Home Office

Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2019 that require a retrospective assessment
Volume 1 (A to M)

Contents

Project	**1. A practical training in microvascular techniques for surgeons**	
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
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
	X	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 licence is to train clinicians in microvascular surgery to improve skills necessary to increase the success rates of microvascular surgery. Due to the complexity of the anatomy in the head and neck region, very often the resulting defects from surgery to cure other conditions, e.g. cancer, require complex reconstructions to optimise function in terms of speech and swallow and quality of life for patients. This is achieved through microvascular “transplantation” of tissue from other parts of the patient's body. The	

	success of this procedure (best published outcomes around 95%) hinges on the critical step of joining (or anastomosing) small arteries and veins under microscopic magnification. This is an extremely challenging and exacting procedure. The consequences of flap failure are devastating for patients. It leads to prolonged length of stay, repeat further operations, worse overall outcomes, infection and in extreme cases can lead to death. Due to the devastating consequences of even very small technical errors in this step it is crucial that training is to an extremely high standard. It is increasingly difficult for trainees to gain practical experience at performing this and consequently developing the required skills. Understandably a learning curve at the expense of patient care is not acceptable and there are growing concerns over the training and experience levels of the next new generation of surgeons and it is now deemed compulsory by the major training committees for trainees to have attended a training course in microvascular surgery.
Retrospective assessment	**Retrospective assessment** Published: 04 May 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?** No work was carried out under this project licence. This was principally due to the Covid epidemic. The first training course was due to run in the Spring/Summer of 2020 which was not possible due to Covid and the pressures on the NHS surgeons who were due to participate. I have now changed job roles and it is no longer within the remit of my work to manage this project and so the project licence has been revoked before its initial expiry date.

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 previously alluded to, increasing the success rates of microvascular surgery would greatly reduce patient morbidity and potentially mortality, in addition to massive cost saving potential for the NHS in terms of length of stay, infection rates and return to theatre rates. This project licence could also improve the number of surgeons who are skilled in the microvascular procedures outined in this licence, of which there is currently a shortage.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use a maximum of 1500 rats over a 5 year period, running approximately 4 courses per year for up to 12 surgeons per course.
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 likely/expected adverse effects are very minimal as all procedures will be carried out under anaesthesia throughout. Animals will be killed at the end of the procedure, whilst still under anaesthesia.
Retrospective assessment	**Retrospective assessment** Published: 04 May 2023 **What harms were caused to the animals, how severe were those harms and how many animals were affected?** There were no harms to animals under this project licence as no work was carried out.
Application of the 3Rs	

1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It is not possible to replicate the complexity of learning to perform anastomoses of pressurised vascular systems in non-animals systems. Several crude simulations exist to introduce basic level trainees to the concepts of suturing small vessels but the reality is that the ultimate success of a vessel anastomosis is judged on whether blood flow is maintained across it. This is also a product of turbulence and clotting factors in blood, which can not be simulated, nor can the precise careful tissue handling and preparation necessary. For these reasons high level training and acquisition of skill can only be achieved through the use of animal models. Alternative learning strategies have not been rejected but instead will form an integral part of the course in which this project licence is used. These include, for example, lectures, videos and computer simulations. We do not consider it possible to replace all teaching demonstrations with on-line workshops as there is no replacement for hands-on experience.
Retrospective assessment	**Retrospective assessment** Published: 04 May 2023 **What, if any, non-animal alternatives were used or explored after the project started, how effective were they and are there any lessons worth sharing with others?** No non-animal alternatives were found during the course of the project licence.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In order to use the minimum number of animals possible, a wide range of blood vessels will be operated on in a small number of rats rather than concentrating on the same vessel, e.g. carotid artery, in a larger number of rats.
Retrospective assessment	**Retrospective assessment** Published: 04 May 2023 **How did you minimise the number of animals used on your project and is there anything others can learn from your experience?** No animals were used under this project licence.

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.	All work being carried out under this project licence will be under anaethesia throughout.
Retrospective assessment	**Retrospective assessment** Published: 04 May 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?** No animals were used and so refinements were not identified during the course of the project. We had looked to refine as much as possible in the planning stages for this project.

Project	**2. Acute Inhalation Toxicology**	
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
	X	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)	The evaluation of test substances (for example industrial chemicals, agrochemicals and pharmaceuticals), for effects following administration via the inhalation route. Methods used will comply with published/accepted test guidelines with methodology utilised not exceeding those specified in the relevant OECD test guideline (OECD 433 or 403 Guidelines which this project covers). Such studies are only required when it can be demonstrated that the substances in question has the ability to present an inhalation hazard to humans and suitable data do not already exist.	

	A retrospective assessment of these aims will be due by 19 November 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 results of these studies allow regulatory bodies to control the manufacture, transport and supply of new products and to make the necessary risk assessment for human and environmental exposure. The results of these risk assessments enable the appropriate risk management strategy to be enacted and this can include the appropriate classification, labelling, hazard communication, transport limitations or banning of substances.
What species and approximate numbers of animals do you expect to use over what period of time?	Rats, and mice and guinea-pigs, approximately 4000 over a five year time 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?	Exposure to test substances can cause a range of effects ranging from mild, transitory respiratory rate effects to mortality, moderate to severe signs of toxicity are expected with the majority of animals exhibiting moderate severity (cumulative) during each study period. Animals that are considered unlikely to survive or are obviously in marked pain and/or in distress will be humanely killed in a timely manner. All humane kills are recorded. Regulation (OECD 433) Criteria for making the decision to humanely kill an animal are characterised by signs of toxicity that may be predicted to culminate in greater than moderate toxicity (from the day after exposure). These may include cyanosis, body weight loss of more than 15% over a 7 Day period, hypothermia, dehydration, absence of voluntary response to external stimuli, coma, major changes in posture and locomotion and respiratory

	distress. If earlier signs of evident toxicity predicting death can be identified these earlier endpoint will be used. Regulation (OECD 403) Criteria for making the decision to humanely kill moribund and severely suffering animals are characterised by severe signs of toxicity. These may include cyanosis, emaciation (body weight loss of more than 25% over a 7 Day period), hypothermia, dehydration, absence of voluntary response to external stimuli, coma (which prevents the animal from reaching food and water for up to 24 hours), severe abnormal changes in posture and locomotion and severe respiratory distress. If earlier signs of evident toxicity predicting death can be identified these earlier endpoint will be used. Exposure Procedure: Restraint during exposure (nose-only exposure) may cause stress and mild discomfort to some animals. Care will therefore be taken to ensure that the minimum possible restraint is used whilst ensuring the animals cannot avoid the test atmosphere. Both food and water will be withheld during the exposure period. Additional Procedures (where required): Measurement of body weights will be conducted as specified in the Study Plan. Animals will be handled according to best practice so as not to cause them any undue stress. It may (on occasion) be necessary to take blood samples, samples will be taken from the suitable blood vessel. The procedure is considered to elicit the same sensation as having a blood test at the doctors or hospital. Animals will be placed in a warming cabinet (for no longer than 15 minutes at 40˚C) prior to the procedure taking place (where considered appropriate) to facilitate bleeding due to widening of the blood vessels which has the effect of increasing blood flow. A series of functional observations designed to assess neurotoxicity may be performed, the procedures conducted may cause minimal to mild stress to some of the animals. Urinalysis may be performed, animals will be held in metabolism cages overnight so that urine can be collected, food will be withdrawn for the duration but only minimal to mild stress is envisaged due to handling. Ophthalmoscopy (assessing the structure of the eye) may be performed. Instillation of Mydriatic solution to the eyes may

	be performed to aid this procedure. Minimal/mild stress may be caused to some of the animals due to the handling/restraint involved. All animals will be humanely killed via a schedule 1 method at the end of each separate study. **A retrospective assessment of these predicted harms will be due by 19 November 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	There are currently no non-animal (*in vitro*) methods suitable for the replacement of these protocols nor are there, as yet, any accepted protocols to enable further reduction in the severity of these studies or in the number of animals used. If the data are not required for formal submission as part of a regulatory package, it is sometimes possible to perform a screening study using reduced animal numbers in order to show that the test item is/is not harmful/toxic. The studies can be used to investigate a wide range of endpoints (including pathological examination of direct tissue or cellular effects in a wide range of target tissues each composed of multiple cell types) and examine mire subtle toxic effects (including behavioural modifications and neurotoxic changes). The complex interactions involved in systemic toxicity are such that these effects can, currently, only be effectively studied in live animals. **A retrospective assessment of replacement will be due by 19 November 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	For all dose groups for the OECD 433 test

Explain how you will assure the use of minimum numbers of animals	guideline, the total number of animals (five animals of one sex per exposure) that are used (in order to determine classification of the test item) is reduced in comparison to the other test guidelines (OECD 403) and as such this test guideline is now the preferred method to be followed within this establishment. When the test item is expected to be relatively non- toxic (not expected to cause death), the OECD 403 test guideline allows the use of a reduced number of animals at the limit test concentration (3 males and 3 females or six animals of the most susceptible sex). Mortality data obtained is processed using validated toxicity software. As this software can utilize many methods to determine an LC_{50}, this allows flexibility with regard to the data required. This can represent a reduction in the number of animals used as a meaningful result can be calculated for a variety of different study designs and may result in additional exposure groups not being required. **A retrospective assessment of reduction will be due by by 19 November 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.	In general, acute inhalation test guidelines specify the use of young adult rats, other species may be used (mouse or guinea-pig) but the rat is the species of choice. The mouse may be used if there is an indication of specific male rat hydrocarbon nephropathy ie alpha 2 mu-globulin nephropathy, or the rat is known to have a species specific susceptibility. The guinea-pig may be used in instances where the metabolic processes of rats and mice render them unsuitable for exposure to some agents whereas guinea pig metabolism is closer to that of humans, an example of which is agents which cause haemolysis in rats and mice but not in guinea pigs and humans. These examples of use of a species other than the rat are extremely rare but are recognised. The OECD 433 test guideline not only uses a

	reduced number of animals but also does not require the use of lethality as an endpoint but instead uses evident toxicity (where moving up to the next highest concentration would be considered to cause death and as such testing would not take place at the higher concentration) in order to classify the test item. For these reasons this test guideline is the preferred method for use in this establishment. The OECD 403 test guideline establishment of an LC_{50} lower than the limit concentrations outlined in the regulations will require exposure of some animals to lethal concentrations of the test item. Mortalities and marked signs of toxicity are, therefore expected in a certain number of studies performed under these protocols, although the aim is to avoid death as an endpoint as far as possible. Monitoring will be such that the expectation is that as few deaths as possible will occur as processes are in place to intervene with animals that are considered unlikely to survive. As studies progress, better estimates on the possibility of recovery from the effects of exposure, based on previous exposure groups can be made and, therefore, if becomes easier to determine humane endpoints. Refinement is also achieved by systems of care and accommodation that enhance the animals welfare. Environmental enrichment by the use of fun tunnels, gnawing material is provided. Group housing of animals is encouraged unless precluded on scientific grounds. Protocol 2 has a severe severity classification due to the potential for animals to die after exposure to the test materials. This protocol covers regulatory requirements mandated by law to classify chemicals which may be hazardous to heath by inhalation exposure in non-European jurisdictions. Sponsors will be guided to use the OECD 433 guideline to achieve these regulatory requirements if possible, as this is a more refined procedure. If, however, an LC50 is required by a regulatory authority, then prospective authority will be sought from the Home Office to run an OECD 403 study, prior to study start.

	A retrospective assessment of refinement will be due by by 19 November 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. Adaptability of the auditory system in 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)	We aim to improve our understanding of the processes that take place in the brain when we listen to speech and other sounds. In particular, we wish to find out how nerve cells in the hearing centres of the brain represent these sounds in a manner that is robust to changes in the soundscape – as we move, for example, from a noisy bar to an empty street. We also wish to find out how these representations are altered as a result of training and learning on different auditory tasks or because of hearing loss. A final objective is to identify the changes in the brain that give rise to tinnitus – persistent ringing in the ears – and whether we can reverse those changes as a potential cure for this debilitating condition.	

	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)?	The principal benefits of this work are advances in scientific knowledge about the neural basis for hearing and listening in life-like situations where background noise or other interfering sounds are present. We expect this work to lead to the generation of better computational models of the brain and to contribute to improvements in the design of artificial speech recognition systems, hearing aids and cochlear implants. Furthermore, this work will help to reveal how the auditory system compensates for hearing loss that affects one ear more than the other and to show whether training can be harnessed as part of the treatment of hearing-impaired people to improve their listening in challenging conditions. Finally, we expect this work to tell us more about what happens in the brain when tinnitus is experienced, which may point the way to potential therapies for this currently incurable and debilitating condition.
What species and approximate numbers of animals do you expect to use over what period of time?	Over the 5 year period of the project, we will expect to use 2,800 mice, 90 rats and 340 ferrets for scientific procedures other than simple breeding and maintenance. We may breed and/or maintain up to 10,000 mice to provide some of the mice required for scientific 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?	Part of the project will involve the raising of genetically-altered mice to allow us to investigate how particular brain cells and circuits contribute to hearing. These animals are expected not to show any harmful condition and to be no different in the way they behave from normal, wild-type animals. The behavioural tests we will use to measure the animals' hearing abilities are painless. In some cases, it will be necessary to motivate the animals to perform these tasks by allowing access to water only during testing. This may

	result in a temporary weight loss, but this will always be monitored carefully and extra food or water provided if this occurs. The availability of modern techniques for monitoring or altering neural activity in particular regions of the brain make it possible to carry out almost all of this work in a manner that should cause only temporary pain or discomfort to the animals in the study. For example, surgical operations for implantation of ultrafine microelectrodes, inserting genes into the brain or making very small lesions will be carried out under general anaesthesia, in aseptic conditions, and with appropriate post-operative care and painkiller medication. The adverse effects that may occur following surgery include transient pain and bleeding, but their incidence is likely to be low and methods of control (e.g. painkillers) and the most refined experimental techniques will always be used to mitigate them. Chronic implants for recording neural activity or for delivering flashes of light to the brain for the purpose of altering that activity are small and light-weight, and do not affect the animal's quality of life or behaviour. Animals will be killed humanely at the end of the experiment or at any point if their well-being is compromised and this cannot be alleviated (e.g. painkillers). **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	Because this project investigates the neural basis for auditory perception and how this is affected by hearing loss, it can only be carried out using *in vivo* approaches. Non-invasive techniques to measure brain activity in humans (e.g. fMRI or EEG) lack the sensitivity to observe changes in the response properties of nerve cells caused by hearing loss or training or alterations in the acoustic environment. Moreover, a key aim of this project is to try and account for changes in auditory perception at a microscopic level in terms of the underlying neural circuitry. This requires the use of post-mortem histological measurements, which would not be ethical or practical to carry out in humans. Using animals to measure the effects of precisely controlled forms of hearing loss also avoids the inevitable variations that would be found among a clinical population. Finally, computer modelling forms an important component of our work, but this relies on the information provided by the animal studies and cannot be used instead. **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	Calculations are carried out to determine the necessary number of animals for each experiment, ensuring significance of our results but also minimizing the number of animals used. We are additionally able to keep animal numbers to a minimum by using cutting-edge methods that yield large amounts of data and experimental designs that allow multiple measurements to be made from each animal. **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.	Mice and rats will be used because they are the lowest vertebrates with an auditory system that is comparable to that in humans. Rodents are unsuitable, however, for some of this work because they are unable to hear the low tones that enable humans to understand speech, hear melodies or localize certain sounds. In contrast, ferrets have an auditory frequency sensitivity that overlaps with that of humans. They can also be readily trained in behavioural tasks that will capture how well they can detect, distinguish between or localize different sounds. Being larger and stronger than rodents, ferrets can carry implants with ease which would be uncomfortably large on a rat or a mouse. Animal welfare costs will be minimized by carrying our procedures in state-of-the-art facilities and using best practice methods. Operations are carried out very carefully under anaesthesia and the animals are given painkillers and closely monitored until they have fully recovered. The earliest endpoints consistent with the scientific aims are applied. The data obtained from these experiments will be used to refine computer models of the brain that will help to guide subsequent experiments, including those in human listeners, and contribute to a reduction in the number of animals needed. **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	**4. Adaptive mechanisms of the ischaemic myocardium**	
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 investigate the changes that occur within the cardiovascular system after a heart attack and during the subsequent development and progression of heart failure. It aims to identify new therapeutic targets and treatments for this condition. **A retrospective assessment of these aims will be due by 10 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)?	Heart failure is a common condition that affects up to 1.5% of adults. There are over 200,000 hospital visits each year due to heart attacks in the UK and this is the commonest reason for the development of heart failure. Despite improvements in early survival after a heart attack, over time the heart continues to decline. The morbidity and mortality of heart failure is unacceptably high despite the use of multiple treatments. Newer and better treatments are therefore required. A better knowledge of the mechanisms that underlie the development and progression of chronic heart failure after heart attack is essential to develop new therapies.
What species and approximate numbers of animals do you expect to use over what period of time?	A total of up to 400 rats and 14,600 mice over the lifetime 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?	The procedures in this licence involve the induction of heart failure by injuring the heart through tying off a blood vessel supplying the heart (in comparison with appropriate control groups). In some cases the injured heart or a control will be placed in the abdomen of a different mouse to determine if the heart responds better when it does not have to do any work. Animals will be assesed by imaging techniques such as those used in the clinic including ultrasound and MRI. Blood pressure will be measured in a small number of cases by surgical placement of a probe into an artery or more commonly by placing a cuff on the tail. Substances that can influence the outcome eg by activating or turning off specific genes either chemically or via gene therapy; by targeting pathways that effect cell function and/ or immune response or are markers to assess heart function will be administered either in the food and/or water or by injection or by surgical placement of a small osmotic pump placed under the skin that can give a continual dose. In rare cases substances may be administered surgically by direct injection into the heart.The majority of animals will experience moderate levels of suffering, indicated by a mild change in breathing rate and/or a mild reduction in mobility. However, it is possible that a minority of animals with heart failure could experience more severe symptoms such as rapid breathing, marked reduction in mobility and weight loss. This will be minimised by regular checking of the

	animals and the use of early humane killing. Rats are used as well as mice in cases where experimental techniques or reagents are better established in this species. All surgical animals are anaesthetised and provided with pain relief as standard. At the end of the study, animals are humanely killed and samples taken for microscopic analysis of the heart tissue to identify changes in cell structure, biochemical and protein changes and changes in genetic markers. **A retrospective assessment of these predicted harms will be due by 10 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	There is no feasible alternative to the use of animal models as heart failure cannot be reproduced in cell systems or by computational modelling as it is a complex condition involving the interactions of several body systems. **A retrospective assessment of replacement will be due by 10 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	We will follow principles of good experimental design to ensure clear answers to the scientific questions being addressed while using the minimum number of animals. We have made a significant reduction in the number of animals that are used by the development of non-invasive techniques that allow for ongoing serial assessment in life eg echo imaging of heart to determine structural changes, size of damaged area after heart attack, functional effects of drugs administered . This is especially valuable when assessing the impact of medicines aimed at preventing or slowing disease progression as effects can be seen in real time in the animal

	concerned . Wherever possible, detailed studies of cellular mechanisms will be conducted in cultured cells (which have been obtained from animals) in the lab rather than in a whole animal. **A retrospective assessment of reduction will be due by by 10 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 study will be performed using mice and rats because all relevant methods and techniques are successfully established in these species, and because of the availability of genetic alterations in these species. These help to study specific biochemical pathways with a view to understanding disease progression. Understanding the effects of interfering with the disease process at specific points in underlying pathways can lead to the development of new treatments for heart disease. All procedures will be conducted by highly skilled, licensed personnel, non-invasive techniques eg imaging will be used where possible. Components of procedures will be the minimum required to be consistent with reaching the scientific objectives. Animals will be closely and regularly monitored during the study. Surgical animals will receive pain relief as standard. Any clinical problems will be dealt with in consultation with the veterinary surgeon. There are strict pre-determined 'humane endpoints' in place, based on clinical signs, at which animals are promptly and humanely killed to ensure that any animal does not suffer unnecessarily. **A retrospective assessment of refinement will be due by by 10 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	**5. ADME and Pharmacokinetic Studies**	
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)	This project licence will provide a service to pharmaceutical and biotechnology companies supporting the development and discovery of new drugs. The aim of studies in the development phase is to meet international regulatory requirements to provide information on the absorption, distribution, metabolism and excretion (ADME) of drugs in the species being used to generate safety data. This information is used to ensure that the safety assessment data obtained in the toxicology studies fully covers human exposure of the drug and its metabolites. ADME programmes are generally conducted using radiolabelled test compounds to	

	provide a simple means of tracking the administered dose within the body. Studies performed in the discovery/research phase are designed to assist sponsors select development candidates based on pharmacokinetic (PK) -absorption, distribution, metabolism and excretion properties. Having obtained initial data using computer modelling and cell-based techniques compounds with good indications are taken forward into preliminary animal testing where the drug candidate is administered to small numbers of animals. Blood samples are analysed for levels of drug and metabolites using sensitive analytical equipment e.g. liquid chromatography-mass spectrometry. These data are then used to calculate PK parameters. **A retrospective assessment of these aims will be due by 16 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 main benefit of the project licence is the identification and development of safe, effective drugs. Discovery/Research studies enable the pharmaceutical industry to efficiently select suitable development candidates and prevent compounds with “poor” PK properties entering the development process (historically one of the main reasons for drug candidate failure). Absorption, distribution, metabolism and excretion information forms a key part of the safety assessment process. The testing enables the pharmaceutical industry to meet regulatory requirements for drugs allowing them to progress through clinical development and meet international regulatory requirements. Patients benefit once the developed products reach the market.

