derived from patients in parallel to investigate those aspects which can be addressed in this way, but these studies are of limited scope in terms of understanding the impact of changes found in patients on muscle and brain function as a whole. To date, the only way of studying these aspects is by using animal models. **A retrospective assessment of replacement will be due by 07 May 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?
2. Reduction Explain how you will assure the use of minimum numbers of	Adult fish between the ages of 3 months to 2 years are required solely for the purposes of breeding and production of embryos (which form the basis of our experimental protocols). There is

animals	no aim to produce adults with a neuromuscular disease. We reduce animal numbers wherever possible by reviewing our experimental data rapidly following an experiment and planning follow up experiments to resolve outstanding experimental questions. In this way the information generated by our research is maximised while experimental animal use is minimised. **A retrospective assessment of reduction will be due by by 07 May 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?
3. 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 protocols on this license are designed to minimise any effects on zebrafish after 5 days of development, where the animals have more substantial capacity for suffering. We aim to restrict any harmful effects where at all possible to the embryonic stages where we can closely monitor the health of embryos and humanely euthanize severely affected individuals before they develop into hatchlings. **A retrospective assessment of refinement will be due by by 07 May 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?

34. Provision of Biological Materials

Project duration

1 years 10 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 aims mentioned in purpose (b)

Key words

Provision, Blood, Biological, Materials

Retrospective assessment

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

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Required at inspector’s discretion

Objectives and benefits

Description of the 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 is to provide a service for the supply of blood and other biological materials for use as controls in diagnostic testing, quality assurance, and research projects requiring biological materials. The samples supplied from the various farm animal species will be used in evaluating scientific tests for a variety of animal diseases. The project is demand driven and all requests for samples will be ethically approved and only supplied once a written case outlining why the samples are required and why no alternative source is possible. Whenever possible blood collection at post mortem will be used. However there are some tests and projects which require fresh blood free from any post mortem changes. The number of animals used will be kept to a minimum by combining the needs of research groups and other users. The samples will be used to further develop and improve animal diagnostic tests related to the required species for a wide variety of farm animal diseases. Blood collection will be from superficial vessels and the techniques used will be of mild severity with no expected adverse effects. This provides control material for a large number of different tests some of which are statutory and also a number of research projects involving farm animal species.

Retrospective assessment

Published: 02 February 2021

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

Yes

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

This work is continuous as there is an on going requirement for the supply of biological materials such as negative control blood. The work will continue to be carried out under a new project licence.

This project is to provide a service for the supply of blood and other biological materials for use as controls in diagnostic testing, quality assurance, and research projects requiring biological materials. The samples supplied from the various species were used in evaluating scientific tests for a variety of animal diseases.

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 main benefits in the licence is allowing peer reviewed research to be undertaken with known control material resulting in a variety of scientific publications and outputs. The tests (including Quality Assurance functions) and products that require blood or sera to underpin vital statutory functions and animal health benefits.

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?

Cattle 50 Sheep 100 Pigs 50 Goats 10 Horses 5 Chickens and Turkeys 200.

These use of these animals will be spread over the 5 year life of the licence with lot of the animals being sampled intermittently over periods of months or years.

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?

Mild severity, the only effect should be insertion of needle to remove the blood sample. Animals will either be killed by a schedule 1 method at the end or kept alive at the Designated Establishment if they are assessed as not suffering or not likely to suffer as a

consequence of the regulated procedures applied. The criteria for this assessment will have been determined by the Named Veterinary Surgeon in advance and records will be kept in a form agreed with the Home Office Inspector. Animals will only be re-used in this protocol if: a) The actual severity of any series of regulated procedures that have previously been applied to the animal, is no more than mild; b) A veterinary surgeon with knowledge of the lifetime experience of the animal or animals has advised that their general state of health and wellbeing is likely to have been fully restored following the application of the previous series of regulated procedures and that the animal is free of adverse effects arising from the previous regulated procedures.

Retrospective assessment

Published: 02 February 2021

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

The actual harms were from the insertion of a needle to take the blood sample. Although all are classified as mild the needle is a larger gauge for large volume blood sampling so potential for more tissue damage.

Pain caused by the insertion of a hypodermic needle according to good veterinary practice, during the sampling.

Stress caused by restraining the animals during procedures. This is particularly the case for pigs which vocalise during restraint. The use of the snare is likely to cause temporary pain although as soon as the pigs are released they appear normal and are rewarded with a treat. We are using clicker training to associate the snare with a treat, and to facilitate moving the pigs to the sampling area thus further minimising aversion and distress. Horses used under this licence are chosen on the basis that they are easy to handle and relaxed about being sampled. There has been no aversion to sampling detected and the sampling is carried out with minimal restraint in their usual field. We had one issue after a horse bleed when the horse developed a medium size haematoma around the jugular puncture site. This cleared up within a few days and the horse never suffered any ill effects nor showed signs of any pain. As a pre-caution the horse had a lengthy break from bleeding and was sampled under direct NVS supervision the next time it was used.