What species and approximate numbers of animals do you expect to use over what period of time?	We use over a five year period approximately 10,800 rats, 3,700 mice, 170 rabbits and 70 guinea pigs. We also use 800 pigs and 130 dogs.
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 do not expect to see any adverse effects in the studies we perform through investigation of the compound prior to a study being initiated. This involves the understanding of the drugs safety in previous studies so carefully selected dose levels are administered minimising the chances of any adverse effects being seen. Dogs and pigs on a re-use basis will kept for the duration of this license depending on their continual health assessments. Dogs and pigs not on a re-use basis will be humanely sacrificed following completion of each project. Following completion of each project rodents and lagomorphs will be humanely sacrificed. On very rare occasions dogs may exhibit an emetic response in the form of vomiting or loose faeces following an oral dose. Vomiting is generally on one occasion and not for prolonged periods. The dogs are very closely monitored if these signs are observed. Surgery under general anesthetic, such as cannulation of a blood vessel, cannulation of the bile duct or cannulation of the small or large intestine is performed under general (isofluorane) anesthesia. All surgeries are performed in a sterile cabinet with each animal being placed on a warming mat to ensure it does not loose temperature during the procedure, aiding recovery. When cannulating a blood vessel the vessel will be clamped prior to insertion of the cannula to ensure minimal blood is lost, following cannulation the cannula is primed with heparanised saline to ensure it remains patent post surgery. When cannulating the small or large intestine care is taken to ensure no gut contents enter the abdominal cavity causing an infection. All of these surgeries involve the animals wearing a purpose made jacket following surgery to ensure the cannula remain in place. The jacket can cause some discomfort at first but this is checked at least one daily to ensure it is at the correct tension, and cannot come off the animal. Post surgery animals maybe lethargic so will be placed into a warming chamber for approximately 30 minutes to ensure they can regulate their own body temperature before going back to their home cage. Analgesics

	will be given to all animals during surgery which can cause slight lethargy in itself. Most animals lose a small amount of weight following surgery but this will be monitored with a daily weighing regimen and moistened diet given to promoteeating. Following surgery the animals will be singly housed to ensure there is no interference with each others jackets and cannula. When a study requires the individual collection of urine, faeces and/or expired air animals will be singly housed in a metabolic cage to enable the separate collection of these samples. During this period the animals may become quieter and less active compared to normal due to the change in type of housing and no direct contact with other animals. To mitigate this the animals will be housed adjacent to each other so they have visual contact at all times. The time period animals are allowed to be singly housed in metabolic cages is strictly controlled. Where possible we use environmental enrichment such as the use of mouse houses to mitigate any adverse effects whilst being singly housed. When a study requires the induction of tumours, via subcutaneous administration of tumour cells, each animal may have a tumour growing on its flank. The tumour size is strictly controlled with a maximum allowable size limit and the growth of each tumour monitored by regular measuring. If the tumour size approaches these limits the animal will be humanely sacrificed. The presence of a tumour on the flank will cause some physiological change to the animal but due to the maximum limit on tumour size the adverse effects on the animal are kept to a minimum. There might be some weight loss observed but the animals will be weighed daily to asses this impact. The number of animals in each cage will be kept to a minimum to minimise any interference with tumours. When orally dosing, animals will be restrained and the drug administered directly into the stomach via a syringe and gavage tube. This may cause a period of discomfort whilst the procedure is being performed. When dosing by the intravenous route rodents and lagomorphs only maybe gently warmed in a warming chamber to enable identification of a blood vessel and aid dose administration. This may cause a period of discomfort as the animals are warmed then a brief period of discomfort as the drug is administered. When administering substances by the intraperitoneal route a needle guard is used to

	ensure the correct depth of each injection is maintained Dose administration via the intra tracheal, intra articulate and intra vitreal routes all require the use of general anaesthesia whilst the procedure is performed. Following intra tracheal dosing care is taken post dose to ensure the animal does not encounter breathing difficulties, if observed, oxygen will be delivered via face mask. Although we have never seen adverse effects following the intra articulate and intra vitreal routes there is the possibility that eye redness or swelling to the knee joint maybe observed. Animals are closely monitored after these procedures for potential signs. The withdrawal of blood samples from superficial vessels, by direct vene puncture, may cause a small period of discomfort as the needle is inserted and blood sample collected. Pressure is applied to the site following collection via cotton wool. **A retrospective assessment of these predicted harms will be due by 16 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	We conduct many research projects involving a wide variety of methods that do not require the use of animals including the use of cell culture and studies using ethically sourced donated human tissue. Nevertheless despite advances in non-animal methods it is still required to use animals where there are no viable alternatives in order to enable additional much needed advances in medical knowledge and development of new treatments **A retrospective assessment of replacement will be due by 16 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	We always use the minimum number of animals

Explain how you will assure the use of minimum numbers of animals	needed to provide the robust scientific data required by international regulatory guidelines for the development of new treatments. These guidelines often specify the numbers of animals that should be used. If required, prior to a study starting, we will consult with statisticians to ensure the number of animals used meets the objectives of a study by the use of power analysis (we control the study power by adjusting the number of animals used). If too few animals are used the experiment may lack power and miss a scientifically important response to the treatment with the possibility of the project having to be repeated. If too many animals are used it is unethical and a waste of resources. Each study will have an adequate sample size, relative to the objectives and the possible variabilities of the study. We ensure the study design is clear and the procedures are defined and use the best possible/available methodologies. Confounding factors that may influence power, such as environmental factors (humidity, lighting and temperature) are strictly controlled to minimise variability in the data produced. All samples are collected exactly on time and to the correct specifications to ensure the data produced is of the highest quality with the least variation between subjects. **A retrospective assessment of reduction will be due by by 16 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 species we use are the most refined with regard to achieving study objectives with over 90% of the animals used being rodents. We always strive to use the lowest order animal first e.g. ensure rats are used prior to dogs. The species of animals used is driven by international regulatory guidelines which are required to be met for new medicines to develop, enter clinical trials and ultimately become medicines to treat human diseases. Our animal facilities are staffed by dedicated

	animal technical specialists who are responsible for the care and welfare of the laboratory animals and their environments. We actively support the 3R's and are innovative in developing techniques to minimise potential harm and enhancing animal welfare. All studies are rigorously reviewed prior to conducting any animal work which ensures the least number of animals are used and least harm caused. **A retrospective assessment of refinement will be due by by 16 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	**6. Application of rodent models of neurodegeneration for the development of novel therapeutics**	
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 disease* is an umbrella term for a range of conditions which primarily affect the neuron cells in the human brain and currently there are no effective treatments for these diseases. This is partly because current knowledge of the causes of these disorders is limited. In this project we aim to study rodent models of human neurodegenerative disease, and to test novel therapeutic approaches in these models. We will specifically study motor neuron disease (MND) frontotemporal dementia (FTD) and Parkinson's disease (PD). **A retrospective assessment of these aims will be due by 02 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 benefits of our studies will be twofold. Firstly we will identify the underlying problems that leads to disease in MND, FTD and PD. Secondly we will test new therapies (preclinical studies) to identify whether or not they are able to reduce symptoms and improve the quality of life for the patient. At present the majority of drugs developed for these diseases fail in man. Greater rigor during preclinical development is necessary to address this.
What species and approximate numbers of animals do you expect to use over what period of time?	This programme of work will involve studies in mice and rats. Over 5 years we expect to use approximately 10000 mice and 600 rats
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 order to understand the disease processes, and to study the effect of novel therapies, mice will inevitably develop features of neurodegenerative disease, including loss or dysfunction of neuronal cells, which will lead to a progressive loss of function that may lead to them becoming paralysed. In this project we will mostly use animals that show early signs of disease with a maximum of moderate severity (85% of mice and all rats). In some experiments (15% of mice) where we need to understand later stages of disease animals will show signs of paralysis and these will be looked after intensively, however will have a severe severity rating. At the end of these experiments we will collect tissues to study hallmarks of disease such as swellings in nerve cell fibres, loss of motor neurons in the spinal cord, and biochemical analysis to investigate levels of specific proteins. **A retrospective assessment of these predicted harms will be due by 02 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	Neurodegeneration is a complex process that

State why you need to use animals and why you cannot use non-animal alternatives	involves numerous cell types. Although we can model some aspects of the disease process in tissue culture, ultimately we rely on animal studies to confirm the relevance of these findings, and particularly to determine the clinical benefits of novel therapeutic approaches. Some experiments could be performed in non-protected animals such as flies. However, flies lack of some of the key genes involved in neurodegeneration. **A retrospective assessment of replacement will be due by 02 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	We consult statisticians to determine the minimum number of animals required to obtain statistically valid results. If we used fewer animals it would give ambiguous results, which are not scientifically valid. **A retrospective assessment of reduction will be due by by 02 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 majority of our work involves mouse models, which are currently the most refined models of neurodegeneration that allow for testing and development of therapeutic approaches. We use animals at early disease stages, avoiding any use of mice with severe symptoms. We have developed refinements and will continue to develop refinements to our models involved in measuring motor function, particularly in relation to MND models. **A retrospective assessment of refinement will be due by by 02 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	**7. Approaches to study Host-Pathogen Interactions in vivo**	
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 parasite Toxoplasma gondii infects a broad range of warm-blooded animals, including humans, and is arguably the most common parasitic infection in man. Toxoplasma has developed various strategies to escape the immune system of the host. The parasite injects a subset of proteins into the host cell to hijack host cellular processes in favour of the parasite. The initial stages of an infection by Toxoplasma (acute phase) generally cause no symptoms in healthy individuals and proceeds silently to the later stages (chronic phase) of infection with the development of a slower growing form of the parasite called cysts that are found in tissues	

	like the brain and heart muscle. Toxoplasma infection is incurable and tissue cysts of the parasite reside in those tissues for the rest of the lifespan of an infected individual. When an individual's immune system is compromised, such as HIV patients or recipients of organ transplant, these individuals are at high risk of infection due to reactivation of the dormant tissue cysts in the brain leading to the development of often deadly disease symptoms. Healthy individuals on the other side can lose their eyesight when infected with specific strains of Toxoplasma and an unborn child can have birth defects if the mother becomes infected during pregnancy. Currently there is no cure or vaccine available. In this project we will identify as yet unknown proteins used by the parasite survive in its host organism, the mouse. We have developed a method to follow individual parasites within complex population. Using this technique we are able to understand how the genetic complexity changes in the course of an infection, and to discover new antimicrobial drugs that can be used to treat infections in the future. Moreover, we will analyse how essential parasite proteins that are the targets of the antimicrobial drugs are required for the parasite to establish or maintain the infection. Together these approaches will allow us to both identify, evolve, and finally validate new drug targets. **A retrospective assessment of these aims will be due by 13 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)?	This project will firstly increase our knowledge about the mechanisms the parasite uses to survive in the host organism. This will ultimately lead to the discovery of new therapeutic targets or pathways that might equally be targeted by other pathogens, causing diseases such as malaria. We will make our results publicly available to the scientific community and beyond using appropriate outreach media. The application of molecular barcoding for antimicrobial drug discovery is a completely new approach for drug-discovery with the potential to revolutionize the process both in terms of time, cost, and success. The approach is broadly generalizable and we anticipate our work impacting upon diverse fields of infection biology and antimicrobial drug discovery.
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 1800 mice will be used over the course of 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?	We will infect mice with Toxoplasma and monitor their health during the infection. For some experiments we can use parasites engineered to emit light, enabling us to image the infection without harming the mice, comparable to imaging methods used for humans in the hospital. Nonetheless, infection with Toxoplasma can cause severe symptoms of illness and result in the unexpected death of an animal. We will humanely kill animals that show severe signs of infections in accordance with established humane endpoints. As Toxoplasma infections are incurable, all animals used in this study will at the end of an experiment be humanely killed and tissue samples will be taken for further analysis. **A retrospective assessment of these predicted harms will be due by 13 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	Once infected with Toxoplasma, all hosts including humans establish a chronic infection that is kept asymptomatic by the host's immune system. If the immune system fails, Toxoplasma can kill the host. We are not able to recreate the complex interplay between different arms of the immune system with state-of the art laboratory techniques, as it relies on the structural integrity and connectedness of organs and tissues. Therefore, we still need to use animals to answer our specific research questions. **A retrospective assessment of replacement will be due by 13 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	We have established a method that allows us to investigate the fates of >100 different Toxoplasma strains at the same time in one mouse. Before, parasite lineages have been tracked one by one. Our method substantially reduces the number of mice needed. This is true for our laboratory, and once we made our method available to the scientific community, laboratories worldwide. Moreover, the results of this study will inform others which virulence factors are worthwhile to study in mice and which ones not. A second advantage is that we can often use Toxoplasma gondii engineered to emit light, thus being able to image the parasite in living animals. Using this method, we can follow the infection in each animal in real time and do not need to euthanize animals to analyse each time point individually. Power calculation is used in our experimental design in order to use appropriate numbers of animals to achieve scientific significance. **A retrospective assessment of reduction will be due by by 13 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 model is currently the only extensively refined animal model of Toxoplasma infection. As we are studying how the intact host organism environment restricts the parasite in both the acute and chronic phase of the infection, we are reliant on a whole organism to understand the complex interplay of different cellular players of host defence. Using the in vivo parasite imaging approach, we are able to administer a minimal dose of the parasite and still be able to assess differences in parasite load between wild-type and genetically altered mice. We have developed mouse body condition scoring sheet aiming for robust monitoring of any adverse effects of infection and prompt intervention where necessary. **A retrospective assessment of refinement will be due by by 13 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	**8. Aquatic Ecotoxicology**	
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)	Purpose – The testing of the safety (environmental risk) of a large range of chemicals, which include pharmaceuticals, biopesticides, industrial and agrochemicals on behalf of companies which develop, manufacture and distribute them. Duration – 5 years The objectives of the project are to provide government regulatory agencies with scientific data, they have stipulated necessary for a balanced assessment of the potential impact of new and existing chemicals on the aquatic environment and its ecology (e.g. fish). The published regulatory guidelines state that	

	manufacturers are obliged by law to provide such data for the two main reasons: 1. Safety to the environment 2. Safety to Human Health **A retrospective assessment of these aims will be due by 13 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)?	To facilitate and enable sound regulatory decisions to ensure that the concentrations of man-made chemicals (i.e. agrochemical, industrial and pharmaceuticals) that will potentially come into contact with our ecosystems (lakes, ponds, rivers) are safe and do not pose any potential risks to our ecosystems, food chains, human health and populations. Further benefits to introducing new chemicals such a pharmaceuticals and pesticides is generally associated to improvements in the quality of life. (i.e. a new anti-cancer drug, saving lives/growing healthy crops to maximum yield for consumption/ and ensuring veterinary medicines do not contaminate meat or pass to humans via consumption)
What species and approximate numbers of animals do you expect to use over what period of time?	A variety of fish species including rainbow trout, fathead minnow, common carp, bluegill sunfish, sheepshead minnow and turbot are expected to be used. The total number of fish to be used over the 5 -year licence period is expected to be approximately 400,000

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 individual studies undertaken involve exposure of groups of fish to varying concentrations of chemicals to assess the effect that the chemicals may have on survival and/or growth of the fish. Adverse effects range from mild discomfort through to death are expected during the project. However, in the majority of exposed fish adverse effects will be mild. The programme of work is designed in accordance with the principles of the 3R,s in order to minimise animal use and severity of procedures. Tiered testing strategies will be implemented, so the results of one study can be used to refine the remaining studies in the programme thus will minimise the severity of any adverse effects. All fish that are exhibiting significant toxic effects, and those surviving to the end of each test, will be humanely killed as soon as possible to avoid unnecessary suffering. **A retrospective assessment of these predicted harms will be due by 13 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	Current regulations e.g. REACH, require the use of fish to assess potential environmental effects. Non-animal alternatives have not yet been sufficiently validated for acceptance by various regulatory authorities and hence cannot be used to replace animal testing in this context. **A retrospective assessment of replacement will be due by 13 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	The number of fish used in regulatory ecotoxicology studies is specified in the relevant test guidelines and is the minimum that is

of minimum numbers of animals	sufficient to allow meaningful data interpretation and submission to a range of regulatory authorities. The use of the specified numbers of fish ensures that the data generated will be acceptable to regulatory authorities and hence the need for subsequent duplication or supplementary testing. Where possible the results of the QSAR predictions, physico – chemical testing and non-animal tests will be used to aid in the prediction of toxicity hence reducing the number of animals required to satisfy the regulatory requirement e.g. by performing threshold tests. **A retrospective assessment of reduction will be due by by 13 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 fish species used have been selected in accordance with the relevant Test Guidelines and the age ranges of the fish are such that they are of the lowest neurophysiological sensitivity that will allow evaluation of the specific endpoints. The species selected are representative of wild species. The data generated is therefore designed to protect these representative species in the environment thereby minimising larger scale environmental effects of tested chemicals. Any fish that are showing a significant departure from the animal's normal state of health or well- being will be identified and humanely killed

Project	**9. Assessment of novel treatments against acute bacterial infections**	
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
	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)	This project aims to develop novel therapies to treat acute infectious diseases. **A retrospective assessment of these aims will be due by 05 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)?	Bacterial infection is a global health problem. The lack of new antibiotics has led to the emergence of many resistant strains. By contributing to the development of new and novel antibiotics, our project will benefit patients' worldwide, enabling more rapid treatment of infections and reduce mortality. By providing high quality services and scientific expertise, we can accelerate the development of new treatments.
What species and approximate numbers of animals do you expect to use over what period of time?	The estimated number of animals to be used over the duration of the project (five years) is 15000. Mice will be used in more than 90 % of studies with rats being used in the remainder. No other species are to be used.
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?	Expected adverse events for acute models involves some pain and discomfort associated with the site of infection. Some animals receive injections into the skin or thigh muscle and may be uncomfortable for a few days. Other animals may develop septicaemia or pneumonia and some of these may die, Some ani-mals may develop diarrhoea. Acute models of infec-tion lead to moderate to severe levels of disease and there is a high risk of animals becoming ill. Frequent checks, every 4 hours, will therefore be carried out throughout all procedures, including overnight, and these will be increased if required. Animals that be-come very sick will be treated or humanely killed. 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 05 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 immune system's response to the presence of pathogens, involves multiple systems, multiple organs and multiple cell types. The complexity of the immune response cannot be reproduced *in vitro*. Experiments on cell lines and tissues as well as insect larvae will be performed where possible. 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 05 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	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. **A retrospective assessment of reduction will be due by by 05 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.	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 lowest possible dose of bacteria 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. We will assess the use of mouse grimace scores as an early indication of pain and possible onset of disease, leading to increased monitoring and possible reduction in duration of disease and reduced suffering as a consequence. Animals are housed in groups and kept in an appropriate environment with plentiful bedding and nesting material and suitable objects 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. Where possible bioluminescent bacteria will be used to reduce number of animals required. **A retrospective assessment of refinement will be due by by 05 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	**10. Assessment of novel treatments against chronic bacterial infections**	
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
	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)	This project aims to evaluate the efficacy of novel therapies to treat chronic infectious diseases. **A retrospective assessment of these aims will be due by 01 November 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)?	Bacterial infection is a global health problem. The lack of new antimicrobial therapies has led to the emergence of many resistant strains. By contributing to the development of new and novel anti-microbial therapies, our project will benefit patients worldwide, enabling more rapid treatment of infections and reduce mortality. By providing high quality services and scientific
	expertise, we can expediate the testing of such drugs and development of new therapies.
What species and approximate numbers of animals do you expect to use over what period of time?	The estimated number of animals to be used over the duration of the project (five years) is 7000. Mice will be used in more than 90 % of studies with rats being used in the remainder. No other species are to be used.
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 order to develop improved therapies against chronic bacterial infections, animals are given doses of bacteria by different routes to induce infections in the lungs, gums, bone, skin or gut. Some of the animals may undergo surgical procedures, resulting in the potential for post-surgical discomfort. This will be prevented through routine use of pain killers. The expected adverse events for chronic models involves some pain and discomfort associated with the site of infection. In general, these models cause only mild to moderate disease, however, some animals may develop more serious infections leading to sepsis. All animals are monitored frequently, and appropriate action taken if they show undue signs of pain or dis-tress, such as the administration of analgesics or in some cases humane killing. 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 01 November 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 immune system's response to the presence of pathogens, involves multiple systems, multiple organs and multiple cell types. The complexity of the immune response cannot be reproduced *in vitro*. Experiments on cell lines and tissues as well as insect larvae will be performed where possible. However, the limitations of these methods do not allow them to replace the use of experimental animals completely: 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 01 November 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	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. **A retrospective assessment of reduction will be due by by 01 November 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.	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 lowest possible dose of bacteria 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. Where possible bioluminescent bacteria will be used to reduce number of animals required. **A retrospective assessment of refinement will be due by by 01 November 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	**11. Assessment of novel treatments and therapies against viral infections**	
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
	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)	This project aims to evaluate the efficacy of novel therapies to treat viral infectious diseases. **A retrospective assessment of these aims will be due by 31 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)?	Viral infection is a global health problem. The lack of new antiviral therapies and vaccines has led to continued outbreaks of disease due to genetic variation and development of resistance leading to treatment failure. By contributing to the development of new and novel anti-viral therapies and vaccines, our project will benefit patients' worldwide, enabling more rapid treatment of infections and reduce mortality. By providing high quality services and scientific expertise, we can expediate the testing of such drugs and development of new therapies.
What species and approximate numbers of animals do you expect to use over what period of time?	The estimated number of animals to be used over the duration of the project (five years) is 7700. Mice will be used in more than 90 % of studies with rats being used in the remainder. No other species are to be used.
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 order to develop improved therapies against viral infections, animals are infected with viruses by different routes to induce infections in the lungs, skin or eyes, this models the infection route experienced by humans. Some of the animals may undergo minor surgical procedures, resulting in the potential for some discomfort. This will be prevented through routine use of pain killers. The expected adverse events for viral models involves some pain and discomfort associated with the site of infection. In general, these models cause only mild to moderate disease, however, some animals may develop more serious infections due to spread of virus to other organs causing more severe illness and respiratory distress. All animals are monitored frequently, and appropriate action taken if they show undue signs of pain or distress, such as the administration of analgesics or in some cases humane killing. 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 31 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 immune system's response to the presence of viruses, involves multiple systems, multiple organs and multiple cell types. The complexity of the immune response cannot be reproduced in vitro. Experiments on cell lines and tissues as well as insect larvae will be performed where possible. However, the limitations of these methods do not allow them to replace the use of experimental animals completely: 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 31 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	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. **A retrospective assessment of reduction will be due by by 31 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.	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 possible will be caused, and studies will be kept

	as short as possible. Animals are monitored frequently for signs of illness, 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 objects 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. We will also carry out a comprehensive (literature) review to enure we are up to date on current research with novel treatments and effects of vaccines and immunomodulatory compounds on mice so we can better predict possible outcome and refine our approach to such studies. **A retrospective assessment of refinement will be due by by 31 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	**12. Atherosclerosis 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)	To understand the link between Atherosclerosis and Inflammation. Why is cardiovascular disease worse in people with other inflammatory diseases? What causes inflammation to start and what happens if it is not resolved? We want to investigate the mechanisms of disease, the molecules and pathways involved in the pathology of cardiovascular disease and understand the complex interactions involved. Atherosclerosis, a build up of fatty deposits within blood vessels, is the main cause of cardiovascular disease and leads to heart attacks and strokes. Cardiovascular disease is the biggest cause of death Worldwide and is a massive social and economic burden on society. Current	

	therapies focus on alleviating risk factors for atherosclerosis such as high-cholesterol and high blood pressure. However despite novel therapies, 70% of events such as heart attacks cannot be prevented. Work over the past 35 years have proven that inflammation is integral to atherosclerosis. A recent clinical trial has shown that reducing inflammation is beneficial in patients with cardiovascular disease. However, targeting the molecule assessed in the trial (IL-1β) increases the risk of infection. Therefore, better therapies that more specifically target inflammation in atherosclerosis are needed. **A retrospective assessment of these aims will be due by 04 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)?	Therapies to treat these conditions would be enormously important. 70% of events such as strokes and heart attacks and aneurysms cannot be prevented. Cardiovascular disease is a huge social and economic burden on society. The ability to identify points of intervention for therapeutic purposes. Either pharmaceutical agents or biologicals or treatment strategies (e.g. prevention) could well be extracted from this work. Increased scientific knowledge could lead to reduced mortality, increased lifespan, improved quality of life and a reduced burden on healthcare systems.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice. Approx. 15,000 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?	Most of the animals in the Mild breeding protocol will experience no adverse effects and will supply the genetically altered stock for other protocols. The Moderate breeding protocol covers situations where a strain is known to have a phenotypic condition, or where completely new cross matings are being attempted. **The Breeding and Maintenance protocols are Standard** The Arterial Injury Protocol involves placement of a small plastic encircling 'collar' on the carotid artery under full anaesthesia. **This alteration of the body, combined with the low level arthritis that may also be initiated can cause bodily disturbances over a period of weeks. If needed, pain relief is administered and any perceived discomforts are mitigated by environmental enhancements and the protocol is designated Severe because of the potential cumulative effects.** **Administration of substances using needles and blood withdrawal may cause transient discomfort for the animal. Where pain may be more likely e.g. formation of an airpouch or collar placement, anaesthesia and analgesia will be used.** **Arthritis may cause swelling of digits and paws. If this causes the mouse distress (e.g. it loses weight or a paw becoming non-weight bearing) the mouse would be killed. However in our strains, arthritis development is known to be milder than in others.** **In some experiments, cardiovascular models may be combined with a model of arthritis. In this scenario the mouse may experience more harm. This increased harm will be mitigated by provision of analgesia or the mouse being killed if it reaches an endpoint.** The Vulnerable plaque Protocol adds a ligation step to the previous protocol to produce a change in the tissue status that we require. Usually recorded as Moderate. Both protocols are used to hasten the disease state progression and allow study of the pathways and affected tissues. The Pharmacokinetics Protocol relates to study of blood levels of therapeutic agents should we find one. This procedure will normally be totally under non recovery surgical anaesthesia and sub threshold. The Tissue preparation Protocol, is where we use the genetically altered mice to provide tissue samples and use them for comparison with 'normal' samples i.e. Wild Type.

	It usually involves killing by an approved method and removal of the required tissues. They may however be subjected to administration of cells from other animals, or agents which stimulate their genetic alteration, or potential therapeutic agents, or irradiation with bone marrow reconstitution. Some experience a sub threshold situation, and others 'moderate'. The Air Pouch protocol is a simple, relatively mild and rapid way to investigate the molecules and pathways involved in inflammation (mild). The Germ free breeding, offers us the option of performing our experiments with a modified gut flora (the biome). The theory is that inflammatory diseases are dramatically altered by changing the microbiological status of an animal. Designated as moderate but very rarely is this the actual experience. Feeding of our type of high fat diet produces no ill effects and no obesity. Irradiation to destroy bone marrow, and reconstitute with the cells of another type of mouse confers characteristics of the donor without lengthy breeding and cross mating programmes. With care and skill the effects are usually returned as moderate. The Angiotensin protocol involves potential mortality and is labelled as Severe. The indications for this are well known and we will monitor intensively to avoid the situation It is the most reliable model for investigating the restructuring of the blood vessels and should help us to characterise our research. Hopefully , comparison to the human situation will allow development of treatments targeted to the mechanisms we discover. The 'glucose test' mid protocol is an injection of glucose and monitoring to see how quickly the body deals with it. It is a measure of adipose tissue function, which plays a key role in atherosclerosis development. At the end of all protocols, the animals will be humanely killed. **A retrospective assessment of these predicted harms will be due by 04 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	Animal models are necessary because no 'test tube' (*in vitro*) techniques emulate the flow characteristics of blood vessels susceptible to atherosclerosis. The 'whole body' inflammatory responses that may influence atherosclerosis are not confined to a single tissue. Rather, the inflammatory processes that occur in arthritis (for example) are dynamic and highly complex, involving movement of cells or agents from distant sites via the circulation. Hence, modelling the effects of treatment on atherosclerosis must at some stage involve whole animals, rather than isolated tissue extracts. **Lower animal models such as Zebra fish are not as complex as mammals and are therefore inappropriate.** A large proportion of our work is *in vitro*. We have exceptional access to clinical material that provides the opportunity to study atherosclerotic cells and tissues from human sources. This is a huge head start in identifying the key pathways and mediators and immediately reduces the need for preliminary screening in animal models. It cannot replace however, the unique whole body scenario. Recently, our work on human derived tissues has suggested a vascular facet which we would like to study in a model of Angiotensin stimulated vascular remodelling. **A retrospective assessment of replacement will be due by 04 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	The same techniques used *in vitro* can be used to study the tissues retrieved from animal models. **To ensure the best experimental design, randomisation and blinding techniques will be applied if possible.** In order to maximise the information extracted from each experiment and to avoid unnecessary repeats, we will measure a variety of parameters: 1) the size of the vascular lesions and the type of cells contained in them will be evaluated by histology or similar techniques; 2) the number and type of cells recruited to draining lymph nodes and spleen will be analysed by a cell analysis method

	called flow cytometry 3) cytokines and other mediators will be quantified at lesion sites, lymph nodes and spleens via histology or genetic analysis; 4) cytokines and other systemic inflammatory markers and lipid levels will be assayed. With the knowledge gained in the laboratory, more targeted studies in animals will reduce the numbers required. The ability to use genetically altered animals to verify the specific objectives will increase our certainty. For experiments deemed necessary, the minimum number of animals required has been determined using power calculations to allow generation of meaningful data within the constraints of experimental variability. Statistical power analysis allows us to predict the numbers of animals that will be needed to detect significant differences. Same sex animals will be used generally throughout the study to reduce experimental variability and enhance reproducibility. The experiments will be performed in a logical sequence and the preliminary experiments will be used to confirm whether reliable and significantly significant data has been recovered using the smallest number of animals possible. Because of our unique position in relation to access to clinical material, and experts in inflammation and atherosclerosis, we feel we can make best use of the facility to study the described models in mice. Our group contains scientists uniquely experienced in this area of in vivo and in vitro work and we expect our methods to continue to develop and refine. **A retrospective assessment of reduction will be due by by 04 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	Mice and rats are the lowest vertebrate groups on which well-established models of atherosclerosis have been developed. Mice are preferable to rats because of the greater availability of genetically modified animals and reagents specific for this species. **Analgesia: Pain relief will be provided as**

(harms) to the animals.	**required except if doing so might significantly affect the experimental outcome. To avoid a potential conflict e.g. by giving an anti-inflammatory in an inflammation model, opiate type analgesia will be considered.** Our protocols are now refined to produce the conditions we require and the minimum possible adverse effects on the mice. Good husbandry, compliance with dosing guidelines, monitoring and defined 'end points', all help to avoid or minimise suffering. Dosing volumes and routes are all specified. **Compliance with LASA aseptic surgery guidelines, Good Experimental Practise, recent improvements of the handling, the living conditions of the mice and their requirements for comfort are all applied.** Substances administered to animals by injection or orally will be made in the smallest volume commensurate with the aims of the procedure. Alternative methods of administration will always be considered. The timing of surgical operations will be selected according to the protocol to ensure maximum observation time of the animal's recovery and the time course of the model. **A retrospective assessment of refinement will be due by by 04 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	**13. Biocide Potency and Efficacy Testing**	
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)	Rodenticides must be effective and have a specific onset time, so their production is carefully monitored to make sure it complies with the regulations set out by regulatory bodies before registration of a product is given. Efficacy testing must be carried out to ensure these products continue to work as intended, within the time frame intended to avoid a buildup of resistance or avoidance. **A retrospective assessment of these aims will be due by 04 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)?	The test procedures detailed in this license form an essential part of obtaining the data required to monitor the efficacy and toxicity of rodenticides. The ability to test these products ensures that they have been produced safely and to appropriate standards. These results provide satisfaction to the relevant Marketing Authority that the tested products perform as intended. The prevention of the tests listed would result in the release of these products being delayed or denied. This could lead to Rodenticides being unavailable where pests are apparent, leading to uncontrolled infestations. Any prevention of testing would have an immediate impact.
What species and approximate numbers of animals do you expect to use over what period of time?	Over the duration of the 5 year project the estimated number of animals used is as follows: Mice 28,000 Rats 43,000
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 aim of the Acute Oral Toxicity Test is to determine the toxicity and onset time of a Rodenticide when a specific dose of an active ingredient is introduced directly to the stomach. Test animals are closely monitored for clinical signs as a result of the poison and time of onset, clinical signs and mortality is recorded. The resulting test data will be used by the manufacturers to assure the products tested work as intended. Any signs of toxicity will be reported to the NACWO, assessed and recorded. Once symptoms persist and the clinical signs can determine impending death humane endpoints are applied. The Acute Oral Toxicity test is also used for liver analysis. As before, the animals receive an oral dose of the Rodenticide directly to the stomach. Onset time and clinical signs are monitored and closely observed. Vitamin K1 is then administered to reverse the effects of the Rodenticide and the animal should return to full health. Blood clotting time is recorded using a Prothrombin Time Test to assess whether the effects have been reversed and observations of the animals continue. At the end of the test the livers are removed and

	sent for analysis to determine the amount of Rodenticide remaining in the organ. This liver analysis is carried out for the research and development of Rodenticides to reduce secondary poisoning to non-target animals. The Efficacy Test or Bait Choice Feeding Trial is designed to test whether a product is effective for the intended use at the intended dose of a manufactured bait (in its final formulation). It is also intended to assess whether the bait formulation is preferred to a challenge diet when both are presented to the animals used for this test. The palatability of a formulated bait is essential in the wild and is designed to be preferable to other sources of food pests have access to in their natural environment. For European submissions a pre-test food intake trial is conducted to ensure the animals are feeding normally. Any animal not eating normally will be removed from the study. For Environmental Protection Agency submissions in the USA a pre-test food intake trial is not required. Instead more animals are used in the test to ensure enough data is generated to submit scientifically valid results. Onset time and clinical signs are observed and recorded along with the amount of each diet eaten by the animals on test. The animals are then observed at least once a day and any signs of toxicity or mortality are recorded. Humane endpoints will be applied to animals showing clinical signs that can determine impending death. Moderate to severe effects need to be allowed to develop to achieve a pass result and prevent a retest (using further animals). If test animals have no pronounced symptoms or adverse effects the test will fail and then testing can be terminated and animals killed. **A retrospective assessment of these predicted harms will be due by 04 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	The most important features of a Rodenticide that contribute to its performance are its toxicity, palatability and time of onset of effect, all of which are largely assessed in the laboratory. The most important factor of Rodenticide regulation is registration and the basic data requirements to achieve registration (i.e. specifications, efficacy and toxicology) is now largely standardised. For the laboratory evaluation of rodenticides, there are no practicable alternative methods to tests involving the use of the target species. The guidelines for registering a Rodenticide include a comprehensive seriesof test methods to be used in the evaluation of the toxicity and acceptability of a Rodenticide. Preliminary screening will take place to assess potential usefulness as a chemical suitable for formulation in a palatable bait before animal tests are considered **A retrospective assessment of replacement will be due by 04 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	Full assessment of toxicology and efficacy is conducted on applications for product authorisations. Toxicology and efficacy studies should be performed with the product to evaluate whether the product is effective for the intended use at the intended doses. Any toxicology and efficacy data from scientific literature are considered only as supportive data and should not replace data obtained from testing, which should be performed according to recognized standards. **A retrospective assessment of reduction will be due by by 04 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.	The animal species used for the various procedures have been established over many years. Testing is species-specific meaning that for a Rodenticides stated intended use the target species should be the test subject. In all cases the species required are specified absolutely by the method as required by legislation and/or the regulatory authorities. Study design is predetermined by regulatory protocol and usually cannot be modified. If modifications are made the statistical significance will be reviewed by a contract statistician who would recommend methodology. While test methods are specified absolutely the equipment used and general husbandry is reviewed for improvement by staff, the NVS and members of the AWERB. **A retrospective assessment of refinement will be due by by 04 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	**14. Bioelectronic Medicines**	
Key Words (max. 5 words)	Bioelectronics, Inflammation, Neuromodulation	
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
		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?	The aim of the project is to develop new types of medicines that change the electrical activity of the peripheral nervous system to treat organ disease – termed 'Bioelectronic Medicine'. **A retrospective assessment of these aims will be due by 10 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?	There is a substantial unmet need for treatments for immune-inflammatory diseases, a group of conditions whose mechanisms and causes are poorly understood. These diseases are due to damaging activation of the immune system and downstream inflammatory pathways, and can lead to severe damage to organs like the kidney which can increase the likelihood of disease or death. Specific immune-inflammatory diseases such as Rheumatoid Arthritis (RA) and Systemic Lupus erythematosus (SLE) affect a large proportion of the human population globally (Cotsapas and Hafler 2013). Existing treatments can be effective in symptom management, but typically don't make the disease better and can cause unpleasant and unwanted side effects. Bioelectronic medicines can provide a different type of treatment with fewer side effects, and without losing their effectiveness over time, which can happen with more traditional medicines and making treatment more precise.
What outputs do you think you will see at the end of this project?	By creating scientific evidence, we will show that our Bioelectronic Medicines for Rheumatoid Arthritis (RA) and Lupus are safe and effective and can be given to patients (clinical trials) in 2020/2021). We will accomplish this by conducting detailed work in live animals (in vivo) under this licence and by using other science within our company and elsewhere. The evidence that our Bioelectronic Medicines work and are safe in rodent (rats and mice) will be used to allow us to discuss with regulatory authorities (who approve new treatments) and doctors whether the treatments can be given to patients and ultimately become a new medicine. We will also share information through scientific journals and seminars to share details of these new treatments with other scientists.
Who or what will benefit from these outputs, and how?	Ultimately our aim is to improve the lives of people suffering from arthritis and lupus, and possibly other serious illnesses. There are

	many patients who could benefit from these treatments. Over 1% of the patient population suffer from RA alone. We expect that it will take several years to achieve our aim, beyond the 5 year duration of this licence but the data obtained under this licence will provide the foundation for development of future treatments. As well as patients, the wider scientific community will also benefit through increased knowledge in this field opening further opportunities in this for innovation in the field of immune-inflammation and neuromodulation.
Will this work be offered as a service to others?	No
How will you look to maximise the outputs of this work?	Studies in animals are only a small part of how we will demonstrate that Bioelectronic medicines are safe and effective. We will use other types of science and a wide range of expert scientists to create as much evidence as we can. Through our collaborations with other organisations such as Contract Research Organisation (CROs) and university researchers focussed on work in animals which mirrors what will happen in humans, improving surgical techniques, device engineering, and using tissues taken from humans, we can quickly assess new ideas and check whether our devices work and are safe.

Explain why you are using these types of animals and your choice of life stages. Typically, what will be done to an animal used in your project?	We will use adult rats and mice in this project licence, some will be genetically modified to show the features of the disease. Adult mice (animal model) have been widely used to study Systemic Lupus Erythematosus (SLE) and have been useful and reliable to study both the causes of the human disease and the potential benefits of new treatments. Mice may develop diseases naturally (spontaneous) or can be treated (induced) to develop the disease (induced), which is similar to the human disease. All animal models share parts of the disease in which occur in humans such as kidney problems (glomerulonephritis), skin problems (lupus flare), and generation of substances which cause damage to tissues (autoantibodies). A rat model (rat collagen induced arthritis (CIA)), where a substance is given to rats to create symptoms, is useful to study treatment of long lasting (chronic) arthritis. CIA is the most widely studied and used model of RA because of the similarity in the causes (pathological and immunological) of CIA and RA. Only adult animals show these signs of the human disease and so only adults will be used. **Protocol 1** We will test changes to the function of the nervous systems (neurological interventions) by providing electrical stimuli (electrophysiology), in animals under anaesthetic, which will not be allowed to wake up. The best methods (anatomy and surgery) will be decided for future longer-term experiments in animals, which will be allowed to come round from anaesthesia and recover from surgery. The experimental period for these studies under Protocol 1 will be no longer than a few hours. **Protocol 2** We will improve device design and functionality, and assess the amount and duration of electrical stimulation, based on

	information from Protocol 1, in rodent models. Animals will undergo surgery to implant an electrode on the splenic nerve, and will be allowed to fully recover over several days. After a period of recovery, the animals will be connected to the stimulation device (animals will be able to move freely in their cage while connected), and electrical stimulation will be applied via the headcap and electrode, and into the nerve. In some instances, substances such as Lipopolysaccharide (LPS) may be administered. The LPS evaluates the animal's ability to respond to an inflammatory stimulus, by testing the body's response to inflammation from markers in the blood. This response is brought on by the production of specialised inflammatory cells which are white blood cells (e.g. monocytes and neutrophils) which are activated and respond to inflammation. Body fluids (such as urine or blood) may be taken to assess the effect on biology. At the end of the study, animals will be humanely killed, and further measurements may be made from blood and/or tissues. These studies will usually last a few weeks to months. **Protocol 3** About 2 weeks before surgery, mice (genetically altered to have characteristics of lupus) will have blood and urine samples taken to provide baseline data. Animals will undergo surgery to implant an electrode on the splenic nerve, and fully recovered over several days. A device (headcap) will be attached to the animal's head to allow connection to to the experimental equipment. In some cases, we will use a purpose developed 'button' through the skin as this technology develops. For 8 to 12 weeks the development of proteinuria (protein in the urine, a sign of kidney damage), lupus flare (skin rash), lymphadenopathy (enlarged lymph nodes), splenomegaly (enlarged spleen) and the production of inflammatory products in the blood will be analysed. Animals will be

	housed on their own during the period of electrical stimulation (i.e. 7 days to 10 weeks) in their home cage. The following may also be carried out: ● Clinical examination, handling animals, and urine collection ● Body weight measurement ● Blood sampling for analysis At the end of the study, typically at 17 weeks of age, the animal will be humanely killed. **Protocol 4** Mice will have blood samples taken and urine collected for baseline analysis of biology. After about 2 weeks, mice that are genetically predisposed to lupus, will be injected once with an accelerator (Pristane), that will speed up the development of lupus. For about 2 months, mice will have blood samples taken and urine collected (no more than once per week) for continued baseline analysis of biology. A few days to weeks later, animals will undergo surgery as described for Protocol 3. For the next 3 to 6 months, the development of proteinuria and the production of inflammatory products in the blood will be monitored. Animals will be singly housed during the period of electrical stimulation (i.e. 1 to 3 months) in their home cage. The following may also be carried out: ● Clinical examination, handling animals, and urine collection. ● Body weight measurement ● Blood sample for analysis At the end of the study, typically 5-6 months after Pristane injection, the animal will be humanely killed. **Protocol 5** Approximately 2 weeks before surgery, mice

	that are genetically predisposed to lupus, will have blood samples taken and urine collected for baseline analysis of biology. Animals will then undergo surgery as described for protocol 3. After full recovery, the animal will be given a single injection of an adeno-virus (Ad-IFN) that triggers the genetic mechanisms of lupus development. Over the course of the next 7 to 9 weeks the development of proteinuria and inflammatory products in the blood will be measured. Animals will be singly housed allowing electrical stimulation (i.e. 4 to 7 weeks) in their home cage. The following may also be carried out: ● Clinical examination, handling animals, and urine collection. ● Body weight measurement ● Blood sample for analysis At the end of the study, typically 7-9 weeks after Ad-IFN injection, the animal will be killed. **Protocol 6** Animals will undergo surgery as described for Protocol 3. After full recovery, rats will be given an injection of type II collagen (producing collagen-induced arthritis (CIA)). This is a typical rodent model to study rheumatoid arthritis. This will be followed up approximately a week later with a booster injection of a pro-inflammatory substance. Over the course of a week to 15 days the animals will start to show signs of joint inflammation. During this time, animals will be in their home cage and have electrical stimulation delivered. The following may also be carried out:

	• Clinical examination and handling animals • Body weight measurement • Blood sample for analysis At the end of the study, typically 21 days, the animal will be humanely killed.
What are the expected impacts and/or adverse effects for the animals during your project?	There may be some pain and stress associated with postoperative recovery which will be managed with analgesics and expert husbandry techniques. In Protocols 3-6, depending on the disease model, animals may present some limb swelling (arthritis), splenomegaly (enlarged spleen), enlarged lymph nodes and skin rash. Stress may be associated with attachment to a tether in the home cage. In the case of arthritis, animals may experience pain, and this will always be suitably managed with analgesics The duration of attachment will depend on the stimulation paradigm and could be from a few hours to 24/7.
What are the expected severities and the proportion of animals in each category (per animal type)?	Protocol 1 is non-recovery (100% of animals), Protocol 2 will be moderate (100% of animals), Protocols 3-5 will be severe (up to 50% of animals may enter severe category, but all attempts will be made to work within the moderate severity), and protocol 6 is moderate (100% of animals).
What will happen to animals at the end of this project?	Killed **A retrospective assessment of these predicted harms will be due by 10 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?	The diseases we are targeting with this therapy are complex conditions affecting many organs/systems in the body and with different causes (including hormonal, genetic, and cellular), which cannot be fully and accurately replicated outside of a whole animal as much about their interplay is unknown. Additionally, animals with a developed peripheral nervous system (PNS) are required to investigate whether electrical stimulation is an effective therapy for immune-mediated inflammatory disease. Through the use of animals, a greater understanding of cause and nature of the diseases and the mechanisms by which the nervous system controls the biological processes can be assessed to support electrical stimulation as therapy for disease.
Which non-animal alternatives did you consider for use in this project?	We use computer modelling, bench testing (non- animal), and human splenocytes (cells from the spleen) and whole spleen tissue work to support this project as well as animal studies.
Why were they not suitable?	Computer modelling is used to predict possible biological outcomes but is not fully conclusive. It cannot replicate the complex biological system of the autonomic nervous system, nor the variability. Electrical stimulation of the autonomic system (responsible for regulating blood flow, heartbeat, digestion, breathing) requires a whole animal to understand the effect. For example, downstream effects such as changes to noradrenaline (a hormone that is produced naturally by the body) outflow from splenic nerve stimulation, allows the measurement of biomarkers to determine whether stimulation works. There are currently no alternative ways of assessing therapy efficacy i.e. the ability to produce a desired or intended result, in models for SLE and CIA without the biological changes seen in these disease models.

	Biomarkers such as protein in the urine (SLE mouse models) and clinical signs of arthritis (CIA models) are only possible in the whole disease state. **A retrospective assessment of replacement will be due by 10 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: 5000 rats: 3000
How have you estimated the numbers of animals you will use?	The number of animals required has been defined by a statistician and based on the estimated number of studies we may carry out each year.
What steps did you take during the experimental design phase to reduce the number of animals being used in this project?	Our animal studies have been based on data generated from ex-vivo, in-vitro and whole human organ work as well as computer modelling and predictive analysis. When planning studies, we carefully considered the following: • **What are our research objectives and how**
	does the knowledge generated advance the field? - any area of research we wish to undertake is based on patient need and the premise of the effectiveness of the therapy is backed up by scientific publications • **The need to use animals / consideration of alternatives:** - we actively engage in the use of computer modelling to understand stimulation paradigms such as current, frequency, voltage, charge density as well as in-vitro work using human cells e.g. splenocytes as well as in- vitro and human

	whole organ research ● **Choice of species of animals to be used:** - we select our animal models by triaging appropriate strains using our animal model selection system. This ensures we have the most appropriate model for the investigations planned thus ensuring we obtain data that is most relevant to the question being asked. ● **Robust experimental design and its justification:** - we have used internal review programmes to assess the validity, robustness and likelihood of success through internal 'animal and project review' forums. These forums are made up of experts from study scientists, statisticians, vets and members of the animal welfare committees. These reviews consider the number of animals and frequency of measurements and interventions to be used, the primary outcomes to be assessed, planned statistical analyses, whether the experimental design meet the objectives of the experiment, what is the minimum number of animals we can use and how will this data impact numbers of animals in future studies as well as all welfare aspects.
What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?	We will use small initial (pilot) studies to understand the optimal approach to surgery, surgical outcomes which will provide opportunities for refinements through power analysis. Pilot studies may be small with 1-3 animals to allow a sampled approach minimising animals numbers used or e.g. larger 6-12 animals where these number may be incorporated into the main study thereby reducing overall animal usage. Larger more definitive studies will always have statistical input in order to optimise the number of animals used by ensuring studies are sufficiently powered to validly answer the question.

	A retrospective assessment of reduction will be due by 10 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?	1. We will take a staged approach to studies, to minimising the risks to scientific objectives and animals: 2. Aspects of the medicine, such as parameters and surgery, are designed and understood in non-recovery studies (Protocol 1). Only once these are fully clarified, we will move into recovery studies (Protocol 2). These recovery studies will start with non-disease animals, to understand aspects of tethering, single housing, dosing, surgical recovery for example. Once this has been refined, disease models will be used to assess the benefits of that refined therapy. 3. Different mouse types (models) for systemic lupus erythematosus (SLE) will be used (Protocols 3,4 and 5); as with lupus a single model alone does not represent the range of disease characteristics and biology seen in people, required to assess and confirm the effectiveness of the treatment. A single model of RA will be used (Protocol 6) as this model is able to provide the required information. 4. The mouse strains we will investigate, develop mild symptoms of lupus spontaneously at approximately 3 and 5 months of age. The disease onsets can be accelerated and aligned to levels of proteinuria similar to the human disease, by using an inflammatory accelerators, allowing us to minimise the period of time for disease development, thereby reducing the

	cumulative harm of disease and treatment period. 5. Collagen-induced arthritis (CIA) is an animal model of rheumatoid arthritis (RA) that is widely used to look at cause and treatments. The experience so far is in rat models. We will limit disease progression to no more than 21 days in a moderate severity, as this will provide required data while preventing unnecessarily prolonged harm to the animals. 6. Harms associated with each disease model have been minimised by reducing the duration of the disease and by setting clear humane and experimental endpoints. 7. The humane endpoints consider all aspects of the physical signs or symptoms associated with the particular disease (including joint swelling, skin flare, lymph and spleen swelling, and general wellbeing). 8. Experimental endpoints are linked in the majority to proteinuria levels, and not directly to the harms.
Why can't you use animals that are less sentient?	The three mouse models for SLE and rat model for RA have been defined by a thorough review as the only in vivo models scientifically suitable for this work. We must use conscious animals in order to allow the progression of a disease and the associated biology in order to measure and test the effectiveness of our therapy.
How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?	We will stay informed by advances of the 3R's from NC3R's website, internal named information officer (NIO). We have a central liaison to share 3R's information and practices across the company. We will implement advances in 3R's though protocol reviews to ensure they have been suitably considered and applied. We will also keep abreast of current as well as new and emerging literature that supports the development of the 3R's.

	A retrospective assessment of refinement will be due by 10 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?