Pigs
Some of the older pigs used for supplying samples got very large (due to age and fat deposition) and became difficult to bleed due to their size; one also became aggressive when restrained. These were culled and replaced by new smaller pigs and a new diet was introduced to minimize the chances of obesity.

Faecal samples can be collected from the floor without affecting the scientific requirement

Cattle
The cattle had been used to provide Bovine Viral Diarrhoea (BVD) antibody-free blood samples The cattle that were previously used were found to have been exposed inadvertently to the virus and became antibody positive. Two new cattle were tested to confirm BVD-free status and used under the licence. To ensure that animals remain antibody-negative we cannot put them out on pasture as this may increase the likelihood to get exposed, so the two cattle may have to stay indoors but it will reduce sourcing of

more cattle to fulfil further requests.

Sheep

The large volume blood samples being supplied in anticoagulant solution to were found to occasionally clot. Refinements have been made to the sampling procedure to prevent further issues. Clotting still happens sporadically. There are ongoing discussions and NVS involvement in the procedures to see what further refinements can be made.

Horses

One horse maintained the status of being a low-level reactor for Equine Viral Arteritis. A serum sample from the first test bleed and a serum sample from the last test bleed were run in tandem. They both returned a result that is negative for import/export reporting but not the flat negative that is required for our purposes. An amendment to the licence was made and a Place Other than a Licensed Establishment (POLE) added because there may be an increase in testing requirements and the EVA negative horse cannot meet the demand alone.

One horse was euthanased due to concerns over his general health and old age (26 years old). The horse had been retired from sampling earlier in the year and was euthanased before the onset of winter.

We had one issue after a horse bleed when the horse developed a medium size haematoma around the jugular area. This has now completely cleared up and the horse never suffered any ill effects. As a precaution the horse had a lengthy break from bleeding and will be bled next under direct NVS supervision.

Replacement

State why you need to use animals and why you cannot use non-animal alternatives.

Blood and faeces are the basis for the majority of laboratory tests involved in animal health and veterinary diagnosis. This licence supplies material to tests that are already established for control purposes or in the process of being developed. Wherever possible sample collected post mortem will be used to supply the need for material, however as these laboratory tests are used to diagnose disease in the live animal, there is often the requirement to use fresh material, to avoid post-mortem changes and contamination issues.

Retrospective assessment

Published: 02 February 2021

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

The only replacement possible on this PPL is the use of blood collected at post-mortem. This is done where possible when large volumes are requested

Reduction

Explain how you will assure the use of minimum numbers of animals.

The number of animals used is minimised by re-using them. This allows a small number of animals to provide control blood across the establishment and other research establishments.

The experimental design is based on matching demand for blood through the request form to numbers of animals maintained to supply the blood.

Retrospective assessment

Published: 02 February 2021

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

This project licence has taken over the supply of blood previously done under another project licence to minimise the number animals used. There were two groups of animals kept, one for each licence, but compiling the supply of control blood under one licence meant one group of animals could be used rather than two with the associated reduction in numbers required. A sampling rota was set up to ensure equal use of the animals supplying the blood.

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 institution and its partner organisation deal with a wide range of farm animal diseases both in a research and diagnostic capacity.

Requests for samples from different groups will be combined whenever possible.

The department has a group of experienced and knowledgeable staff who are able to perform the necessary techniques quickly and efficiently. Having licence holders who are proficient at the techniques required is essential for good sampling practice, which minimises any animal stress and keeps animal handling to a minimum.

Analgesia/anaesthesia will be used where appropriate.

The severity limits of this protocol are mild.

Retrospective assessment

Published: 02 February 2021

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

The pigs used for the purposes of this licence have an outside paddock that they have access to during the day. This has led to a reduction of foot issues/lameness and greatly

improves their enrichment. It also allows for increased activity level and socialising. Clicker training is used to facilitate sampling and avoid aversion.

A number of large volume equine bleeds were requested – 1000ml. The project licence holder negotiated with the client and these samples were split into two smaller samples. Refinements have been made to the sheep sampling for to prevent the clotting and contamination of the samples. The sheep are now sampled indoors to prevent wind blowing debris into the sample pots. The sampling technique has also been improved, from allowing the blood to flow into the sample pot using a needle connected to tubing and gravity to using a syringe and needles to allow the blood to be taking quicker to help prevent the blood from clotting during the extraction process as this may have been a potential cause.

Some of the older pigs used for supplying samples got very large (due to age and fat deposition) and became difficult to bleed due to their size; one also became aggressive when restrained. These were culled and replaced by new smaller pigs. Also a new diet has been introduced to keep their weight to the minimum it should be for their age on the advice of the NVS, as they were carrying too much fat making it difficult to elevate the head enough for blood sampling, as well as the potential to cause joint and foot problems; this has already resulted in being able to get blood from the largest oldest pig that was kept. Also on occasions the pigs sit down which can cause difficulty in obtaining the blood, it can then be challenging for them to get back to standing due to the slipping even when straw or shavings are under foot, so we have decided to try standing them on a rubber mat to prevent slipping and scuffing their hocks. The mats have made an improvement to the sampling procedure.