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?	**Surgery** We will refine surgical protocols through pilot studies (protocol 2) to ensure the most optimised approach is developed, that will minimise harms and confirm and optimise surgical outcomes. The electrical stimulation therapy will be fully assessed in non-recovery experiments (protocol 1), where we can define parameters that are well below any pain activated threshold. **Husbandry, procedures, housing and environment** We will examine and adapt our handing, restraint, husbandry and procedural techniques to best suit tethered animals. For some of our studies, solo housing is the only option due to the electrical stimulation requirements, meaning an animal will sometimes need to be attached to a tether 24/7. We will investigate ways to pair house animals with tethers, as currently none exist. We will provide an enriched environment that reduces solo housing stress. This may also be achieved through the use of 'buddy' animals where appropriate or specially adapted cages. We will limit single housing to the minimum period necessary for the study, and we will provide visual, auditory, olfactory and tactile contact where possible. Skin button and implantable wireless devices if wireless systems become available, they will be assessed as a viable alternative to tethered systems. Ultimately

	part of the company's remit is to design a fully implantable, miniaturised or wireless device. This will provide a refined system of electrical stimulation in disease models, for use in this licence, and the wider scientific community.
What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?	The following is a list of the best practice guidance that we routinely follow. Prescott MJ, Lidster K (2017) Improving the quality of science through better animal welfare: the NC3Rs strategy. Lab Animal 46(4):152-156. Olsson IA, Hansen AK and Sandøe P. Animal welfare and the refinement of neuroscience research methods - A case study of Huntington's disease models. Lab Anim. 2008 Jul;42(3):277-83 Review of harm-benefit analysis in the use of animals in research - Report of the Animals in Science Committee Harm-Benefit Analysis Sub-Group chaired by Professor Gail Davies Nov 2017 NC3R's - Responsibility in the use of animals in bioscience research: Expectations of the major research council and charitable funding bodies LASA - Guiding principles on good practice for Animal Welfare and Ethical Review Bodies Sep 2015 Wei Li *et al.,* (2017) An update on lupus animal models, Curr Opin Rheumatol. Sep; 29(5): 434–441 Diehl KH et al., (2001) A Good Practice Guide to the Administration of Substances and Removal of Blood, Including Routes and Volumes: JOURNAL OF APPLIED TOXICOLOGY 21, 15–23 Morton DB et al., (1993) Removal of blood from laboratory mammals and birds Laboratory Animals 27, 1-22

Project	**15. Brain metastasis: Imaging 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)	The aims of this project are to understand how the local environment in the brain contributes to the growth of brain tumours. In particular, we are interested in secondary tumours in the brain which arise from primary tumours elsewhere (mainly breast, skin and lung). Currently, it is very difficult to diagnose these secondary tumours in the brain early enough to effectively treat them. At the same time, the specialised barrier between the brain and the blood prevents many potential therapies from accessing these tumours. Again, this reduces the effectiveness of treatment. Consequently, the prognosis for patients with secondary tumours in the brain is extremely poor. Under this project, we aim to	

	identify factors in the response of the brain to the presence of tumours that either help or prevent tumour growth. In this way we expect to identify new targets for therapy. We are also developing new approaches to enabling potential therapies to cross the barrier between the blood and the brain in order to enable effective treatment of brain tumours. Finally, we are developing new imaging methods that we believe will both help to diagnose secondary tumours in the brain earlier and also report on processes within the tumour that may impact on therapy effectiveness, such as blood flow, acidity and amount of oxygen. **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)?	Up to 40% of all cancer patients will suffer tumour spread to the brain. Owing to improved treatment of primary tumours, the incidence of secondary brain tumours is increasing. There is currently no cure, and life expectancy after diagnosis is generally only a few months. It is critical, therefore, that we develop new methods for earlier diagnosis and improved treatment. We anticipate that this work will enable development of new therapies and/or allow better application of existing therapies in patients with known or suspected brain metastases. We will also develop imaging methods that we believe will enable earlier diagnosis and, consequently, more effective therapy in patients with brain metastases.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use 5000 post-natal mice and 1000 post-natal rats in this work over a period of 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?	The expected adverse effects may include drug toxicity, vascular damage or uncontrolled bleeding following injection, local pooling of blood in the tissue following blood sampling and incomplete wound healing or infection following surgery. Some animals will undergo repeat imaging sessions, which can lead to an

	increasing aversion to induction of general anaesthesia as the number of sessions increases; induction is achieved as rapidly as possible through introduction of the animals into a chamber pre-filled with anaesthetic gas. In addition, deaths under general anaesthesia cannot be prevented and occur in a percentage of animals; this is more common in animals with higher tumour burden as respiratory and cardiac function may be compromised. With some of our tumour models, there is a moderate likelihood of either neurological symptoms or compromised health owing to the nature of the models. In all surgical cases, it is also expected that animals may experience a degree of pain. Some animals will undergo irradiation of their tumours, which can result in irritation and soreness to the skin overlying the tumour. This is only expected at the very highest doses to be used, and most animals will experience little or no pain or discomfort associated with irradiation. For the above reasons, we monitor the animals very closely and always seek advice from the NACWOs and vets. We use analgesia wherever required and all surgical procedures are performed under general anaesthesia. Although there is a possibility of animals experiencing moderate harm from any tumour model, they are often returned as actual mild severity. Based on our previous experiences and the balance of work between the protocols on this licence, the likely actual severity will be mild or lower in approximately 70% of animals, moderate in approximately 30% of animals and severe in <0.5% of animals; there is a very low risk of a severe outcome as some deaths cannot be predicted regardless of how closely we monitor the animals. At the end of the experiment all animals will be killed painlessly according to an approved procedure. **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	Metastasis is a clinical feature of cancer that depends upon tumour cells spreading

and why you cannot use non-animal alternatives	throughout the body. As such, it is a phenomenon that requires both a circulatory and immune system. At this point in time there is no tissue culture or modelling system that can duplicate or predict the process of secondary tumour spread in the mammalian system. We will support our animal studies with cell-based investigation of tumour-brain cell interactions, but this does not fully model the intact mammalian brain where multiple cell types are present in a complex 3-dimensional geometry and are supported by, and interact with, both the circulatory and immune systems. Consequently, whilst such models may allow modelling of specific elements in isolation, they cannot be used to model the entire system and, thus, do not represent a viable alternative to animal models. It is also not possible to fully test new imaging techniques in cell-based or non-biological systems, since the MR signal characteristics are not the same as those obtained from the brain in situ. All of our new contrast agents are tested extensively in phantoms and cell-based systems first, and only progress to animal studies once efficacy has been established in our standard assays. Nevertheless, since the overall goal of this work is to develop new techniques and agents that can be applied clinically a period of pre-clinical testing in animal models is essential and unavoidable. A considerable body of data is available on the neurobiology of rodents and they are widely used in models of brain diseases. It is recognised that many of the processes that we study in rodents are directly relevant to understanding of the physiology and pathology of the human nervous system. It is not possible to conduct meaningful experiments in this area in anything other than mammals since the development of the brain and immune system is unique to this class. Although a small number of immune system and metastasis studies have been conducted in zebrafish, it is widely agreed that our understanding of the extent to which zebrafish sufficiently reproduce and predict the behaviour of human cancer and metastasis remains unproven. For this reason, we do not believe that zebrafish represent a viable alternative to rodent studies at the present time. Therefore, this work will use experimental models in rats and mice, in which we have a substantial body of prior work on which the current work is based. **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 number of animals	Much of our work involves imaging in animal models, and as a consequence of being non-invasive this allows us to study temporal events in the same animal. Thus, the use of imaging, and MRI in particular, for animal experiments represents significant reduction. In addition, many different types of data can be obtained from each experiment, which further reduces the number of animals required. The number of animals in this licence has been chosen to be sufficient for statistically reliable data, based on previous results, the intrinsic variability of in vivo models and imaging data, and the magnitude of the expected changes. We consult extensively with the departmental statisticians as new studies begin to ensure that the optimal number of animals is used to obtain meaningful results and also kept to a minimum. Appropriate control groups are included and specified in each protocol and will be essential for proper statistical analysis and evaluation of observed effects. In all cases brain tissue will be used following the in vivo experiments, for immunohistochemical and molecular analysis, in an attempt to use the minimum number of animals possible. **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)	Much data is available on the biology of rodents and they are widely used in models of experimental neuropathology. It is recognised that many of the cellular and intracellular events we study in rodents are directly relevant to understanding of the physiology and pathology of the human nervous system. It is not possible to conduct meaningful experiments in this area

to the animals.	in anything other than mammals since the development of the central nervous system and immune system is unique to this class. In most cases we will use both rats and mice and we have a substantial body of work in these species on which the current work is based. For the tumour models, we have spent considerable time refining these models to allow a longer time span for investigation of the brain tumours in symptom-free animals. Moreover, although a small number of animals will be taken to the latest time points possible in many cases we are more interested in the early stages of tumour development, when the tumour burden is too small to cause distress to the animal. In order to better understand the contribution of inflammatory pathways to brain tumour development we will use various strategies to modify inflammatory pathways in some animals. We will use the most refined approach possible to modify aspects of the inflammatory response (e.g. up or down-regulation of single effector proteins) in order to minimise both adverse effects and additional suffering in these animals. Indeed, this is scientifically important since it allows us to dissect out specific contributions to the process. Wherever possible, without compromising the scientific outcomes of our studies, analgesia will be administered as required. If the use of analgesia will compromise the scientific outcomes, the experiment will be ended and the animal killed painlessly according to an approved procedure. Need to specify that analgesia will be provided for surgical procedures and that aseptic technique adhered to in compliance with LASA Guidelines on aseptic surgery. All substances will be administered by the most refined route possible. For example, where long-term treatments are required these will be given via a subcutaneous mini-pump if possible, thus reducing the handling and associated distress to the animal. We work closely with the NACWOs and vets, and have a strong track record in reviewing and refining our methods regularly as new approaches become available.

	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	**16. Causes and consequences of corvid intelligence**	
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
		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)	Recent findings suggest that corvids, members of the crow family, possess sophisticated cognitive abilities comparable to those of primates (including reasoning and planning for the future). This project will further our understanding of the evolution of intelligence by addressing how corvids' cognitive abilities develop and what benefits these abilities provide in their natural environment. Most research on corvids has been conducted in captivity, limiting our understanding of the factors favouring the evolution of corvid intelligence in nature. This project will use observations and behavioural experiments on wild corvids to: (1) Assess the cognitive processes corvids use to overcome social and ecological	

	challenges. The need to navigate the challenges of life in complex and dynamic societies is thought to be a key driving force behind the evolution of primate intelligence. The project will use experiments to test whether corvids, like primates, recognise multiple individuals, keep track of the relationships between them and benefit from their investments in social relationships. It will also examine the potential benefits of forming strong social bonds for individual health, ageing and reproductive success. (2) Investigate how learning from others affects corvids' behaviour. Experiments will examine how information about predators and new foraging techniques spreads through groups by learning. (3) Assess the causes and consequences of variation in cognitive ability. Corvids will be given a variety of cognitive tasks to determine (i) why individuals vary in their cognitive abilities and (ii) how this variation influences the ability to survive and reproduce. Hormone analyses (from feathers and blood plasma) will allow assessment of stress (corticosterone) and its relationship to cognitive performance. **A retrospective assessment of these aims will be due by 22 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)?	1) Advancing fundamental understanding of how and why cognition evolves. This will be of widespread importance to biologists, psychologists and anthropologists 2) Revealing how social bonds influence individual health, ageing and cognitive performance in natural populations. In addition to its immediate contributions to the scientific literature, this will contribute to the development of evidence-based interventions to promote human health and well-being 3) Conservation and wildlife management: insights into animals' responses to environmental changes will contribute to the development of effective, evidence-based policy. 4) Public

	engagement and science education. Findings will be widely disseminated through public workshops and exhibitions, tv, radio, press and social media, fostering understanding and appreciation of the scientific process and our native wildlife.
What species and approximate numbers of animals do you expect to use over what period of time?	Approx 2500 wild jackdaws and 200 wild rooks 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?	With the exception of the withdrawal of feathers (for hormone analysis) and small blood samples (for analyses of hormones, parasite levels, ageing and the inheritance of social traits), the project is entirely non-invasive. Blood and feather sampling will be conducted using aseptic technique, taking only the small volumes necessary for laboratory analyses. These procedures are of mild severity and no adverse consequences are anticipated. Birds will generally be released immediately following sampling to avoid undue stress. To understand how corvids' social structure is maintained throughout the year, a small number jackdaws and rooks will be fitted with a gps tag mounted on a harness. This will be removed within 18 months and birds released back into the wild. The severity of gps tagging is mild: extensive prior research shows that tags cause no discomfort or ill-effects. **A retrospective assessment of these predicted harms will be due by 22 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	There is no alternative to research on non-human animals if we are to understand of the factors favouring the evolution of intelligence. Without conducting research on animals, it is impossible to understand the benefits that being intelligent may provide. Mathematical models can be useful in examining the theoretical efficacy of cognitive processing, but cannot explain how animals use their cognitive abilities to solve challenges under

	natural conditions. In this project, statistical analyses of field data (controlling for confounding factors) will determine (a) how corvids respond to challenges in their environment and (b) how these responses may provide benefits for health, ageing and rearing young. **A retrospective assessment of replacement will be due by 22 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	All proposed experimental and observational protocols are based on previous successful research conducted by myself and others. Sample sizes for experiments will be determined using power analysis to determine the minimal number of animals needed to detect statistically meaningful effects. Specialised statistical techniques will allow any confounding factors to be accounted for, maximising the possibility of detecting meaningful patterns in the data. As male and female corvids look alike, it is necessary to take small blood samples to determine their sex accurately by examining the sex chromosomes. Blood samples will also give accurate measures of current levels of stress hormones, parasite levels and telomere lengths [protective caps on the ends of chromosomes that shorten with age, providing a measure of ageing] and allow genetic analyses to determine if offspring inherit parents' characteristics. Feathers provide a long-term measure of stress throughout development. The total number of individuals sampled will depend on the rate of population growth, but numbers are likely to be fewer than 500 jackdaws each year and 200 rooks in total. The number of gps tagged individuals will be kept to the minimum necessary (<40) to establish if social bonds persist year-round. **A retrospective assessment of reduction will be due by by 22 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.	Corvids have large brains and possess sophisticated cognitive abilities, making them ideally suited for studies of the evolution of intelligence but most previous research has been conducted in captivity. The use of wild corvids represents a significant ethical advance, given that captivity can cause substantial stress. Blood sampling will be performed rapidly and to avoid unnecessary suffering. Small blood samples (< 100µl) will be taken from superficial blood vessels. The area will be swabbed with ethanol before and after sampling to prevent infection, and any bleeding will be controlled with gentle pressure. The birds are small enough to be gently restrained by hand and will be released immediately following sampling. Existing evidence shows handling and sampling does not affect nestling survival or provision of care by parents. One feather will be removed rapidly immediately following blood sampling of jackdaws. Central tail feathers will be taken as they do not affect flying ability and will re-grow. GPS tags are extremely lightweight and will be fitted using a soft harness held snug to the body, minimising any risk of the harness becoming tangled. **A retrospective assessment of refinement will be due by by 22 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	**17. Chemotherapy, Immunology and Pathogenesis of Trypanosomatid group infections**	
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)	Three of the major neglected tropical diseases (Chagas disease, human African trypanosomiasis and the leishmaniases) which cause death and suffering in humans worldwide, are caused by trypanosomatid protozoan parasites. They are threatening a total of more than 400 million people worldwide and collectively cause approximately 150 000 deaths per year. There are no human	

	vaccines available for any of these diseases and current drug therapy is unsatisfactory due to toxicity, resistance, cost and methods of administration. The aims of this project include the creation of more predictive and refined animal models for these infections, better understanding of the mechanisms whereby the parasites cause disease symptoms and the role of the immune response in causing pathology, identification of preclinical candidate compounds capable of curing the infection or alleviating the pathology and the identification of novel drug targets or vaccine candidates. **A retrospective assessment of these aims will be due by 10 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 expected benefits of this project include: i) Characterisation of how anti-leishmanial and anti-trypanosomal drugs work in vivo and how/when they are best used, e.g. optimal dosing or route of administration. ii) Assessment of the roles played by the parasite and the immune response in the development of clinical disease and identification of mechanisms involved. ii) Characterisation of how a disease process can affect drug action or how drugs can affect disease processes. iii) Contribution to the identification, evaluation and development of new medicines, therapies, vaccines or diagnostic tools. iv) Refinement of animal models and their use in studies to develop medicines.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mainly mice and potentially a small number of hamsters. Over 5 years we estimate to use a total of 7775 mice and 290 hamsters. This number of animals is shared by research groups working across the department.
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 infected with Leishmania parasites that invade internal organs (viscerotropic) show no clinical signs of infection and those infected with cutaneous Leishmania parasites, although developing a lesion on the rump, show no signs of discomfort or distress. Hamsters infected with viscerotropic Leishmania parasites will progress to clinical disease, but endpoints are set to not exceed a moderate level. Mice and Hamsters infected with Trypanosoma parasites will show

	clinical signs of disease such as piloerection and hunched posture for a short period during the acute phase of infection or under immunosuppression. Endpoints are defined to not exceed a moderate level of severity except in the three protocols with a severe limit which will only be used under defined circumstances. A small percentage of animals may experience a higher severity limit, e.g. in cases when new strains of parasite are introduced, for which the progression of infection cannot be known in advance. Different strains of parasite may show different tissue locations which could affect the type of symptom the animal will develop and this may not be predictable. Where animals are showing clinical symptoms that may hamper feeding/drinking for short periods the food and water will be provided within the cage such that it is within easy reach at all times, and the animals will be monitored more closely. In experiments where drugs are being tested against the brain stage of African trypanosomiasis a small percentage of animals may find accessing food difficult because of weakness in hind legs. The worker will ensure such animals have ready access to both food and water at all times. This symptom is not predictable but where it appears permanent, the animal will be humanely killed. At the termination of each experiment all animals will be humanely killed by a Home Office approved method. **A retrospective assessment of these predicted harms will be due by 10 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	The programme of work aims to identify new treatment and vaccination strategies, to assess pathology and host-parasite interactions in disease models and to identify tools to monitor treatment responses. These studies require the use of animals to answer specific questions, which cannot be investigated in isolated cell culture. Examples of such studies include the distribution and effect of a drug in a multicellular organism or the response to a treatment taken by mouth

	measured in blood or other tissues. Pathology is tissue or organ specific in each of these diseases and requires an intact immune response, so cannot be studied in isolated cell cultures. **A retrospective assessment of replacement will be due by 10 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	Experiments are planned in order to maximise data output with the least number of animals used and to minimise bias by allocating animals to treatment groups in random order. Where possible data is evaluated by a researcher unaware of the nature of treatment given to each group. In each experiment and at all stages of the work, control groups of mice (e.g. untreated or PBS treated animals in studies evaluating drug activity) will be included and where possible animal numbers will be limited by sharing control groups or using control groups for analysing multiple outcomes. As an example blood samples may be collected from animals undergoing experimental procedures such as evaluation of drug activity to look for prognostic markers. The number of animals will be significantly reduced by our development and usage of non-invasive imaging technologies. Use of dual reporter bioluminescent/ fluorescent parasites also maximises the biological data that can be obtained from each animal thereby reducing numbers again. **A retrospective assessment of reduction will be due by by 10 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 is the host species of choice for Leishmania and trypanosomes and will be used wherever possible. Mouse models have been used and validated extensively and provided much of our knowledge to date on these infections. In some experiments, hamsters are necessary to recapitulate elements of human pathology that are not adequately reproduced in murine infections

	(for example visceral leishmaniasis or digestive Chagas disease). The parasite strains we use for infections are well characterised with respect to the disease they cause and all new parasite strains we use are assessed for their infection kinetics and virulence in small groups of animals and, prior to this, in cell culture. The use of non-invasive imaging for trypanosome or Leishmania infections will reduce the total number of animals used and in some cases determine an endpoint for experiments to limit disease progression. The use of non-invasive heart monitoring in mice with specific symptoms may allow intervention prior to cardiac failure. **A retrospective assessment of refinement will be due by by 10 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	**18. Circuits for Cognition: Cells, Networks and Neuropharmacology**	
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
		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 will study how the brain allows us to perform to plan our everyday life. How do we make informed choices? We do this by looking at the options available and choose the ones that help us to get what we want. To do this, we sometimes need to plan multiple things. If I want to go on holidays, I may need to save up, I may need to book a flight well ahead, I need to check my passport, as the journey approaches I need to pack my bag, make sure the cat gets fed, ... For all of these actions, I need what is called attention, memory, make decisions, and take actions. In the past these have been studied in isolation. But, to plan my holiday, I need to activate	

	them simultaneously. I need to attend to the computer monitor to make my booking, remember where and when I want to go, make decisions about the best flight option, and hit the right keyboard keys. How the brain achieves this is still a mystery. The brain is composed of many different parts, that help us attend, memorise, decide, act, but we do not know how they interact in these real life scenarios. Many different brain chemicals are involved, but we do not really understand how they act, when they act, and what their exact function is. We will study how the building blocks of the brain (the neurons) interact when animals perform tasks that involve attention, short term memory, decision making, and selection of specific actions. Specifically we ask: which parts of the brain are important for which aspects of the task, and which brain chemicals are important for which aspects of task. Understanding this is important, as most psychiatric diseases are diseases where the interactions between neurons, between different parts of the brain, and brain chemicals is dysfunctional. To do so we will record with very different techniques, which all provide complimentary information. Firstly we use small devices (microelectrodes), and study what single neurons do in one part of the brain, and across multiple parts of the brain, and what brain chemicals do in one part of the brain, in other parts of the brain, and how they help brain areas to communicate with one another effectively. The latter requires to selectively influencing the brain chemicals (the so-called transmitter systems). These studies are unavoidably invasive and can only be done in awake task performing animals, not people. The minimally invasive technique of functional brain imaging (fMRI) will also be used on animals whenever possible and every attempt will be made to compare these findings to equivalent studies done non-invasively in humans It is not possible to perform these studies in isolated cells, or even in brain slices in a test tube, as it is the interactions between individual cells and the rest of the brain that is of importance in these studies. **A retrospective assessment of these aims will be due by 11 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)?	Proper understanding of the mechanisms of attention, working memory, decision making and learning is crucial to understand psychiatric and cognitive diseases (such as Schizophrenia, Attentional Deficit Hyperactivity Dysfunction, Alzheimer's disease and Lewy body dementias and stroke of the parietal cortex). Once we understand how this is achieve by the intact brain, we can better understand what goes wrong in the psychiatric and cognitive diseases mentioned above. This in turn makes it easier to develop a targeted approach to treat these disorders. The research therefore may have long-term clinical implications. Moreover, ultimately it will yield a proper understanding how attention, learning and memory are implemented in the brain. • We will learn which brain chemicals (and their docking stations) are involved in cognition and goal directed behaviour. • We will learn which brain areas are involved, and how different cell types enable these. • We will learn how different brain chemical orchestrate the complex interactions between different brain areas,. • Crucially, we will obtain a thorough understanding how specific cognitive dysfunctions (such as Schizophrenia, attentional disorders, dementias) may arise, and thereby our studies will inform psychopharmacology (the treatment of mental disorders with specific drugs). It may in the long-term aid in the development of better drug design for the treatment of a variety of cognitive dysfunctions. These are long-term goals, where progress has been made over the last 30 years, but where many crucial questions still remain unanswered.
What species and approximate numbers of animals do you expect to use over what period of time?	Non-human primates (NHP, up to 64 macaques over 5 years) Rodents (up to 512 mice over 5 years). Macaques are chosen due to the evolved visual system, their advanced cognitive capacity, and the

	similarity of prefrontal areas to those found in humans. They can be trained to attend, memorize, and devise strategies to decide near optimally to harness rewards, within trials and across trials. Rodents do not have such an evolved visual system and rich visually based behavioural repertoire, and while they are able to navigate their environment, their decision-making relies at least in parts on different circuits and mechanisms. However, rodents can still be an excellent model system to understand how specific cell types contribute to cognitive functions due advanced genetic tools available for them and certain techniques currently being more suited for rodent work. We will therefore also use rodents for parts of the projects, to gain insights that are currently not possible in non-human primates.
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 applied methods are routinely used by most NHP and rodent labs worldwide. Non-human primates Monkeys bred for research purposes will be slowly acclimatized to accept placement in primate chairs for up to 5 hours. Cranial surgeries will be performed to implant a head post and recording chambers, Some animals will have recording electrodes implanted which will allow long term recording of selected neuronal populations. The head post allows the animal's head to be stabilized while recording neuronal activity, without causing discomfort. This is necessary, as it allows for stable, undisturbed recording of brain activity using neuroimaging or electrophysiology while they perform a behavioural task. Monkeys are motivated to perform the task by having controlled access to fluid (they receive at least 20ml/kg/day during training/testing sessions but in most cases more during the working week), In some animals will have controlled access to food instead of fluids (in which case they receive their main food in the form of a 'slurry' in the testing setup during the working week). Fluids are provided to the monkeys as reward for correct task performance. Mice are motivated to perform tasks by controlled access to water. Fluid (and if necessary food) control is carefully regulated individually for each animal to provide optimal motivation to the individual animal ensuring at the same time good health and wellbeing. Fluid (or food) restriction can be complemented (or even replaced) by electrical microstimulation of reward centres. The expected adverse event with immobilisation is transient initial

	distress, which is minimized during the acclimatizing procedures, so that it is mild if not absent in well-trained animals. Similarly, initial distress may also be present during initial brain imaging sessions caused by the restricted space and loud noise of the scanner. Acclimatization here similarly results in calm animals performing many trials without obvious signs of distress. Access to brain neurons for recordings, microscopic imaging or experimental interventions requires surgical implantation of a post, which is used to stabilise the head, and of recording chambers. Multiple chambers need to be implanted for delineating neural communication between areas. Animals may have very small areas of the brain temporarily inactivated by physical, chemical or optogenetic methods, partly involving substances injected into the brain in tiny amounts. These procedures should have no or only barely noticeable effects on the animals in everyday life. A potential adverse event is infection associated with the implant, which could in the worst-case result in negative welfare effects for the animal, where in the worst case scenario (e.g. seizures, monoplegic/hemiplegic paralysis, encephalitis) animals will be humanely killed. Significant efforts are made to prevent or minimise infection by further developing and optimizing implantation methods and by regular care and treatment of wound margins. Inserting probes or cannulas into the brain to record or manipulate neuronal activity is associated with a certain risk for intracerebral accidents, such as infections, bleeds or seizures. Although such incidents are rare, they have the potential to result in negative welfare to the experimental animal if they occur. Important methodological and diagnostic steps including MRI are taken to prevent this with clearly defined action and end points. At the end of the experiments or under exceptional conditions when untreatable adverse effects occur that result in prolonged suffering of the animal that cannot be easily ameliorated, the animal will be humanely killed. The brain will be extracted to allow detailed investigation of brain tissue that is not possible from living organisms. We will assess physiological markers for stress throughout the study period to identify biomarkers for the animals' cumulative experience during the study. When the scientific endpoint is reached for an animal, the animals will be killed humanely by an overdose of anaesthetic. To maximize the use of the animals, some of them will be anaesthetized and other types of brain recordings made, then after

	keeping them anaesthetized for several days, they will be killed without having allowed them to recover consciousness Most of the individual procedures that are applied to the animals are of moderate severity, but the overall severity banding of protocol 1 is severe. Rodents (mice) Mice will be trained to perform specific tasks, in a laboratory setting. Cranial surgeries will be performed to implant a head post and recording chambers, Some animals will have recording electrodes implanted which will allow long term recording of selected neuronal populations. Some animals will receive intracranial injections of viral vectors to allow for optogenetic manipulation of neuronal activity. The head post allows the animal's head to be stabilized while recording neuronal activity, without causing any discomfort. The chambers will be used to access the brain for short-term electrode recordings and investigation of the neurotransmitter systems involved in attention, learning and memory. All of these techniques are designed so that after the surgery the animals make a full recovery, and can then be studied in ways that cause minimal distress. In order for the animals to be motivated to perform the various tasks, the amount of fluid they are given each day will be restricted, and they will be given fluid as a reward when they complete a task successfully. The degree of restriction will be very carefully monitored, and kept to the minimum needed to motivate them to perform their tasks (absolute minimum at least 40ml/kg/day). When the scientific endpoint is reached for an animal, the animals will be killed humanely by an overdose of anaesthetic. Some animals may be anaesthetised and other types of brain recordings made then they will be killed without having been allowed to recover consciousness. The overall severity banding of protocol 2 is moderate. **A retrospective assessment of these predicted harms will be due by 11 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	Cognitive functions do not exist outside the animal kingdom and even there the abilities are only properly evolved in birds and mammals. While imaging studies in people can reveal the basic anatomical aspects of cognitive control, it does not allow insight into the underlying mechanisms. Computational modelling can make predictions regarding the implementation of certain functions and I have a well-established tradition of close collaboration with computational scientists to use this approach. However, these modelling studies require experimental validation, which in this case requires invasive animal work. Whenever possible, use will be made of mice, since some specific investigations can be done in this species. In order to minimise the numbers of animals used, especially the numbers of primates, a series of linked studies which build upon each other for one aim are carried out in each animal, and the maximum information is obtained by conducting a final study under anaesthesia. During this final study tissue will also be taken for other, in vitro, investigations. **A retrospective assessment of replacement will be due by 11 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 current standard of the field in neuroscientific studies involving NHP is to report effects in 2-3 animals per publication. This is in part, because the effects from intracranial recordings have much higher signal-to-noise ratios than non-invasive methods. Monkeys work on cognitive tasks several hundreds to thousands of trials every day, giving a high number of trials and statistical power. Comparison to a second or third animal ensures reliability and reproducibility of the applied methods and discovered effects, while at the same time minimising the number of used animals. Our methods provide a high yield of data from each experiment. We often use multiple electrode systems, which gather data from multiple sites simultaneously.

	The same overall principles also apply for the research involving and mice. However due to smaller effect sizes and nature of experiments with less trials, effects are typically tested on 6-8 mice (determined by power calculations), keeping the number of tested rodents to a minimum. **A retrospective assessment of reduction will be due by by 11 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.	For the largest part of this programme, NHP are the species of choice and without alternatives to investigate the neuronal mechanisms of complex, visually guided cognitive behaviour. The reason for this is that the brain of NHP resembles the one of humans in its organization more than that of any other species. The primate sensory system and the areas involved in cognitive control differ in some key aspects from rodents (e.g. the existence of a dorsolateral and ventrolateral prefrontal cortex and a frontal pole in primates, which is absent in rodents). These areas are probably the areas that are most involved in cognitive control in humans. For our results to be applicable to human patients, it is necessary that much of the work is carried out in old world primates, such as macaques. Research over the past decades from many labs throughout the world has established that macaque monkeys can be well trained on various cognitive tasks involving controlled eye movements. This is not possible to the same extent in non-primate species. Whenever possible, we will use rodents to test all aspects of the project that do not require primates, specifically the function of certain cell and circuit mechanisms for the interareal communication, and the global underlying neuropharmacology that shapes these interactions. Here, cell-type specific manipulations are available for rodents that currently do not exist for non-human primates. We will also work on improving the welfare for experimental animals by i) developing strategies to increase bone and skin integration of cranial implants and ii) the identification of biomarkers of animal experience in relation to research events. Our institution is one of the few centres in the UKand

	Europe that is fully committed to and equipped for highest quality experiments in NHP and rodents. This includes a dedicated veterinarian team, a dedicated NHP MRI scanner, excellent housing facilities, dedicated and unique experimental labs. We are constantly evaluating and improving our husbandry standards and implantation methods to ensure the wellbeing of our animals. All surgeries will be done under general anaesthesia with post-operative pain relief provided for as long as is necessary (we will aim to provide this in a way to enable the animals to self-administer the analgesics). We are very experienced in training monkeys so that they gradually become accustomed to working with us on their cognitive tasks whilst being head restrained and in unfamiliar surroundings such as MRI scanners or when their brains are being imaged using very specialised microscopes. If necessary animals will also be temporarily given sedatives or drugs to reduce anxiety during early phases of training. If a particular animal is unable to adapt to the training despite these careful training methods it will be transferred to a more suitable protocol for this individual following consultation with the home office, or it will be humanely killed using an anaesthetic overdose. Devices such as head restraint posts and chambers for accessing and recording from the brain are implanted in the skull and are cleaned and examined typically several times a week to minimise chances of infection developing. Animals are also monitored daily during the week so any changes in their weight, body condition, behaviour or demeanour can be rapidly detected and any necessary changes to their schedules or veterinary treatments made promptly. All animals will be housed with one or more compatible cage mates at all times except for when medical, or welfare reasons or cage-based testing make temporary single housing unavoidable. Macaque monkeys will be used in experiments that will take several years to complete. Training to learn the complex visually based tasks takes often many months in macaques. Here, 2-4 main surgeries (over the scientific lifetime of an animal) are needed to implant a head restraint device and recording chambers onto the skulls to record, image or manipulate neuronal activity repeatedly throughout the duration of the project. Additional minor surgeries (8-16) may be needed to maintain the recording chambers in adequate conditions to achieve the scientific purpose. These steps are needed to measure how the activity of identified neurons relates to stimulus conditions and behavioural reactions, including

	perceptual reports, and to delineate how these microscopic measures of single neuron activity relate to more global, non-invasive measures of brain activity (such as EEG or fMRI that are routinely used in humans). For non-recovery procedures done at the end of an experiment, animals will be kept under anaesthesia for the duration of the procedure, which can last for up to 5 days and may involve giving agents that can paralyse the muscles. They will always be attended by an anaesthetist experienced with the use of these agents and will have key vital signs monitored at all times to ensure they remain under full anaesthesia and that a surgical level of unconsciousness is maintained. Mice: Mice are becoming the prevalent mammalian species in biomedical research, as only in mice is it possible to use extremely powerful techniques of genetic manipulation and targeting. The techniques of simultaneous recordings described above rely heavily on these techniques. We take multiple steps to minimise welfare costs. We will use appropriate anaesthetic and analgesic regimes for pain relief during surgery, and some techniques in subsequent experiments are non-invasive, as they involve imaging. When we do insert probes into the brain, these are fine probes, and they cause no pain as the brain lacks that sensation. We further minimise discomfort by choosing light-weight head attachments, so that the mice can move normally, by carefully controlling the minimum amount of water received every day, to prevent dehydration, and by progressively acclimatising the mice to the behavioural environments, so that they do not experience stress. **A retrospective assessment of refinement will be due by by 11 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	**19. Control of EHV-1 in the horse**	
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)	Equine herpesvirus type 1 and 4 (EHV-1/4) cause respiratory disease, abortion and neurological disorders in horses. Protection afforded by existing vaccines currently in use is suboptimal. There is still no vaccine that is licensed to protect against the neurological form of the disease and abortions still occur in highly vaccinated horse populations. The purpose of this project is to improve protection afforded compared to currently available products by preventing viraemia, and thereby reducing the risk of abortion or neurological disease that can follow. **A retrospective assessment of these aims will be due by 22 January 2021** The PPL holder will be required to disclose:	

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Retrospective Assessment	**Retrospective assessment** Published 30 July 2020 **Is there a plan for this work to continue under another licence?** Unfortunately, the PPL had to be revoked early, the intention is to apply for a further Project Licence in the future. **Did the project achieve its aims and if not, why not?** The objectives of this Project Licence were to monitor antigenic changes in equine influenza viruses, assess suitability of virus strains for challenge studies, determine whether vaccines generate an immune response or not, determine whether vaccines protect against challenge or not and provide a service for regulatory studies. This included ensuring that vaccine studies were carried out with an appropriate challenge strain. Two pilot studies were carried out successfully to assess virus strains for challenge studies. One of these was carried out with a strain that caused widespread outbreaks in the UK during 2019 and provided the added benefit of useful sera for antigenic characterisation of viruses. One vaccination/challenge study was carried out using this recent strain and was used to assess immunological response and efficacy. Although a small number of studies were completed in less than two years, all of the objectives were met to some extent.
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 will improve the understanding of EHV-1/4 disease and develop and test the safety and effectiveness of new vaccines, which will directly improve the health and welfare of horses worldwide.
What species and approximate numbers of animals do you expect to use over what period of time?	Up to a total of 155 horses will be used in this project over a five-year period which will include both grant funded work and work funded by vaccine development companies under contract. A maximum of 10 horses will be used to provide blood for isolating primary equine cells that will be used to select only those vaccines that are most worthy of further

	investigation. This will enable us to reduce the number of horses required in subsequent experiments. Up to 20 animals will be used to test new challenge strains and to improve the challenge models to get the best data that will be applied to the next step. Up to 50 horses will be used to test the safety of new vaccines and establish which dose of vaccine is likely to be the most effective prior to challenging horses with equine herpesviruses. A maximum of 80 horses will be used to test the effectiveness of vaccines in studies that will lead to the registration and launch of products to prevent the serious clinical signs induced by EHV-1 and 4 in horses.
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?	Uninfected animals are kept at grass. Taking of blood samples for the isolation of primary equine cells for the cultivation and characterisation of live attenuated vaccines will lead to transient discomfort and no lasting harm. These are routine procedures conducted by experienced staff. Horses will be maintained on site and may be rehomed at the end of the procedure. Animals that are vaccinated with killed, live attenuated or subunit vaccines may experience mild local reactions such as swelling or soreness. We expect any reactions observed will be no worse than those seen following the administration of current commercially licenced EHV-1 vaccines that are used in the general horse population. Horses that receive killed or subunit vaccinations may be rehomed at the end of the procedure. Horses that receive live attenuated vaccines in the early stages of research cannot be rehomed, but could be maintained on site. Once a dossier of data has been compiled for each vaccine, this will be reassessed to determine whether these horses could be safely rehomed. Animals that are exposed to wild type EHV-1 or EHV-4 are housed with a containment building. Animals typically have a self-limiting and mild illness. Unvaccinated horses typically develop a fever from day 2 post-infection which lasts for 2-3 days and may develop nasal discharge. Naïve horses can sometimes develop secondary bacterial infections after virus challenge and any affected animals will be treated with antibiotics. There is a possibility (<12%) that horses infected with EHV-1 may develop neurological disease as a result of damage to blood vessels in the brain or spinal cord,

	similar to that seen in some outbreak situations in the field.. In most animals the mild clinical signs resolve without intervention within approximately 10 days of onset. Any affected animals will be observed closely for the duration of the clinical signs and appropriate supportive therapy administered. If they fail to respond to treatment or the severity limit is likely to be exceeded they will be euthanased in order to minimise any suffering. EHV-1 infected animals cannot be rehomed as they are thought to carry the virus for the rest of their lives. This virus may reactivate later in life, spread to other animals and cause disease.EHV-4 causes much more mild clinical signs than EHV-1 and poses much less of a risk to other animals. Therefore these animals can be rehomed if it is in the best interests of the animal and they have been through the establishment's re-homing scheme. Animals that are fully recovered at the end of procedures may be kept alive at the establishment (with agreement of a vet) with a view to their reuse on procedures if appropriate and licenced. Otherwise animals will be killed humanely using an approved method.
Retrospective assessment	**Retrospective assessment** Published 30 July 2020 **What harms were caused to the animals, how severe were those harms and how many animals were affected?** Eleven ponies were used for challenge studies, all of which experienced respiratory signs including pyrexia for at least one day, cough for at least one day and nasal discharge. Overall severity level assessed as moderate due to multiple clinical signs. Twenty eight ponies were used for a vaccination/challenge study, of which seven were unvaccinated controls and experienced the same respiratory signs indicated above. Overall severity level assessed as moderate due to multiple clinical signs. The remaining twenty one ponies displayed reduced clinical signs compared to the unvaccinated controls. Ponies experienced mild discomfort due to vaccination, blood sampling and the taking of nasal swabs.

	Overall severity assessed as moderate due to the cumulative experience of repeated sampling combined with mild clinical signs.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	During the preliminary stages of the vaccine development program we will use cell lines and horse cells isolated from blood to avoid the need to infect animals. These studies will enable us to select only those vaccines that are most worthy of further investigation in horses. Unfortunately the use of horses for our research is essential. EHV-1 and EHV-4 cause disease that is unique to horses and we need to measure how the horse's immune system responds to the virus and vaccines in order to maximise the level of protection achieved by the vaccines. For pharmaceutical companies to licence and market vaccines commercially they need to be tested according to regulations determined by the competent authority. These regulations state that the vaccines must be tested in the target species, in this cases equines.
Retrospective assessment	**Retrospective assessment** Published 30 July 2020 **What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?** It is not possible to replace the natural host in vaccination/challenge studies for the purpose of registering new vaccines for equines.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Data generated from *in* vitro experiments will enable us to select only the most appropriate virus strains or vaccines for further testing in the horse, reducing the number of horses required. The design of all studies will be checked by a statistician to ensure that the smallest numbers of horses are used in order to achieve statistically significant results. Infection studies typically use between 4-6 horses. Vaccine studies have typically 6-8 animals per group.

Retrospective assessment	**Retrospective assessment** Published 30 July 2020 **How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?** Use of individual nebulisation rather than room nebulisation enabled us to use smaller groups of ponies for the vaccination/challenge study as all ponies showed obvious clinical signs during the pilot challenge studies. Previous group sizes have typically been 10, we used 7 per group.
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 only way to accurately determine how well an EHV-1/4 vaccines work in the horse is to use the natural host. Basic models have been developed that help us characterise live attenuated virus vaccines in the laboratory before we use them to vaccinate horses, thereby reducing the number of animals used for testing. However, we cannot model the complex interaction of the virus with the horse immune system so we must use horses to determine whether newly designed vaccines are safe and work. The procedures will be undertaken by experienced staff on site thereby reducing the amount of stress suffered by the animals. Generally the more the horses are handled, the more familiar they become with the routine and suffer less stress as a result. Extensive experience of animal handling and regulated procedures suggests that serious adverse effects of repeated blood sampling, vaccinating and EHV infection are rare. Horses that undergo vaccination only are kept at grass in groups in grass paddocks with freedom to roam with shelter if they require. The research being addressed by the programme will contribute to the welfare of horses in the long term by providing new and improved vaccines. In order for pharmaceutical companies to obtain marketing authority for commercial vaccines they must be tested in the target species, in this case equines.

Retrospective Assessment	**Retrospective assessment** Published 30 July 2020 **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?** Every effort was made to handle the ponies as much as possible prior to starting procedures, which made the challenge phase of each study run smoothly and with minimal stress for the ponies. Ponies were kept in their original weaned groups to reduce stress. Ponies were exposed to virus challenge using individual nebulisation and acclimatised to the apparatus one day prior to challenge. This worked well and the nebuliser was tolerated very well by the ponies during actual virus challenge. Environmental enrichment was provided in the form of a variety of vegetables, with different ponies showing a preference for different vegetables. Some ponies enjoyed playing with water during room-cleaning and this was turned into an apple-bobbing game with the pony handlers. Scratching brushes were placed around the rooms and were utilised by the ponies. Day and night footage were captured on camera to determine whether any further improvements in environment were needed, but pony behaviour ,including lying down, mutual grooming and play interaction, was normal. No negative or stereotypical behaviours were displayed by the ponies. During the second challenge study numerous photographs were taken to generate an improved scoring system for assessing nasal discharge. This was blind-tested on a panel of scientists and led to revision of the clinical scoring system used for the vaccination/challenge study and an improvement in the quality of the data generated. This also led to an agreement with the sponsor to reduce the total time spent in the containment facility prior to challenge.

	A talk on the conduct of pony studies was presented in the 3Rs session at LASA by the NACWO.

Project	**20. Cortical and sub-cortical control of movement**	
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 has the overall aim of improving our understanding of the different nerve centres in the brain and spinal cord involved in the control of movement, and translating the knowledge into improvements in treatments for patients recovering from injury, such as after stroke or spinal cord injury. Specific objectives are: 1. To understand the relative contributions of different parts of the nervous system to movement control in healthy animals 2. To understand how these different parts	
	of the nervous system contribute to recovery after damage (e.g. after a	

	stroke), and how their interactions lead to deficits seen in patients who have recovered from damage, such as spasticity 3. To understand how connections between and within these parts of the nervous system allow information processing to coordinate the control of movement 4. To understand how abnormal connections or information processing by these neural centres can generate movement disorders such as dystonia or tremor 5. To understand how non-invasive methods, which we use in human subjects, activate different parts of the nervous system 6. To understand how connections between cells in different parts of the brain strengthen or weaken based on their activity 7. To understand how novel protocols, which we have developed to stimulate neural pathways non-invasively in humans, can act on these cellular processes to strengthen or weaken connections 8. To understand how rhythmic activity in different parts of the nervous system is generated by neural circuits in both health and disease (e.g. in tremor), and what the function of this activity might be for information processing and motor control 9. To develop a novel primate model of motoneuron disease, and to use this to address key outstanding questions about how the disease progresses 10. To test whether biological measures of cellular health, and brain imaging, can provide information about the welfare of our experimental animals **A retrospective assessment of these aims will be due by 05 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)?	This project will deliver new understanding of how different neural centres work together to control movement, and how this changes in disease. This will advance our understanding of fundamental science. Stroke is currently the leading cause of disability in the UK. There are >100,000 new strokes annually, one quarter in individuals aged under 65. The UK has 1.2m stroke survivors, around half of whom live with a disability that affects their everyday life (all figures taken from The Stroke Association). Treatment options for improved rehabilitation are limited, especially for hand function – one reason for this is a poor understanding of the scientific basis for control of movement, and the processes underlying its recovery after injury. The information gained by this project will allow us to devise principled new treatments to improve rehabilitation. If this leads to even small improvements in function, it will translate into major social and economic impact. Motoneuron disease is a rapidly progressing, fatal disease which produces degeneration of the cells in brain and spinal cord which control movement. Current animal models for this disease use rodent models, but these lack an important connection seen only in primates, which may be critical to the biology of the disease. We will test a new technique to produce a primate model of motoneuron disease. If successful, this could provide important new details about how the disease spreads, as well as provide a way of testing disease-modifying treatments in future. Essential tremor is a common movement disorder, which produces excess shaking of the limbs. Recent results from human patients have suggested that the disease arises from problems in a part of the brain called the cerebellum. In this project, we will test whether selectively damaging a particular set of connections in the cerebellum in primates can generate a model of essential tremor. Again,

	this will elucidate important features of the disease, as well as potentially aid the development of novel therapeutics in future.
What species and approximate numbers of animals do you expect to use over what period of time?	109 macaque monkeys over 5 years and 250 rats over 5 years. This licence covers multiple different projects, which investigate different nerve centres, and different hypotheses in the healthy state, and how these change in different diseases. Each project will use between 2 and 5 animals, depending on the detailed experimental protocol; the numbers given reflect the requirements of the multiple projects which will run under this licence for its 5 year span.
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?	Monkeys will be trained to accept some restraint (a neck collar), and to perform a behavioural task. They are motivated to perform the task by having restricted access to food, and occasionally fluid; food and fluid rewards are then given for correct task performance. After training is complete, they are surgically implanted with a headpiece to allow head stabilisation and electrodes to record muscle activity from the forelimb. Recordings will then be made from the central nervous system in the conscious state, whilst the animal performs the task. Animals may then be implanted with a chamber over the spinal cord, and further neural recordings made from spinal neurons during task performance. The most common adverse effects are associated with wound infections associated with the long-term implants. In a small proportion of animals, a specific small area on one side of the brain will be damaged using surgical techniques. Some animals will have lesions in two specific small areas. In the days immediately following, these animals may need nursing help with feeding due to impaired movement ability, which may include a loss of movement in one limb. However, as in human stroke patients with little damage they often show a rapid recovery, although there is likely to be a residual impairment in limb function on one side. In other monkeys, viruses will be used to insert genes to produce excess levels of protein thought to be involved in clinical disease, such as motoneuron disease. This will cause loss of motoneurons and weakness in the targeted muscle, which may they spread to other muscles, although the experiment will be

	terminated before this impacts significantly on welfare. Animals will typically undergo a maximum of three major surgeries, although some may undergo up to eight. Rats may be prepared for recording by a surgery to inject tracers or novel genetic material into the nervous system, after which they are allowed to survive for a few weeks. Subsequent experiments are carried out under anaesthesia, and involve making electrophysiological recordings or removing brain samples for analysis in vitro. The animal is killed before it can recover from this anaesthetic. Recovery from the initial surgery is unlikely to show adverse effects, and no adverse effects can be experienced in the final terminal procedure. The macaque experiments will have moderate severity, although the licence limit of 'severe' may be reached for short periods in some animals associated with the period immediately after surgery-induced brain damage . Rat experiments will be of moderate severity. At the end of experiments, all animals are humanely killed. **A retrospective assessment of these predicted harms will be due by 05 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	This project investigates the complex interplay of nerve circuits in different regions, and as such must be carried out in intact organisms. The laboratory does run a substantial programme of experiments in healthy human volunteers and patients; however, these can only produce indirect data. Detailed understanding at the level of single nerve cells and their connections can only be achieved using the invasive approaches possible in animals. I maintain a knowledge of the literature by checking PubMed and Web of Science databases, to ensure that I am aware of any replacements if they are developed.

	A retrospective assessment of replacement will be due by 05 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	This project is investigating fundamental processes of how the brain controls movement and how that control goes wrong in disease. When we measure how cell activity relates to different movements, or the strength of connections between cell pairs, variation between neurons dwarfs inter-individual variation between animals, so the experimental unit is a recorded single neuron rather than an animal. We use sophisticated multi-electrode recording methods, which ensure that the maximum of data is gathered from each animal. Experiments in awake monkeys therefore often yield sufficient data for publication from just two animals. The convention in the field is that this allows us to check that results are consistent between individuals. Using two animals also reduces the experimental risk: one animal might produce less data than expected, but this can be made up by the second monkey, ensuring that the project can still succeed. Experiments under terminal anaesthesia use advanced anaesthetic methods to maintain the animals in good condition for extended periods (around 70 hours for macaques); this again enables us to gather extensive datasets from each animal, although even with recordings lasting several days, it is usually not possible to gather as much data per animal as using chronic methods with many daily recording sessions. For this reason, experiments under anaesthesia often use 4-6 animals to generate sufficient data. In this case, animal numbers are minimised by performing analysis mid-way through an experimental series, ensuring that only sufficient data is gathered as required to address the scientific question. Power calculations are used where appropriate to inform experimental design and numbers of animals used. Where we do not have the key data required to perform such a calculation,

	such as the variability expected across cells, as often we are the first to make a particular recording, estimations will be based on previous experience. Again, data analysis mid-way through an experiment can allow us to determine how many animals are likely to be required to answer a question. **A retrospective assessment of reduction will be due by by 05 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.	Some basic nerve circuit properties can be investigated in rats. However, the nerve centres and connections controlling movement differ in key aspects in primates compared with non-primate species. These connections underlie a highly developed ability to produce fine control of small groups of muscles, especially those of the hand; this provides primates with their high level of manual dexterity. It is essential to use such Old World primates such as macaques to ensure results will translate to human patients. In addition, the intelligent, inquisitive and social nature of macaques means that they are capable of learning complex behavioural tasks. Only one part of the motor pathway is known to differ between humans and macaques; part of this project will investigate how this difference can contribute to differences in recovery after damage between humans and monkeys. Our techniques have been refined over many years, and we continually seek to improve them – for example, by improving the design of our headpiece and electrode implants to make them more tissue-friendly, which allows better healing of skin margins and integrates wires which run under the skin into the headpiece structure. We have also recently improved the way in which we repair wounds after spinal cord implant surgeries, which has reduced the incidence of wound complications. All recovery surgeries are carried out under full aseptic conditions, with advanced anaesthetic regimes which produce rapid and uneventful recovery. Full programmes of post-operative pain management are in

	place. Analysis methods are constantly being optimised: for example, by advanced statistical methods, we can achieve the same scientific benefit with shorter durations of recordings and fewer trials performed. **A retrospective assessment of refinement will be due by by 05 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?

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Project	**21. Detecting markers to investigate and diagnose diabetic vascular disease.**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	2 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)	**Aim 1**) We will establish a range of identifiable 'biomarkers' from blood vessels correlating with early stages of pre-diabetic and diabetic disease. **Aim 2**) We will confirm the presence of 'biomarkers' in samples of vessels with more established diabetes, verifying our 'REDACTED sampling' method is effective for later 'biomarker' identification. As one target is to identify 'biomarkers' of diabetes before the disease actually develops, we need to use a model of the disease where the blood vessels are initially healthy but are	

	certain to be developing diabetes. For this reason, the first study objectives will be carried out using a genetic model of diabetes in mice, as identifying what the 'advance warning' biomarkers are in humans is not feasible. With this mouse model of diabetes, we will collect blood vessel tissue and sample it using our 'REDACTED sampling' procedure, while also analysing changes to blood and vessel walls, to correlate the appearance of biomarkers with disease stage. Next, we will use 'REDACTED sampling' to analyse blood vessel samples from patients with and without diabetes, to confirm the presence of biomarkers that indicate more advanced disease (expanding on the mouse study). In addition, we will match up the markers from blood vessel sampling by 'REDACTED sampling' with changes in blood markers and disease changes seen to blood vessel structure.
Retrospective Assessment	**Retrospective assessment** Published: 27 September 2022 **Is there a plan for this work to continue under another licence?** No **Did the project achieve its aims and if not, why not?** We successfully achieved the aim of generating a series of tissue samples from mice with and without a model of Type 2 diabetes mellitus (T2DM), and also carrying out our novel SMA sampling technique on these tissue samples. From this we generated a series of protein-containing samples from each category (with and without T2DM), which have also successfully undergone early analysis: this analysis identified a series of proteins unique to the T2DM samples. We were awarded follow-up funding to carry out analysis on human samples, due to our progress with this mouse work. This work re-started in April 2022 (a long delay due to awaiting an Ethics permission amendment,

	unconnected to our completed work). What was achieved in the mouse work was limited however, as the planned 'timeline' of samples was severely reduced due to lockdown in March-April 2020 (we sought and obtained permission to continue the work from the University's lead for research, but a team member had suspected Covid-19 so collections had to be cancelled).
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 study will identify specific markers of diabetes, which REDACTED can collect, to identify diabetes earlier than currently possible. Detecting a 'biomarker' of change to blood vessels due to the pre-diabetes state will allow intervention to prevent diabetes in that individual, before disease progress has gone too far to fully reverse. Our aim is for the 'biomarkers' found in this study to ultimately provide diagnosis tools for clinical use, after further technique development. From a scientific perspective, the application of our cell sampling technique to a specific disease process will allow our REDACTED method to advance from first discovery towards obtaining markers for ultimate clinical use. We will also link the markers which we obtain with changes to blood and the structure of human blood vessels. A broad gain of this project is that earlier detection of either imminent diabetes or its complications allows potentially huge savings in healthcare costs, as earlier treatment to prevent these is far less expensive than managing them once they develop. Further wider benefits from this technique come from its potential for application to human tissue in other contexts: this may be useful for researchers in other fields (e.g.
	cancer markers).

What species and approximate numbers of animals do you expect to use over what period of time?	A total of 200 animals: we have calculated that we will require 49 tissue samples, obtained after confirming the correct genotype in the animals used for tissue samples. The remainder of the number is to provide animals for breeding, providing numbers required for successful project completion (and cover a safety margin of numbers in case of sample losses). The project will run for approximately two 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?	We will breed the animals and maintain them: they are genetically modified to develop changes that model diabetes, so they will not undergo any other procedures beyond normal maintenance. Tissue samples will be collected at a range of time-points as these changes occur, with the animals sacrificed painlessly. The post-mortem tissue samples obtained for analysis in the laboratory using our novel technique. Weight gain is a known feature of these mice, with a typical weight of 50-55g, compared to around 30g in normal mice. Any animals that are over 60g will be humanely sacrificed. Regarding diabetes complications, these mice can develop reduced wound healing, liver fatty tissue change and nerve damage. Liver fatty change does not cause animals distress within the age range to be used for mice in this study (up to 14 weeks). The main effect of both reduced wound healing and nerve damage would be the risk of developing ulcers on the body, which will be identified by observation (these mice need bedding material changed often, so this gives plenty of opportunity to check for ulcers). If ulcers are significant (too large to heal or cause distress), the animal will be humanely sacrificed. Blood vessel wall pathology will be developing in these mice, but not far enough to cause active cardiovascular disease in the time period of this study.

Retrospective assessment	**Retrospective assessment** Published: 27 September 2022 **What harms were caused to the animals, how severe were those harms and how many animals were affected?** The animals harms were due to the genetic modification (GM) model of T2DM only, in this project they were simply maintained in normal animal facilities, then sacrificed with tissue samples collected post-mortem for analysis. During life, mice with the GM model developed significant weight gain, which stayed within the limit set during our study (weights were monitored and no animal came close to the limits set). Although the GM model can also cause symptoms due to liver and vascular disease, this did not happen at the earlier ages in which all mice in our project were, again these were monitored for and no such complications were seen in the mice in this study. The total number of animals used in this project was 51, with 16 having undergone moderate and 35 non-recovery procedures.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Although as much background work as possible has been achieved using cells or tissue in the lab, this study's specific target is identifying the appearance of disease markers over the course of diabetes progression, from normal tissue through early diabetic states to fully-developed disease. This simply cannot be achieved using human subjects at an even vaguely realistic cost. We have plans to carry out work focusing on specific some aspects of diabetes and technical aspects of our new sampling procedure (quantifying sampled material relative to the amounts within cells): both of these studies will be carried out using cells in the lab. Hence we have avoided animal-based research for as many aspects of our research with suitable replacements as we can. However for this project, the planned animal work is essential for completion.

Retrospective assessment	**Retrospective assessment** Published: 27 September 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?** We did not use any replacement beyond that already in the study design when we started (specifically, using human tissue samples for all parts of the work other than those specifically needing markers of early diabetes). The study minimised animal use and we are unaware of any developments since that would have allowed us to fully replace this.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use the minimum number of animals to achieve our research objectives, and tissue samples taken for this work will be used for multiple analyses, so the minimal number of animals will be used for maximal research data.
Retrospective assessment	**Retrospective assessment** Published: 27 September 2022 **How did you minimise the number of animals used on your project and is there anything others can learn from your experience?** We were able to reduce the number of animals used from discussion with colleagues, as we were advised that purchasing adult animals already known to have the GM change in place (rather than breeding such mice ourselves then testing them) would allow fewer mice to be used overall. We had been hesitant to do this initially, due to the perceived risk of losing these highly-expensive mice in transit, but we were advised that the difficulties with breeding these mice could lead to comparable problems (with limited resources for the project, we needed to

	minimise the risk of failure and thus needless animal sacrifice). In practice, the direct delivery of the adult mice with T2DM was very successful and allowed our project work to commence without difficulty. We would recommend therefore that for a relatively small study such as ours, direct purchase is a more feasible means than aiming to establish an in-house breeding programme.
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 ability to follow the complex changes caused by diabetes in vascular tissue, makes this a suitable model for a screen of disease 'biomarkers' that can be obtained with our new technique. For comparison, we will use normal mice, as recommended by the breeder of the mice. This study has also been designed so that the samples for sampling will be obtained without causing the animals any distress, pain or suffering due to this. Other than the impact of the disease development, which is of course an unavoidable aspect of these animals' normal life, there will be no other causes of distress or variation from normal laboratory animal maintenance and care. All staff handling the animals, including the research worker assigned to this project, will be fully trained in animal handling and care, including recognising signs of distress due to illness, and the ability to quickly and humanely end suffering if necessary.
Retrospective assessment	**Retrospective assessment** Published: 27 September 2022 **How did you minimise the number of animals used on your project and is there anything others can learn from your experience?** We were able to reduce the number of animals used from discussion with colleagues, as we were advised that purchasing adult animals already known to have the GM change in place (rather than breeding such mice ourselves then testing them) would allow fewer mice to be used overall. We had been

	hesitant to do this initially, due to the perceived risk of losing these highly-expensive mice in transit, but we were advised that the difficulties with breeding these mice could lead to comparable problems (with limited resources for the project, we needed to minimise the risk of failure and thus needless animal sacrifice). In practice, the direct delivery of the adult mice with T2DM was very successful and allowed our project work to commence without difficulty. We would recommend therefore that for a relatively small study such as ours, direct purchase is a more feasible means than aiming to establish an in-house breeding programme.

Project	**22. Detection of Bacterial Toxins**	
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
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The aim of the project is to provide a diagnostic service for patients with suspected botulism or tetanus. This project also helps with the effective investigation, surveillance and control of outbreaks of disease, including the rapid detection of food contamination. **A retrospective assessment of these aims will be due by 27 November 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)?	Results of the tests for botulism and tetanus are returned to clinical/medical colleagues involved with the management of diseased patients and allow confirmation of the clinical/medical diagnosis and evidence for their most appropriate medical/clinical management and treatment. Results of tests for botulism are also used for the identification of sources of infection and is of special importance to those involved with control of the food chain i.e. environmental health officers and staff from the food standards agency. These results provide vital informed and evidence-based information for the identification of toxic food and allow its removal from the food chain to prevent further cases of the disease. This has far-reaching health benefits for both humans and animals.
What species and approximate numbers of animals do you expect to use over what period of time?	Potentially 60-100 mice could be used over a year. However, due to the use of non-animal alternatives such as Polymerase chain reaction (PCR) we have found that we use far fewer animals than what we estimate.
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?	On separate occasions, mice will be injected with either blood serum, human stool or food extract to diagnose botulism or tetanus. If the test is found to be positive the animal will experience clinical signs; in cases of botulinum these will be - Piloerection/staring coat, wasp-like waist, laboured breathing and paralysis (i.e. loss of the use of limbs) and in cases of tetanus, these will be; progressive paralysis, a curvature of the tail (towards the side of the injection), limping and stiffness of limbs, total paralysis and spastic paralysis of limbs (i.e. loss of use of limbs). When the test is positive the animal will suffer considerable pain and distress and the severity category for positive tests is severe. If the test is found to be negative, the animal will experience a brief departure from its normal state of health and wellbeing. All animals are euthanized/put to sleep humanely at the end of the test. Animals are closely monitored throughout the duration of the test to ensure that as soon as the disease can be diagnosed the animals are euthanized/put to sleep humanely. Out of 500 tests carried out over the last 5 year project 75 mice were found to be positive which is about 16-20 per cent of the tests.

	A retrospective assessment of these predicted harms will be due by 27 November 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	Currently, the mouse bioassay (MBA) or mouse test is the only laboratory test authorised for the detection of biologically active C. botulinum and C. tetani nerve toxins. Over the years, there have been several tests developed to detect botulinum toxins from clinical specimens and food. Until recently, none of them have been sensitive enough to replace the mouse bioassay (MBA) completely. Scientists in the USA have carried out trials using ELISA test, However, this initial ELISA test was not accurate and it lacked sensitivity, especially when used with clinical/medical specimens and did not identify whether the toxin was biologically active, which is essential for food testing. More recently, another ELISA test was described but it was still not sensitive enough and could be used only for preliminary screening of foods. A MALDI-TOF test has been described, but this test was designed to identify C. botulinum organisms from cultures, not the toxin. None of these tests are commercially available and if they had been used instead of the MBA there would have been serious consequences for patients because these tests are not sensitive enough to make an accurate diagnosis. The Endopep-MS test has been under development for some time in the USA . This test's specificity and sensitivity have been constantly improved over the years with the aim of reaching results comparable to the mouse test. We have made considerable progress in the use of the polymerase chain reaction (PCR) as an alternative to animal tests, but these detect

	the presence of neurotoxin genes and the potential of strains to produce neurotoxin; they do not detect the presence of active neurotoxin itself. Thus, there is an occasional need for the mouse test to detect the actual toxin in clinical specimens, foods, and cultures as the PCR may not detect organisms with unusual toxin types or toxin variants. We regularly check the National Center for Biotechnology Information (NCBI) database for novel/new toxin gene sequence variants and ensure our PCR detection tests are continually updated so that all known novel/new toxin variants can be detected. A new PCR test detecting the ntnh protein gene which is present in botulinum, tetanus and bacterial strains that produce a toxin, including those producing unusual toxin types or toxin variants and in toxin-producing strains of toxin producing bacteria is being developed. This should allow PCR detection of all strains capable of producing neurotoxin regardless of toxin type or if it is a new toxin variant. Regarding the Endopep-MS (MALDI-TOF) test described above, we are currently exploring the possibility of using such a test which has the potential to reduce and possibly replace the mouse test in the future. However, this will depend on the approval from the relevant authorities and the commercial availability of the test. **A retrospective assessment of replacement will be due by 27 November 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	Since 2004 we have been using in-house, real-time PCR tests to detect neurotoxin genes cultures of botulinum and tetanus. This has resulted in a 90 per cent reduction in the use of animal tests. However, as some clinical/medical samples e.g. serum do not

	contain bacterial cells, and the PCR test does not detect biologically active toxin and therefore diagnosis of the disease by mouse tests cannot be eliminated completely. We use the mouse tests rarely and have several strategies to ensure animals are only used when necessary. The decision to use animal tests will depend on the high degree of suspicion of botulism/ tetanus in the patient. Therefore, prior to testing, the clinician is contacted to gather the necessary medical information on the patient and to provide advice on the most suitable specimens to be taken to maximise the chance of detecting toxin if present. In addition, the mouse test will be performed only in the absence and unavailability of any specimens suitable for a non-animal testing method such as PCR which we always prioritise. REDACTED The later step helps to prevent the use of animals for specimens unlikely to be positive. In suspected cases of tetanus, the immunity to the disease in the patient is also checked prior to the mouse test. If the patient is shown to be immune, then the mouse test is not performed. When possible, we always prioritise alternative samples for PCR, however, in situations when toxin detection is the only option, the mouse test is unavoidable. In food samples, where botulism toxin detection is essential, PCR is also used to identify the type of C. botulinum present which reduces the number of animals otherwise used for toxin typing by further neutralisation tests. We are currently developing a PCR test to detect the ntnh gene (the gene that is carried by botulinum and tetanus bacteria). This will allow the detection of variants of strains otherwise not detectable by our current PCR tests. **A retrospective assessment of reduction will be due by by 27 November 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 is the animal of choice for the test and this species has been proven historically to be the most appropriate species for this test. This means all validated tests for botulinum and tetanus diagnostic purposes use mice. All animals will be housed and tested in pairs and never used alone. The test is carried out by staff who are highly trained and participate in regular competency checks to ensure they are proficient in carrying out regulated procedures, recognising the signs and symptoms of positive cases of botulism and tetanus at the earliest possible time. All animals will be observed frequently to assess whether they are showing signs of botulism or tetanus. If signs of botulism or tetanus are observed, then the tests will be halted at the earliest opportunity. All animals are made as comfortable as possible for the duration of the test. Moistened food will be provided for animals that may have difficulty accessing food or water in a conventional manner and extra bedding is always provided to keep the animals warm. **A retrospective assessment of refinement will be due by by 27 November 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. Determining the mechanistic actions of FOXA1 related pathways in breast, prostate and 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)	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)	FOXA1 is a protein involved in binding DNA and drives the formation, spread and resistance of breast, prostate and pancreatic cancer. Currently, patients are prescribed treatments based upon the type of cancer they are diagnosed with, however not all cancers of the same type respond well to the same treatment. This means that many patients receive no benefit from a treatment but still experience the negative side-effects, or a patient may not be prescribed the best possible treatment for their tumour. This study will identify at a molecular	

	level which genes and proteins are driving cancer progression and treatment resistance. This knowledge will not only allow for better selection of available treatments for particular groups of patients, but also inform the development of new treatments which target these genes and proteins. **A retrospective assessment of these aims will be due by 05 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)?	This project will make a significant contribution to the field of cancer research by identifying the mechanisms by which FOXA1 and its associated factors facilitate the growth, survival and resistance to treatment of breast, prostate and pancreatic cancer. This study will identify new targets for future drug development and targets for which existing drugs could be used
What species and approximate numbers of animals do you expect to use over what period of time?	Mice; approximately 4200 over 5-years. We require the use of mice in this study because in order to implant human tumours into an animal, we must use an animal with a weakened immune system (immunodeficient), otherwise the human tumour would be rejected by the host. The mouse NSG model is currently the best available immunodeficient host for this purpose. In cases where immunodeficient models are not required, we also need to use mice because the implanted tissue is derived from mice and therefore will only grow in mice genetically similar to the donor. We require this number of mice in order to be confident in our results, as it is necessary to have enough mice in each treatment group for an experiment to give a definitive result and this has been determined by an expert statistician.

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 have minor surgery to implant human derived tumours either just underneath the skin or into their breast tissue. It is expected that 100% of mice will recover very quickly from these surgeries and they will be given painkillers and anaesthetic similar to those used for humans during surgery. The mice will be treated with drugs that we have identified as potential cancer treatments. Generally, these will be medicines that are already used to treat human patients with cancer or other conditions and we will use amounts that are already known to be safe. It is possible that there could be adverse effects such as complications from surgery or side effects of the treatments, these would likely be weight loss or a deterioration in body condition. The majority of mice in this study will only have one surgery to implant cancer cells or a small piece of a human patient's tumour, these mice are expected to recover rapidly from this surgery and should not experience any pain or discomfort during the project. Mice that are used to study metastasis may start to experience discomfort or pain as metastases develop, these mice will be humanely killed if they are likely to exceed the severity threshold established in our protocol. An experienced veterinary surgeon is available to advise. At the end of the studies, each mouse will be humanely killed and then the tumour and other tissues will be removed for analysis. **A retrospective assessment of these predicted harms will be due by 05 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	Tumours and the way they develop and respond to treatments are greatly influenced by the surrounding environment. Currently, there is not the technology to accurately mimic the human environment and the complex interplay between bodily systems without using an animal. We are using mice for our experiments as we require a mammalian host species to be a good representation of the human body.

	A retrospective assessment of replacement will be due by 05 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	An expert statistician has been consulted to determine the minimum number of mice which will need to be used in the study using a power analysis. This will ensure the minimum number of animals are used whilst also ensuring that the results of the study are scientifically valid. **A retrospective assessment of reduction will be due by by 05 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.	To implant a patient's tumour, we need to use an animal that has a greatly weakened immune system, or the tumour would be rejected. NSG mice are readily available animal models which lack a strong immune system. Care of NSG mice is well established at our facility and others worldwide. The techniques used are well supported by colleagues internally and externally. We are strictly limiting the size of tumours in the animals to 14mm mean diameter. When required, mice will be anaesthetised or given pain relief to prevent or minimise discomfort. **A retrospective assessment of refinement will be due by by 05 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	**24. Development of Therapeutics for Inflammatory 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)		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)	Inflammation plays a major role in a number of acute illnesses that affect the heart and lungs and the whole body, that have few or no treatments available. Inflammation of the heart muscle (myocarditis) is an abnormal response that can follow direct injury to the heart such as caused by Coxsackie viral or Chagas disease infection, lack of oxygen as happens in a heart attack, or following cancer treatments such as immune checkpoint inhibitors or doxorubicin. It also sometimes occurs without any discernible underlying triggering cause. The inflammation can further damage the heart muscle setting off a chain of events that leads to scarring, heart failure, heart rupture and death. No effective treatment yet exists to tackle this problem. The problem is important: myocarditis is estimated to cause ~10% cases of unexplained heart failure in Europe. In	

	Latin America, Chagas disease affects between 5-18 million individuals, 20-30% of whom will develop myocarditis. Myocarditis progresses to heart failure in ~30% of patients, with between 20-80% of patients dying in two years. Heart attack results in heart failure and death, and is more common with approximately 490000 cases per year in UK. Inflammation of the lung occurs in a condition called acute respiratory distress syndrome. This can follow lung infections such as sepsis, pneumonia, influenza, or smoke inhalation. It affects about 10% of patients in the ICU, with death rate of about 30-40%. Inflammation of many organs (heart, lungs, kidney, liver) can also result from a condition called systemic inflammatory response syndrome or "cytokine storm" that occurs as a complication of bacterial sepsis, and of viral infections such as influenza and SARS. Again this has a high mortality rate due to multiple organ failure. Inflammation is driven by chemokines, substances produced by the body to attract white blood cells.Ticks block inflammation at the site of the bite so that they can keep sucking blood for days to weeks. We have discovered potent anti-inflammatory substances from tick saliva (evasins) that block chemokine action, and could be developed as new treatments for heart muscle inflammation or sepsis or respiratory distress syndrome arising from different causes. The advantage of these substances is that they would block the culprit chemokines rather than the responding cells and the immune system as a whole and thus be specific in their action. We have demonstrated that some of these evasin agents potently inhibit inflammation in a short-term model (air-pouch) of inflammation, and these studies have allowed us to establish the optimal route, dosing regimen, and frequency of administration for some of these agents. The major aim of this project is to develop these substances into new treatments for heart, lung and systemic inflammation. We will identify the most appropriate dose and route of administration and demonstrate that these substances are effective in reducing inflammation in well-characterized models of inflammation (sterile peritonitis, air-pouch inflammation), and heart inflammation - myocarditis and heart attack, and models of lung, and systemic inflammation. As we have over 40 different evasins, with differing chemokine binding specificities, our goal is to create and test different combinations of the evasins to precisely target

	only those chemokines active in the different inflammation models. **A retrospective assessment of these aims will be due by 10 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 short-term benefits will be to prove that the new substances are effective in models of inflammation, myocarditis and heart attack. The medium-term benefit will be the translation of the proof-of-concept into “first-in-man” studies. The long-term benefit the therapeutic use of these substances in patients with orphan diseases such as myocarditis, and in other major diseases such as myocardial infarction, acute respiratory distress syndrome and sepsis. Although our present research is focussed on heart, lung and systemic disease, there is potential for these evasins to be used in other chemokine-driven conditions such as stroke, pancreatitis, inflammatory bowel disease, atherosclerosis and arthritis for instance.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice, approximately 10000, 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?	New anti-inflammatory substances will be studied by injecting normal mice with different doses through different routes to determine which dose and route combination is best for giving adequate circulating levels of the drug. In these experiments, mice will experience mild to moderate harms including discomfort and transient pain at the site of injection, and at the site of drawing blood to measure the level of the drug in the circulation. New anti-inflammatory substances will next be studied in short term models of inflammation. These are sterile peritonitis, created by injecting an irritant into the peritoneal cavity, and the air-pouch model created by injecting an irritant into an air pouch under the skin. Mice used for the sterile peritonitis model will experience moderate harms including abdominal discomfort and transient pain from peritoneal irritation. Mice used for the air-pouch inflammation model will experience moderate harms such as local discomfort and transient pain at the site of the air pouch creation and subsequent inflammation. They will be then injected with the new anti-inflammatory substances, and will also experience further moderate harms from injection and blood sampling including pain and discomfort. Once new anti-

	inflammatory substances are shown to work in a short-term model of inflammation, and the dose and route of administration established, we will study their effect in myocardial inflammation and in heart attack models. Myocardial inflammation is produced either by injecting Complete Freund's Adjuvant under the skin (which induces an immune reaction to heart muscle) or by using modified mice where heart inflammation develops naturally e.g. as a result of a gene modification, or by treating mice with a drug such as a cancer checkpoint inhibitor that causes myocarditis in humans. Mice will experience moderate harms including discomfort and pain at the site of injection of Complete Freund's Adjuvant or a drug that causes myocarditis. They will also experience harms (pain and discomfort) associated with injection of the new anti-inflammatory substance, and blood sampling, and with other non-invasive and invasive tests such as echocardiography needed to determine the effect of the new anti-inflammatory substances being administered. They may infrequently experience severe harms including skin ulceration, joint swelling, loss of appetite, weight loss, and lameness, and effects of myocarditis such as heart failure which would cause shortness of breath, loss of appetite, lethargy and loss of weight. Suffering is minimised by humanely killing mice that show these features. Heart attack is caused by a surgical procedure where under anaesthesia a heart artery is ligated. Mice are kept for short term (up to 7 days) studies or longer-term studies (4-8 weeks) to allow for the process of recovery from heart attack to be observed. During this time, animals are generally free of adverse effects, show normal levels of activity, and are housed in social groups. Mice will experience moderate to severe harms, including discomfort and pain of surgical procedures. They also experience harms (pain and discomfort) associated with injection of the new anti-inflammatory substance, and blood sampling, and with other non-invasive and invasive tests such as echocardiography needed to determine the effect of the new anti-inflammatory substances being administered. Occasionally they may develop breathing difficulty and suffering is minimised by keeping these mice under regular observation and humanely killing any that show these signs. Mice may also die suddenly during the surgical procedure or suddenly after the procedure due to irregular heart beat or rupture of the heart. As death occurs quickly, suffering is minimised. On the whole the most animals are killed humanely at the scientific endpoint without experiencing any adverse effects. Organ and systemic inflammation

	are created by administering drugs and agents that are known to have these effects. Mice will experience moderate to severe harms, including local discomfort and pain. They also experience harms (pain and discomfort) associated with injection of the new anti-inflammatory substance, and blood sampling, and with other non-invasive and invasive tests such as echocardiography needed to determine the effect of the new anti-inflammatory substances being administered. Occasionally they may develop breathing difficulty. Suffering is minimised by keeping these mice under regular observation and humanely killing any that show these signs. Mice may also die suddenly. These potential for these harms will be reduced by using the smallest dose and the shortest duration possible to detect a reduction in inflammation with a new therapeutic. Breeding of mice for the above experiments results in mild harms if mice are genetically altered, or moderate harms if mice have a genetic alteration that makes it likely that they may develop myocarditis or heart failure. The animals will be killed at the end of the study or if a genetic line is important we will freeze embryos or sperm in case future studies are necessary. **A retrospective assessment of these predicted harms will be due by 10 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	Mouse models are necessary for validation of anti-inflammatory agents as cell-based or computer methods or non-protected animal alternatives cannot recapitulate the complexity of inflammation that happens in mammals. We have considered the use of non-mammalian models such as zebrafish, for our studies, but these animals typically regenerate the heart following damage (something that mammals do not do), rendering them unsuitable as a model for inflammation-mediated non-regenerative heart muscle disease in humans. Importantly zebrafish do not have lungs, which is a major organ damaged during sepsis and the systemic inflammatory response, and in the acute respiratory distress syndrome. Moreover, the ticks from which we have isolated the evasins parasitize mammals (including humans) and are specialised against mammalian and human chemokines and inflammation pathways. Zebrafish chemokines are very different from human chemokines, and we do not expect that ticks would have adapted to neutralize zebrafish chemokines as they do not parasitize fish.
	We are actively engaged in replacement by extensive use of cell-based methods where possible in the discovery process. In particular, in the discovery of tick evasin genes we identified these genes using computer methods, cloned synthesised genes into yeast and identified the relevant ones using yeast surface display, all performed without the use of animals. This process "Bug-to-Drug" is now used extensively in our laboratory to identify potential therapeutic agents from tick saliva. In a more traditional approach tick colonies would have been maintained on mammals such as mice and rats and rabbits, tick saliva extracted, and analysed to discover the new therapeutics. Ex vivo alternatives – e.g. ex vivo lung and cardiac perfusion models are being established in collaboration with colleagues where suitable expertise is available. **A retrospective assessment of replacement will be due by 10 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 take care to breed the minimum required for our phenotyping experiments. We routinely use statistical power calculations to guide our experimental design so that the minimum number of animals are used. We use cell based and computer studies extensively to predict the best candidates for animal studies, thus maximising the likelihood of success. We use approaches of good experimental design including randomisation (e.g. where we randomly allocate animals to treatment and placebo control groups), and blinding of the observer, so that results are not susceptible to observer bias, and thus are reproduced more easily, thus reducing animal use. Animal use is further reduced by freezing embryos and/or sperm to reduce breeding numbers. **A retrospective assessment of reduction will be due by 10 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.	We use mice because disease models of autoimmune myocarditis, and heart attack, that are sufficiently similar to man have been developed in the mouse. We use moderate severity short-term models such as sterile peritonitis and skin air-pouch inflammation to evaluate drug efficacy and only if these are successful do we proceed to long-term and severe models. In this regard, an aim in this application is to establish the skin air pouch model for evasin therapeutics as it is a more refined model of short-term inflammation than the sterile peritonitis model. We have monitoring regimes developed with the advice of the Vet and NACWO with humane end-points defined to keep within the severity limits. We keep careful records of expected effects, and this document is made available for consultation by animal care staff. Animals

	are provided with analgesia to minimize pain experienced. Animals exhibiting any unexpected harmful effects incompatible with the severity limit are euthanased and we will commit to developing early end points for all cardiac phenotypes at risk of sudden death. All adverse effects are documented and periodically assessed in order to detect sporadic unexpected events. Animals are routinely maintained in a barrier environment and group housed whenever possible. We use minipumps to deliver drugs rather than repeated injections. These are surgically implanted. Infections are minimised by performing recovery surgery under aseptic conditions. Pain and discomfort post-surgery is minimised by providing medications, analgesia, warmth, access to water-softened chow, and fluids (as required). Pain due to injection of complete Freund's adjuvant is minimised using anaesthesia, and complications such as ulceration minimised by using aseptic technique and the smallest adjuvant dose possible. Pain from sterile peritonitis and air-pouch inflammation is minimised by using the smallest doses of inflammatory substance that generates inflammation. We routinely use aseptic technique for myocardial infarction models, use that least risky background strains to reduce risk of cardiac rupture, and habituate animals to blood pressure monitoring. For lung injury models we will use the nebulised aerosol route in preference to nasotrachaeal/oropharyngeal/intratracheal routes which require anesthesia. **A retrospective assessment of refinement will be due by by 10 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	**25. Development of veterinary medicines for equines**	
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/clinic al needs being addressed)	The overall aim of the programme of work is to provide efficacy and safety data for products for the control and prevention of disease in horses. Disease control products are continually being developed, but it is a legal requirement for these to be fully tested for safety and efficacy prior to them being marketed. This licence will enable studies to be carried out on behalf of pharmaceutical companies to satisfy these legal requirements. Some work of a supportive nature may also be conducted, for example validating challenge models in advance of an efficacy study being conducted. **A retrospective assessment of these aims will be due by 05 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 overall aim of the programme of work is to develop safe and effective means of controlling disease in horses. Ill health caused by disease in horses continues to be a worldwide welfare concern. This problem is being exacerbated by the rising levels of resistance to various products. The results of studies conducted under this license will be incorporated into dossiers submitted to the regulatory authorities to allow new veterinary medicinal products to be marketed. This will allow more effective and safe veterinary medicines to be available to veterinary surgeons and farmers. This will result in more effective and safe means of controlling / preventing disease in horses. There are approximately 950,000 equines registered in the UK, with the majority being pleasure horses (companion animals) with significant emotional and financial engagement. The horse racing industry turned over £229 million on on-course and £48 million off course betting last year (2018). The failure to prevent disease (e.g. equine flu) or provide effective medicines (e.g. antibiotics, anti- inflammatories) would have a significant impact on the health of the UK herd, with knock-on effects on the attendance at competitions or race meetings. The British Equestrian Trade Association's National Equestrian Survey 2015 reports that the economic value of the equestrian sector stands at £4.3 billion of consumer spending across a wide range of goods and services each year. This has increased from £3.8 billion in 2011.
What species and approximate numbers of animals do you expect to use over what period of time?	Horses, 650

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expecte d level of severity? What will happen to the animals at the end	For a typical safety study, animals are administered the veterinary medicine and observations made. The administration will usually be by injection. Adverse effects are generally mild and can include injection site reactions, increase in temperature and reduced activity. For an efficacy study without challenge, a range of blood samples are usually taken before and after treatment to allow efficacy to be determined. For an efficacy study with challenge, animals are usually challenged with a mild form of the disease to determine the efficacy of the treatment. Symptoms of the disease are monitored closely, and continuously (including overnight) for some of the more severe challenges. Symptoms are only allowed to progress to a point that allows proof of efficacy to be determined, this is very often dictated by European guidelines. In the majority of cases the adverse effects are likely to be minimal or mild. Where animals are challenged in order to test the efficacy of a product, then the disease model will be the least severe available in order to satisfy European guidelines. In addition, the animals will be monitored frequently, with appropriate intervention when adverse effects are observed. Continuous monitoring, including overnight, combined with treatment / humane euthanasia will be implemented to try to ensure that severity limits are not exceeded. Where at all possible, animals will be returned to local farms following certification by a veterinary surgeon that they are fully recovered. Where this cannot occur (an unregistered veterinary product for example), animals will be humanely euthanased and the carcases will be incinerated. The veterinary medicines under investigation are likely to be curative, so only the untreated "control" animals, specifically required by the regulator, are likely to experience clinical symptoms of disease. **A retrospective assessment of these predicted harms will be due by 05 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	In order to get a marketing authorisation for a veterinary medicine, efficacy and safety data for that medicine must be provided to the regulator. European guidance documents stipulate that the target species of animal is used to produce this efficacy and safetydata. Legal requirements and the use of non-animal alternatives will be kept under constant review. **A retrospective assessment of replacement will be due by 05 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	Where there is a European guidance document detailing the requirements, we will comply with these. Guidance documents usually stipulate the design to be used and the mnimum number of animals. Where there is no guidance document, we will take the advice of a statistician on the design and the minimum number of animals required to maximise the chance of achieving the desired result. The principles of good experimental technique will be implemented, and will include sourcing even / representative animals, allocating treatments randomly, blinding of study staff to treatments administered, accurate data collection and prompt checking / processing / analysis of data. **A retrospective assessment of reduction will be due by by 05 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	The animal species we propose to use are as dictated by European guidance documents. In most cases the adverse effects are likely to be minimal or mild. Where adverse effects are anticipated, horses will be monitored regularly to ensure that severity limits are not exceeded. Where severity limits might be exceeded, we will intervene to treat the animal. Animal husbandry is well above commercial standards, with animals kept in smaller numbers, monitored very closely by experienced stock people and with frequent

take to minimise welfare costs (harms) to the animals.	inspection by veterinary surgeons. Animal accommodation is substantially better than Defra code of recommendations for the welfare of equines and is compliant with A(SP)A codes of practice. Each individual study is reviewed ethically before commencement, paying regard to the methods proposed. Where possible animals will be penned in groups of similar animals. Where individual penning is required, animals will be wthin sight and sound of compatriots. Environmental enrichments will be provided and tailored to the requirements of the horses. Horses are usually examined by a veterinary surgeon or experienced stockperson on their farm of origin. There is usually an acclimatisation period to allow animals to become accustomed to their new surroundings prior to the commencement of preocedures. Following acclimatisation, and before the start of regulated procedures, there is usally a further examination by a veterinary surgeon or experienced stockperson to ensure that the animals are healthy and fit for study. Samples may be taken before the commencement of a study to check that the animals are healthy and within normal reference ranges i.e. blood chemistry and haematology. Prompt veterinary attention is provided to ill animals, which are observed closely until resolved. For disease challenge models, observations are at a frequency to try to ensure that animals do not progress beyond their severity limit. Interventions, treatment or euthanasia, will be as soon as study requirements are achieved and well in advance of severity limits. Where possible space allowances are well in excess of Home Office minimum space allowances, we have found this to be beneficial to the welfare of the horses. As part of the acclimatisation process, before studies commence, we put horses through a simulation of the handling / sampling they will experience during the study. This usually involves moving the horses through the handling setup and simulating the sounds and handling they will

	experience during procedures. As part of this, food treats are offered to encourage and reward the horses. We have found this helps reduce the stress on the animals when it comes to conducting procedures. **A retrospective assessment of refinement will be due by by 05 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	**26. Development of veterinary medicines for farm animal species**	
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 overall aim of the programme of work is to provide efficacy and safety data for products for the control and prevention of disease in farm animals. Disease control products are continually being developed, but it is a legal requirement for these to be fully tested for safety and efficacy prior to them being marketed. This licence will enable studies to be carried out on behalf of pharmaceutical companies to satisfy these legal requirements. Some work of a supportive nature may also be conducted, for example validating challenge models in advance of an efficacy study being conducted. Where studies are in support of obtaining a marketing authorisation, the design, minimum number of animals	

	and success criteria are very often specified in guideline documents, and there is minimal leeway for change. In the absence of a guideline document study designs are usually based on previously successful and a qualified statistician advises on the number of animals and study design. **A retrospective assessment of these aims will be due by 24 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)?	The overall aim of the programme of work is to develop safe and effective means of controlling disease in farm animal species. Ill health caused by disease in farm animal species continues to be a worldwide welfare concern. This problem is being exacerbated by the rising levels of resistance to various products. The results of studies conducted under this license will be incorporated into dossiers submitted to the regulatory authorities to allow new veterinary medicinal products to be marketed. This will allow more effective and safe veterinary medicines to be available to veterinary surgeons and farmers. This will result in more effective and safe means of controlling / preventing disease in farm animals.
What species and approximate numbers of animals do you expect to use over what period of time?	Cattle 1200; Sheep / Goat 1000; Pigs 1200; Chicken 21600; Turkey 550; Rabbit 800

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 a typical safety study, animals are administered the veterinary medicine and observations made. The administration will usually be by injection. Adverse effects are generally mild and can include injection site reactions, increase in temperature and reduced activity. For an efficacy study without challenge, a range of blood samples are usually taken before and after treatment to allow efficacy to be determined. For an efficacy study with challenge, animals are usually challenged with a mild form of the disease to determine the efficacy of the treatment. Symptoms of the disease are monitored closely, and continuously (including overnight) for some of the more severe challenges. Symptoms are only allowed to progress to a point that allows proof of efficacy to be determined, this is very often dictated by European guidelines. In the majority of cases the adverse effects are likely to be minimal or mild. Where animals are challenged in order to test the efficacy of a product, then the disease model will be the least severe available in order to satisfy European guidelines. In addition, the animals will be monitored frequently, with appropriate intervention when adverse effects are observed. Continuous monitoring, including overnight, combined with treatment / humane euthanasia will be implemented to try to ensure that severity limits are not exceeded. When efficacy testing of rabbit haemorrhagic disease vaccine, European regulations require that at least 80% of unvaccinated animals die with signs of the disease within 120 hours of challenge. Animals displaying symptoms will be monitored continuously to try to ensure that we can intervene at the appropriate time to humanely euthanase the rabbits and minimise suffering. Where at all possible, animals will be returned to local farms following certification by a veterinary surgeon that they are fully recovered. Alternatively, animals may be sent directly for humane slaughter in the same manner as other farm animals. Where this cannot occur (an unregistered veterinary product for example), animals will be humanely euthanased and the carcases will be incinerated. **A retrospective assessment of these predicted harms will be due by 24 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	In order to get a marketing authorisation for a veterinary medicine, efficacy and safety data for that medicine must be provided to the regulator. European guidance documents stipulate that the target species of animal is used to produce this efficacy and safety data. Legal requirements and the use of non-animal alternatives will be kept under constant review. **A retrospective assessment of replacement will be due by 24 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	Where there is a European guidance document detailing the requirements, we will comply with these. Guidance documents usually stipulate the design to be used and the mnimum number of animals. Where there is no guidance document, we will take the advice of a statistician on the design and the minimum number of animals required to maximise the chance of achieving the desired result. The principles of good experimental technique will be implemented, and will include sourcing even / representative animals, allocating treatments randomly, blinding of study staff to treatments administered, accurate data collection and prompt checking / processing / analysis of data. **A retrospective assessment of reduction will be due by by 24 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,	The animal species we propose to use are as dictated by European guidance documents. In most cases the adverse effects are likely to be minimal or mild. Where adverse effects are anticipated, animals will be monitored regularly to ensure that severity limits are not exceeded. Where severity limits might be exceeded, we will

having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	intervene to treat the animal. Animal husbandry is well above commercial standards, with animals kept in smaller numbers, monitored very closely by experienced stock people and with frequent inspection by veterinary surgeons. Animal accommodation is substantially better than Defra code of recommendations for the welfare of livestock and is compliant with A(SP)A codes of practice. Each individual study is reviewed ethically before commencement, paying regard to the methods proposed. Where possible animals will be penned in groups of similar animals. Where individual penning is required, animals will be wthin sight and sound of compatriots. Environmental enrichments will be provided where possible and these are tailored to the requirements of the species. Dust baths, perches, nest boxes, toys and auditory stimuli (radio) will be provided for poultry. Toys will be provided for pigs and nesting material for farrowing pigs. A variety of enrichments will be provided for rabbits, including multi-levels, tunnels, toys, chews, nest boxes and offset barriers. Animals are usually examined by a veterinary surgeon or experienced stockperson on their farm of origin. There is usally an acclimatisation period to allow animals to become accustomed to their new surroundings prior to the commencement of preocedures. Following acclimatisation, and before the start of regulated procedures, there is usally a further examination by a veterinary surgeon or experienced stockperson to ensure that the animals are healthy and fit for study. Samples may be taken before the commencement of a study to check that the animals are healthy and within normal reference ranges i.e. blood chemistry and haematology. Animal husbandry is to a very high standard, by experrienced staff following up to date guidelines and regulations. Prompt veterinary attention is provided to ill animals, which are observed closely until resolved. For

	disease challenge models, observations are at a frequency to try to ensure that animals do not progress beyond their severity limit. For example, following challenge with Rabbit Haemorrhagic Disease virus, rabbits will be monitored continuously. Interventions, treatment or euthanasia, will be as soon as study requirments are achieved and well in advance of severity limits. predictive and ensure death is not an outcome. **A retrospective assessment of refinement will be due by by 24 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	**27. Diagnosis of Statutory and Endemic Avian Viral 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)		Basic research
	X	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)	1) - To maintain diagnostic capability and meet statutory requirements as laid down by international agreements and standards. To give a service of enhanced diagnostic testing and to supply high quality diagnostic reagents for identifying notifiable agents. 2) –To maintain the capability of the REDACTED to respond to all cases of confirmed notifiable poultry viral disease, and to provide an accurate and reliable diagnostic service for potential disease problems within the UK poultry industry. This work covers UK disease investigations and also import/export requirements (proving freedom from disease) as laid down by export regulations.	

	3) – To look at any possible new and emerging viral diseases in poultry and influenza in pigs and potential other mammals of significant interest to the REDACTED. **A retrospective assessment of these aims will be due by 01 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 provision of an effective, timely and reliable laboratory service for the diagnosis of viral diseases of birds has a number of potential benefits. In relation to outbreaks of disease in commercial poultry, the rapid detection of viral pathogens in submitted samples assists government, the private veterinarian and/or poultry company in deciding on appropriate action. This in turn will reduce the impact of the disease on the health of the poultry population under investigation, for notifiable disease this will involve slaugh-ter and eradication to protect the national flock. As well as helping protect the UK food chain, the control of notifiable disease is necessary for international trade in poultry and poultry products. Some diseases such HPAI (highly path-ogenic avian influenza) are zoonotic and can infect hu-mans so is also a benefit of protecting human health in the accurate and timely diagnoses of these diseases. As well as the UK situation, the identification and charac-terisation of Newcastle disease and avian influenza virus-es submitted to the International Reference Laboratory, gives these benefits in many parts of the world. In addition these results provide information on the global situation regarding these two very important transboundary viruses and enable governments and industry to implement ap-propriate control measures to limit the spread of the dis-ease, improve the health and welfare of animals and pro-tect public health and food security.

What species and approximate numbers of animals do you expect to use over what period of time?	Chicken eggs 200,000 Turkey eggs 1,500 Duck eggs 5,000 Goose eggs 1,500 Pigeon eggs 200 Pheasant eggs 200 Partridge eggs 100 Ostrich eggs 50 Quail eggs 100 Chickens 8,000 Ducks 100 Geese 100 Pheasants 100 Partridges 100 Pigeons 100 Ostrich 100 Turkeys 500 Quail 50 The licence is demand lead by amount of diagnosis required, these figures represent maximum numbers that may be used during the five-year span 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?	The majority of the animals will not experience any adverse effects other than those associated with the infrequent administration of an injection (usually only once at the start of a protocol), or the collection of a blood sample (for example once a week). Topical or systemic anaesthesia is used to reduce stress and pain associated with the procedure. The procedures listed are mild in severity, although it is recognised that for a small number of birds will be used under two protocols which are categorised as severe due to the potential pathogenic nature of the viruses used. These tests are required under OIE international regulations for characterising highly pathogenic avian influenza and Newcastle Disease viruses. The severity of these clinical signs will vary from mild to severe depending on the strain of virus that is being diagnosed. Steps will be taken to prevent unnecessary distress. These include an increased number of observations of the animals and use of clearly defined clinical score recording to assess

	severity and/or progression or recovery. **A retrospective assessment of these predicted harms will be due by 01 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	A complete biological system is required for the production of diagnostic material and to meet statutory requirements for diagnosis of infection. Throughout the life of the previous licence we have reduced usage of protected animals by developing alternative in-vitro systems (for example cell culture). However for many tests prescribed by international standards for avian disease control there is still a statutory requirement for the use of animals. **A retrospective assessment of replacement will be due by 01 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	The numbers of animals used on this licence is primarily demand-led by the diagnostic need, with the numbers of animals used for in vivo diagnostic tests being dictated by international standards. Molecular methodology has been developed to reduce the number of times in vivo tests have to be carried out (e.g. only for the index case of a notifiable disease outbreak to enable initial full virus characterisation). In addition, animals from one of the protocols may be re-used in a second protocol to reduce numbers of birds producing antisera. **A retrospective assessment of reduction will be due by by 01 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 main animal model used within this licence is the egg. The embryonic form is used and most of the diagnostic tests are concluded before the embryos reach 2/3rd incubation (sentience). The production of antibodies and antisera, and the collection of blood products use birds as these are dictated by the tests that these biological products will be used in. Chicks and young birds are used for the two statutory in-vivo diagnostic tests (intravenous pathogenicity index (IVPI) and Intracerebral pathogenicity index (ICPI). These tests must be undertaken in high disease containment facilities where it is impossible for continuous direct observation and checking. Birds are observed and checked frequently for clinical signs and if they indicate they will not recover from the infection they are euthanased. This proportion of birds that have to be euthanased varies with the pathogenicity of the virus , the lower pathogenic virus are around 20% but the highly pathogenic viruses it is around 70%. Due to the unpredictable and quick nature of the highly pathogenic viruses some birds may die between observations. Molecular methods for determining pathogenicity are being developed and may replace the statutory *in-vivo* tests used in this licence during the life time of this licence but these tests can only be fully replaced once the technique has been accepted in to international legislation. **A retrospective assessment of refinement will be due by by 01 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	**28. Dissemination and immune subversion of Toxoplasma gondii**	
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
		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 parasite *Toxoplasma gondii* infects a broad range of warm-blooded animals, including humans, and is arguably the most common parasitic infection in man. Toxoplasma has developed various strategies to escape the immune response of the host. The parasite injects proteins, so called virulence factors, into the host cell that divert cellular processes in favour of the parasite. The acute phase of infection generally causes no symptoms in healthy individuals and proceeds silently to the chronic phase of infection with the	

	development of cysts in tissues like the brain and heart muscle. *Toxoplasma* infection is incurable and tissue cysts of the parasite reside in those tissues for the rest of the lifespan of an infected individual. People with a non-functional immune system, such as HIV patients or recipients of organ transplant, are at high risk of suffering from re-infection due to reactivation of the dormant tissue cysts in the brain leading to the development of often deadly inflammation of the brain. Healthy individuals on the other side can lose their eyesight when infected with specific strains of *Toxoplasma* and an unborn child can have birth defects if the mother becomes infected during pregnancy. Currently there is no cure or vaccine available. In the proposed work we aim to identify yet unknown virulence factors used by the parasite to tamper with the hosts' immune defence. We have invented a method to test 200 parasite proteins at the same time and to further analyse how essential they are for the parasite to establish (acute phase) or maintain (chronic phase) the infection. This analysis will allow us to identify and access new possible drug targets. **A retrospective assessment of these aims will be due by 29 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)?	This project will firstly increase our knowledge about the mechanisms the parasite uses to modulate the immune response to its benefit. This will ultimately lead to the discovery of new pathways that might equally be targeted by other pathogens, such as bacteria, or viruses, causing diseases such as malaria or tuberculosis. We will make our results publicly available to the scientific community and beyond using appropriate outreach media. Next to providing insights into the molecular mechanisms regulating the immune response to Toxoplasma and how the parasite is able to escape or modify the immune response, this study will pave the way for follow-up studies in humans to design

	counter-measures or a vaccine strategy.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mice in our study as they are the natural host of the parasite. We determined 5000 animals to be sufficient in the 5-year period of this project to re-sult in reliable results. We will make sure to use the minimum amount of animals per experiment to achieve results reliable 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?	We will infect mice with Toxoplasma and monitor their immune status during the infection. For some experiments we can use parasites engineered to emit light, enabling us to image the infection without harming the mice, comparable to imaging methods used for humans in the hospital. Nonetheless, infection with Toxoplasma can cause severe symptoms of illness and result in the death of an animal. To avoid unnecessary suffering we will treat animals with antibiotics where appropriate and closely monitor their health status. We will euthanize animals that show severe signs of infections in accordance with established humane endpoints. As Toxoplasma infections are incurable, all animals used in this study will at the end of an experiment be euthanized and tissue samples will be taken for further analysis. **A retrospective assessment of these predicted harms will be due by 29 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	Once infected with *Toxoplasma*, all hosts including humans establish a chronic infection that is kept under control by the host's immune system. If the immune system fails, *Toxoplasma* can kill the host. We are not able to recreate the complex interplay between different arms of the immune system with state-of the art laboratory techniques, as it relies on the structural integrity and connectedness of organs and tissues. Therefore, we still need to use animals to answer our specific research questions. Toxoplasma does infect warm blooded animals, rendering the use of alternative models, such as fruit fly and wax worm difficult. Zebra fish larvae

	can be used as alternative if studying the early phase of infection, but will be limited to only one part of the immune system present in humans. Also, fish are not a natural host of Toxoplasma and the results obtained by its study may not reflect the biology of host-pathogen interaction. **A retrospective assessment of replacement will be due by 29 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	REDACTED method that allows us to investigate 200 different Toxoplasma virulence factors at the same time in one mouse. Before, investigated virulence factors were analysed one by one instead. Our method substantially reduces the number of mice needed. This is true for our laboratory, and once we made our method available to the scientific community, laboratories worldwide. Moreover, the results of this study will inform others which virulence factors are worthwhile to study in mice and which ones not. REDACTTED we can often use *Toxoplasma gondii* engineered to emit light, thus being able to image the parasite in living animals. Using this method, we can follow the infection in each animal in real time and do not need to sacrifice animals to analyse each time point individually. To ensure we obtain meaningful scientific results with the minimum amount of mice, we calculate hwo many mice are required before the onset of an experiment. We routinely review our breeding strategies and cryopreserve any strains of mice not being actively investigated **A retrospective assessment of reduction will be due by by 29 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	The mouse model is currently the only extensively refined animal model of *Toxoplasma*

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.	infection. As we are studying how the immune system restricts the parasite in both the acute and chronic phase of the infection, we are reliant on a whole organism to understand the complex interplay of different cellular players of host defence. Using the *in vivo* parasite imaging approach, we are able to administer a minimal dose of the parasite and still be able to assess differences in parasite load between wild-type and genetically altered mice. We have developed mouse body condition scoring sheet aiming for robust monitoring of any adverse effects of infection and prompt intervention where necessary. **A retrospective assessment of refinement will be due by by 29 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	**29. Disuse osteoporosis and novel therapies for promoting bone growth**	
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)	People with spinal cord injury develop weakening of the bones (osteoporosis) in their paralysed limbs. The aim of this project is to develop a novel therapy for spinal cord injury related osteoporosis. Mechanical stresses and strains imposed on bone by weight support and locomotion are important signals. Specialised cells at the centre of the bone (bone marrow) sense these signals and stimulate them to form hard or mineralised bone. These constant mechanical signals are necessary for normal bone turn-over (breakdown and replacement of bone), keeping bones strong and healthy. The	

	weakening of bones that occurs in the absence of these signals is referred to as disuse osteoporosis, and the consequence of this in spinal cord injured individuals is that they have a higher chance of suffering fractures. These can result even from simple everyday activities such as transferring from a wheelchair to a bed or getting out of a car. Furthermore, the consequences of fractured bones are more serious for people with a spinal cord injury than in healthy able-bodied individuals. This is because the bones are much slower to mend – the same processes that leads to weakening of the bones, means that new bone is much slower to form and fractures are slow to heal. Complications from these fractures lead to increased morbidity, mortality and increased healthcare costs. Currently, there are no drugs or therapies for preventing the occurrence of this disuse osteoporosis in spinal cord injury. During normal turnover of bone (continuous process of resorption and replacement), new bone is formed by specialised cells found within the hollow centre. These cells are a type of stem cell that are able to change into cells that form either bone, fat or cartilage. These cells can be grown and studied in a dish (in vitro), and it has been found that when subjected to a particular type of vibration, these stem cells are stimulated to become bone forming cells. The type of vibration that is effective is of very low amplitude (a tiny fraction of a mm) and very high frequency (more than 1000 times per second). So far, the bone stimulating effect of this type of vibration has only been investigated in the dish. The aim of our study is to determine whether applying this vibration directly to paralysed limbs could prevent or even reverse spinal cord injury induced osteoporosis. This will be investigated in a rodent model of spinal cord injury, in which we have shown that there are rapid alterations in the structure of bone that can be readily measured using a specialised type of high resolution x-ray technique, which is performed on the bones after they have been removed from the animal. We will develop devices that can be used to apply the vibration to the hindlimb, and verify using instruments that measure vibration (interferometry), that the

	appropriate vibration is reaching the bones. We will then use this therapy applied regularly to the limbs of the spinal cord injured animals, to investigate how the bone responds to these vibrations. We will investigate whether the therapy can prevent bone loss when applied early after the injury, and whether it can reverse established bone loss if therapy is delayed to mimic cases of chronic spinal cord injury.
Retrospective Assessment	**Retrospective assessment** Published: 28 October 2022 **Is there a plan for this work to continue under another licence?** No **Did the project achieve its aims and if not, why not?** Yes, we were able to show that a vibration device we had designed delivered vibration to bone in an animals leg at the amplitude range intended. We were then able to carry out experiments that used the device to investigate whether this therapy could prevent or reverse changes in the bone (e.g. loss of bone) that occur after spinal cord injury. We did not see evidence of this in our experiments. The duration of the vibration therapy that it is possible to use in this model is limited and may have limited the effectiveness of the treatment.
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 show whether the application of low amplitude, high frequency vibration directly to the limb is able to alter the course of the detrimental changes that occur in bones of the paralysed limbs after spinal cord injury. This information will provide proof of principle that this type of therapy should work in humans if appropriate vibration can be successfully delivered. The animal work will inform the immediate clinical translation of the approach in a Spinal Cord Injury Unit. There is currently no effective intervention for the prevention and/or reversal of the bone loss seen in this patient group. Therefore, this will represent a significant advance in maintaining their quality of life. If we

	can demonstrate effectiveness in this form of disuse osteoporosis, then it is likely that the same approach will be of benefit in other forms of osteoporosis, or where there is need to stimulate bone growth. This might include maintaining bone in astronauts undertaking long space missions or promoting the healing of complex bone fractures.
What species and approximate numbers of animals do you expect to use over what period of time?	The project is estimated to require the use of 350 adult rats over the course of 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 most of the animals used in this investigation (about 80%), it will be necessary to completely cut or transect the spinal cord. This is a severe procedure. These animals have a temporary inability to empty their bladder and need to be regularly assisted. They also permanently lose the use of their hindlimbs, however, this is what is essential in order to model disuse osteoporosis. Because they do not regain use of the hindlimbs as in other spinal cord injury models, the osteoporosis is consistently seen and is permanent. This means we have the opportunity to test whether a therapy like the one proposed here can prevent it. Although they lose the use of their hindlimbs, the animals retain good mobility through the use of their forelimbs, and are able to move rapidly around their cage, groom normally and obtain food and water. They do not exhibit signs of pain as can commonly occur in spinal cord injured patients. These animals also lose feeling from their hindlimbs, and as a result will not feel discomfort during the application of the device to the limb. We therefore expect the therapy itself to be well tolerated by the animals. The animals will only have the vibration device attached to the limbs periodically for treatment sessions, which will last a maximum of 2 hours. The duration of treatment will be no longer than 6 weeks and the overall period that animals will be on procedure will typically be 8 to 12 weeks. All animals will be humanely killed at the end.
Retrospective Assessment	**Retrospective assessment** Published: 28 October 2022 **What harms were caused to the animals, how severe were those harms and how many animals were affected?**

	Thirty-three rats used under this licence received spinal cord transection surgery as a model of spinal cord injury in which the resultant paralysis leads to the types of changes in bone that happen in spinal cord injured patients. This is a severe procedure. Animals require extra husbandry and in particular they require bladder care for a period after the injury until spontaneous emptying of the bladder resumes. The hindlimbs of the animals are permanently paralysed by this procedure. The animals are able to groom, move around their cage and obtain food and water and show no signs of distress. Six rats underwent sham spinal cord transection surgery (the same operation as those being injured but without the spinal cord transection) and these show a quick and full recovery. Thirteen of the spinal cord transected rats underwent a vibration treatment. This involved the attachment of a device producing vibration to a hindlimb, for twice daily 2 hour sessions for 5 days a week, for a total of 6 weeks. The animals were fully familiarised with wearing the devices and so tolerated this well. The design of the device and the method of fitting were such as to minimise any potential trauma or abrasions to the limbs and no sores or problems with the skin were seen.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The objectives can only be achieved by experiments using live animals because the complexity of the biological systems to be examined (bone metabolism), the need to see a response to perturbation of the system (disuse resulting from paralysis) and the need to apply the experimental therapy over time. These experiments cannot be reproduced in vitro or modelled in other ways.
Retrospective Assessment	**Retrospective assessment** Published: 28 October 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?** No non-animal alternatives were used or explored during the project. The aim of the

	project was specifically to conduct in vivo testing of an effect that had previously been observed in vitro. The rat model of complete transection of the spinal cord at a low thoracic level is the most suitable rat model for determining the efficacy of non-pharmacological intervention that requires the attachment of a vibration device directly to the hindlimb to deliver a targeted (as opposed to whole body) stimulus for long periods. This is due to the paralysis and resultant loss of sensation below the level of injury. Other animal models would not tolerate well the attachment of such a device. This particular model therefore produces the osteoporosis to be investigated but also makes possible the method of delivering the vibration.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The numbers of animals to be used in each part of the study will be the minimum required to provide reliable results (avoiding as far as possible inter-animal and technical variability). The results will be analysed statistically for significance. The rapid nature of the changes in bone metabolism in this model means that the numbers of animals required for significant results are minimised. Where data is available, we will use power calculations to determine the group sizes required to provide statistically significant answers. Current information based on the use of juvenile rats, and assuming that
Retrospective Assessment	**Retrospective assessment** Published: 28 October 2022 **How did you minimise the number of animals used on your project and is there anything others can learn from your experience?** The model is a severe model of osteoporosis which we have experience with. Based on this knowledge we set up the minimum number of animals we considered would give reliable results. The novelty of the investigation meant that power calculations were of limited benefit. For the rats that underwent the vibration intervention, only one hindlimb was vibrated and the limb on the other side could therefore used as a control. This design enabled the number of animals used to be reduced. we look at the effects of the therapy at a time point two weeks after injury, suggests we will be

	able to detect an effect of 10% due to the vibration intervention, we would need group sizes of 8 to 10 rats. Experimental design is continually reviewed and our studies carefully executed in order ensure use of minimal animal numbers consistent with the experimental aims, and to maximise information obtained from each animal. In the investigations of vibration, we will apply the therapy to one limb and the opposite limb will act as an internal control, so halving the number of animals that would be required for separate treatment groups.
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.	**Species** Rodents are the most appropriate species as they are the least sentient animals that could be used. The bladder care required and the scale of the rat skeleton would make the project difficult to conduct on mice and most of the previous literature has used the rat as the experimental animal. **Models, methods and minimising suffering** We have considerable experience of the spinal cord transection model and the animal husbandry required to minimise adverse effects. We have introduced a monitoring system for animal welfare which also incorporates built in end points which trigger immediate humane killing. Spinal cord transection animals will be housed in cages which are larger than the standard rat cage and provided with appropriate environmental enrichment and as far as possible, animals are group housed.
Retrospective Assessment	**Retrospective assessment** Published: 28 October 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?** For this project, the choice of animals could not have been improved. As noted above, the main risk for harm (other than the direct effect of the spinal cord transection injury) was considered to be the process of attaching the vibration device to the limb for relatively long periods of time. Harm to the animals was minimised by training the rats before the start of the intervention by incrementally increasing the duration for which

	the device was attached. Great care was taken when attaching and removing the devices to ensure the animal was not injured by this process. The vibration devices were optimised on cadavers to avoid hard edges that might lead to skin damage.

Project	**30. DNA Damage Responses 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)		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?	The aim of this project is to elucidate the molecular mechanisms by which DNA damage results in genome instability and disease **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?	

Why is it important to undertake this work?	This work has the potential to identify and develop novel therapeutic strategies, and ultimately treatments for human diseases associated with unrepaired DNA damage.
What outputs do you think you will see at the end of this project?	The outputs of this work will be increased knowledge of the links between defects in DNA repair and human disease and, based on this knowledge, the identification of possible new therapeutic opportunities. It is expected that all of the key outputs will be published in peer reviewed international journals, including where possible the highest impact journals such as Nature, Cell, and Science.
Who or what will benefit from these outputs, and how?	The primary beneficiaries in the short term (1-5 year) will be the relevant scientific community, who will benefit and inform their own experiments based on our studies. In the longer term (beyond 5 year), the beneficiaries will be individuals with rare genetic diseases in which disease results from established genetic mutations that disrupt DNA damage sensing and repair. This is because the knowledge gained from this project will inform better diagnosis and clinical treatment of such patients, of which there are currently tens-of-thousands, world-wide.
Will this work be offered as a service to others?	No
How will you look to maximise the outputs of this work?	*The outputs of this work will be disseminated at leading international conferences, in the highest quality and impact publications (in line with ARRIVE & PREPARE guidelines), and through enhanced and extended collaborations with other research groups, clinicians, and with Pharma.*
Explain why you are using these types of animals and your choice of life stages.	Mice are required for experiments where the impact of DNA damage on human disease is under investigation, because these are the least sentient mammal that recapitulates the complexity observed in humans in DNA damage responses, and the disease pathology that arises if such responses are defected. The use of mice is thus critical to our understanding of how DNA damage induces human disease, and to identify new therapeutic opportunities to prevent or cure such disease.

Typically, what will be done to an animal used in your project?	We may alter the DNA of mice to inactivate DNA damage response genes of interest by gene editing early stage embryos generated by superovulation. Unmodified or modified embryos may be developed to term by surgically placing them into a female rendered pseudo-pregnant by mating with a surgically vasectomised male. We may breed the (genetically appropriate) mouse offspring and compare the phenotype of the mutant mice with normal littermate controls, to address the impact of the mutated gene in question on health and disease. In some cases animals may be treated with chemicals that induce DNA
	damage to identify which genes/biochemical pathways protect organisms from environmentally relevant DNA damage, or may be treated with potentially therapeutic chemicals to identify novel opportunities for curing DNA damage-associated diseases. During the course of the project, if at any stage an animal experiences poor health that cannot be ameliorated, it will be killed humanely and in a timely manner. All animals that have reached the end of their study will be killed.
What are the expected impacts and/or adverse effects for the animals during your project?	Most animals (>90%) will experience little or no discomfort and any discomfort will be transient with no long lasting harm. Some animals (<10%) may experience moderate discomfort associated with DNA damage related phenotypes (e.g. ataxia) and in such cases the mice will be monitored frequently for health and humanely killed if this becomes poor and/or the humane endpoint is reached. Occasionally, animals (<0.5%) will experience a more severe discomfort (sporadic/transient seizure), but only in "therapeutic" experiments when we are trying to cure this phenotype with novel therapeutics. In these experiments, animals will be humanely killed once the time-to-onset of first seizure is been ascertained, thereby minimising suffering. When in doubt about the health of an animal, advice will be sought from the named veterinary surgeon (NVS).
What are the expected severities and the proportion of animals in each category (per animal type)?	Most animals (>90%) will experience mild or no discomfort, with <10% experiencing short term moderate discomfort associated with surgical procedures or with phenotypes associated with their DNA damage-response defective genetic status (e.g. ataxia). A small number of animals <0.5% may experience short term discomfort associated with a more severe disease-associated phenotype (seizure), which we occassionally need to measure to examine the curative efficacy of putative therapeutic compounds.

What will happen to animals at the end of this project?	used-in-other-projects, killed **A retrospective assessment of these predicted harms will be due by 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?
Why do you need to use animals to achieve the aim of your project?	Mice are required for experiments where the impact of unrepaired DNA damage on disease-related phenotypes is under investigation. These questions are critical to our understanding of how DNA damage induces neurological disease and/or cancer predisposition.
Which non-animal alternatives did you consider for use in this project?	Where possible, we use microorganisms or human cell lines for our studies. For example, we dissect mechanistic aspects of the biohemical pathways that repair damaged DNA using such in vitro model systems. This both limits the need for in vivo models such as mice, and guides our choice of mouse model where the later are necessary.
Why were they not suitable?	Microorganisms and human cell lines cannot recapitulate human disease, and so the experiments that are needed to understand the links between DNA damage and disease must be conducted in vivo. **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?
Enter the estimated number of animals of each type used in this project.	mice: We anticipate using a maximum of 23,600 mice
How have you estimated the numbers of animals you will use?	The numbers estimated here are based on our average actual usage over our previous project, factoring in that the new project is significantly smaller than the current project. There is a significant reduction (~30%) in estimated mouse usage, compared to the current project.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?	Mouse numbers are kept to the minimum required to maintain the relevant colonies and carry out the necessary experiments. Mice will be genotyped early after birth to facilitate breeding and minimise colony size. Where appropriate, group sizes are determined by standard power analysis (e.g. SISA). Typically, we maintain colony sizes of 4-6 breeding pairs per strain, and when not needed these strains are maintained for future use as frozen sperm/embryos thereby preventing needless breeding programmes. Also, where possible, we reduce mouse numbers using optimal/appropriate statistical approaches. For example, in experimental terms, we minimise mouse numbers by designing multi-parameter experiments in which we can test several hypotheses simultaneously (e.g. measuring the impact of a particular gene loss on multiple cell types in multiple tissues), thereby allowing us to reduce not only the number of mice in the test groups but also the number of control groups.
What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?	We employ small-scale pilot studies to determine the likely impact of an experimental parameter, and where possible we utilise tissues from individual animals for multiple experimental purposes. Also, as mentioned above, we minimise mouse numbers by designing multi-parameter experiments in which we can test several hypotheses simultaneously. **A retrospective assessment of reduction will be due 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?
Which animal models and methods will you use during this project?	We will use DNA damage response-defective mouse strains to understand how DNA damage causes human disease. Only in those cases where critical questions cannot be addressed by other methodology do we utilise mice. Under all circumstances we will minimise animal suffering by frequent health and behaviour monitoring, and by humane killing of animals in poor health. Occassionally (<0.5% of animals) mice may experience a transient but more severe phenotype (seizure), if we are attempting to cure this human disease-associated pathology. In these cases we ensure that we measure time to onset-of-first seizure, to

	minimise repeated suffering, rather than seizure frequency. Where in doubt, advice will be sought from the Animal Care manager (NACWO) and staff, named veterinarian (NVS), and if appropriate the Home Office Inspector.
Why can't you use animals that are less sentient?	Where possible, we use human cell lines for our studies. However, mice are required for some experiments where the impact of unrepaired DNA damage on disease states is under investigation. These questions are critical to our understanding of human DNA repair-defective human diseases. Mouse is the least sentient mammalian model system available to us for this purpose. Whilst non-mammalian species (e.g. fish, amphibia, nematodes, flies etc) do have DNA repair pwthays, the proteins involved in the pathways we are interested in are not typically conserved e.g. XRCC1 protein in these species lacks activities/domains that are present in the mammalian protein.
How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?	I am in close contact with the NACWO and NVS, who regularly inform project and personal licence holders of recent developments in the 3R's, including frequent appraisal of 3Rs-associated sites such as NC3Rs & FRAME. **A retrospective assessment of refinement will be due 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?
How will you refine the procedures you're using to	Under all circumstances we minimise animal suffering by frequent (e.g. daily/twice daily) health and behaviour

minimise the welfare costs (harms) for the animals?	monitoring, including the use of health score sheets, and by humane killing of animals if the humane end point is reached. Appropriate pain management and post-operative care is in place, as detailed in each of the relevant protocols. In addition, we will introduce appropriate refinements to our protocols when and where possible as new techniques advance and become available e.g. we will attempt to refine the embryo transfer protocol using non-surgical methods to reduce animal discomfort. Under all circumstances, if in doubt, advice will be sought from the Animal Care manager (NACWO) and staff, Veterinarian (NVS), and the Home Office Inspector.
What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?	We utilise a variety of online, published, and local sources to inform our experiments including previous scientific literature, web resources such as the NC3R Experimental Design Assistant, and local statisticians and animal facility/welfare staff.

Project	**31. Dynamics of transcription regulation and its impact during ageing 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
		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 human body is composed of over two hundred unique types of cells with specialized roles and yet every cell contains the same genetic information called the genome. The differences between types of cells are largely determined by master regulators which direct how the genome is ‘read’ in each cell. The broad goal of this project is to use a systems biology approach to discover the rules by which the mammalian genome operates. We want to decode the regulatory elements in the genome which dictate how, when and where genes are expressed. These mechanisms ultimately	

	control the creation of specific cells, tissues and species. We will use our knowledge of the normal mechanisms controlling gene expression to inform cancer research. We are specifically interested in identifying the features of the normal transcriptional regulatory landscape of a cell which make its genome susceptible to mutations and results in the development of cancer. We have chosen to use liver cancer as our model, in part because it is one of the most lethal human cancers. We also aim to explore the impact of ageing on the stability of transcriptional regulatory mechanisms. Ageing is a complex molecular process and we want to investigate if a gradual, random increase in transcriptional instability is one of the hallmark features of ageing in mammalian tissues.
Retrospective assessment	**Retrospective assessment** Published: 20 December 2022 **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 license was entirely unused since its issuance, and no mice have been ordered nor have any procedures ben performed on it. The reason my laboratory requested this license was just in case our experiments on the previous lab held license required repeating. Fortunately, none were necessary.

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 expected from this project are novel, fundamental insights at the genome-wide scale into the mechanisms which control transcription. The project is expected to contribute to an understanding of how these regulatory mechanisms can influence the development of cancer and how they may be associated with normal, healthy ageing. Such information is of potential use to scientists working in pre-clinical research. For example, we will provide knowledge that could improve the ability to link genetic differences between individuals with variations in disease susceptibility. However, any pre-clinical benefits are beyond the scope of this immediate project.
What species and approximate numbers of animals do you expect to use over what period of time?	This project uses mice as the experimental animal. We expect to use fewer than 8,800 mice during the five year project 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?	Many of our studies use tissue samples collected from mice which have no expected adverse phenotypes and which have been killed by a humane procedure. Some studies use mice carrying mutations which may cause impaired liver function or growth retardation. We also use mice treated with a carcinogen and then allowed
	to age to develop liver tumours. Some experiments need samples taken post-mortem from old mice and these aging mice can show a variety of adverse health conditions associated with getting old. We intend to humanely kill experimental mice before or at the onset of signs of illness. We expect to be able to intervene in this way for the vast majority of mice; however, we anticipate that a small number of mice will deteriorate so rapidly that they die. Experimental animals are killed by a humane method at the end of the study.

Retrospective assessment	**Retrospective assessment** Published: 20 December 2022 **What harms were caused to the animals, how severe were those harms and how many animals were affected?** This license was entirely unused since its issuance, and no mice have been ordered nor have any procedures ben performed on it. The reason my laboratory requested this license was just in case our experiments on the previous lab held license required repeating. Fortunately, none were necessary.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Post-mortem tissue samples from animals are essential for our studies to understand normal tissue-specific transcriptional regulatory networks. Non-animal approaches such as cell culture are of limited use for our research since the transcriptional regulatory systems found in cultured cells have invariably drifted substantially from those in the original tissue from which they were derived. For our experiments investigating transcriptional regulatory systems during tumour evolution, mammalian tumour samples are crucial for similar reasons. Tumour biology is profoundly influenced by its environment within the body and tissue culture methods are unable to mimic this complex interplay.

Retrospective assessment	**Retrospective assessment** Published: 20 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?** This license was entirely unused since its issuance, and no mice have been ordered nor have any procedures ben performed on it. The reason my laboratory requested this license was just in case our experiments on the previous lab held license required repeating. Fortunately, none were necessary.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Animal numbers are minimised by designing breeding strategies to produce experimental animals as efficiently as possible and by the use of statistical analyses to determine the minimum number of animals required without compromising the scientific aims of the experiments. We collect multiple samples from experimental and control animals to form a tissue repository for future *ex vivo* experiments. If possible, we use tissue from this repository rather than use new animals. This repository also allows us to use samples from the same animal in multiple studies.
Retrospective assessment	**Retrospective assessment** Published: 20 December 2022 **How did you minimise the number of animals used on your project and is there anything others can learn from your experience?** This license was entirely unused since its issuance, and no mice have been ordered nor have any procedures ben performed on it. The reason my laboratory requested this license was just in case our experiments on the previous lab held license required repeating. Fortunately, none were necessary.

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 is the experimental animal of choice for this project. Many evolutionarily distinct inbred strains exist, which are crucial for our comparative functional genomics studies. There is reliable technology to genetically manipulate mice and an extensive inventory of readily available genetically modified mice. This resource allows us to assess disruptions caused by altering specific components of transcriptional regulatory networks. Our choice of a carcinogen-induced model of liver cancer is based on the experimental needs to introduce a high mutational burden in the liver cell at tumour initiation and to use evolutionarily distinct inbred mouse species, some of which are not amenable to genetic engineering. Appropriate health monitoring protocols, for example increased close monitoring, are implemented for mice expected to have an adverse phenotype, for example caused by a genetic modification which results in impaired liver function; caused by developing liver tumours; or caused by ageing. When appropriate, suitable anaesthetic regimes are used to minimize suffering or the animal is killed by a humane method.

Retrospective assessment	**Retrospective assessment** Published: 20 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?** This license was entirely unused since its issuance, and no mice have been ordered nor have any procedures ben performed on it. The reason my laboratory requested this license was just in case our experiments on the previous lab held license required repeating. Fortunately, none were necessary.

Project	**32. Dynamics of transcriptional regulation and its impact on human susceptibility to cardiovascular 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
		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 human body is composed of over two hundred unique types of cells with specialized roles and yet every cell contains the same genetic information called the genome. The differences between types of cells are largely determined by master regulators which direct how the genome is ‘read’ in each cell, thus determining the range of functions a particular cell type and tissue performs. Focusing on the cardiovascular system, the broad goal of this project is to use a global approach to study the rules by which the mammalian genome is read in	

	cardiovascular cell types such as the heart muscle. We want to decode the regulatory elements in the genome which dictate how, when and where genes are expressed (e.g. in the heart). These mechanisms of gene regulation ultimately control the creation of specific cells and tissues, and determine how they respond to changes in their environment such as cellular stress. We will use our knowledge of the normal mechanisms regulating genes to inform cardiovascular research, with a focus on susceptibility to these diseases in humans. We are specifically interested in identifying the features of gene regulation in a heart cell which make some human individuals more susceptible to cardiovascular disease. We have chosen to use the heart muscle as a model: (i) because heart disease is very common worldwide; and (ii) because several aspects of disease predisposition in the heart (such as the development of an enlarged heart or an irregular heart beating) can be experimentally studied in both human cellular models (in a dish) and animal models. **A retrospective assessment of these aims will be due by 19 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 potential benefits expected from this project are novel, fundamental insights into the mechanisms of gene regulation in the cardiovascular system. The project is expected to contribute to an understanding of how these regulatory mechanisms can influence the development of disease, and how they may be associated with susceptibility to specific forms of heart disease - such as impaired heart muscle function or irregular heart beating. Our work will also generate reference datasets made available to the research community, which can and have often been used to address complementary questions by other research groups. The information provided by our work is of potential use to scientists working in pre-clinical research. For example, we will provide knowledge that could improve the ability to link genetic differences between individuals with disease susceptibility. However, any pre-clinical benefits are beyond the scope of this immediate project.
What species and approximate numbers of animals do you expect to use over what period of time?	This project uses mice as the experimental animal. We expect to use fewer than 2,500 mice during the five-year project licence. The majority of these animals will be used for breeding and maintenance of research colonies, with only around twenty percent of mice being used in experiments associated with adverse effects.

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 our studies use tissue samples collected from mice which have no expected adverse effects and which have been killed by a humane procedure. Some studies use mice carrying mutations which may cause impaired tissue function or growth retardation. For these mice, we control mutation dosage to restrict adverse effects to a mild severity, and closely monitor mice for any unexpected defects during early post-natal life. We also use mice treated with specific substances or subjected to cardiac surgery to modify cardiovascular function, and then allowed to develop impaired heart function. Some of these mice are also subjected to imaging studies to measure changes in cardiovascular physiology, or exposed to a low oxygen environment (to mimic the cellular environment associated with heart failure). During this work, we conduct studies at early time points and the mildest possible severity, thereby minimising the adverse effects animals may experience. We also provide best care to prevent pain (with anaesthesia and analgesia) and avoid animal suffering. However, adverse effects such as development of an enlarged heart, an irregular heart beating, abnormal weight loss, reduced mobility or altered respiratory rate are likely to be unavoidable to meet our scientific objectives. Some animals may experience rare post-operative complications which are not part of our scientific goals such as neurological, metabolic, or digestive dysfunction, infections or build-up of abdominal fluid. These mice are immediately euthanised to avoid suffering. We intend to humanely kill experimental mice before or at the onset of moderate signs of illness. Because animals will be intensively monitored, we expect to be able to intervene in
	this way for the vast majority of mice. Experimental animals are killed by a humane method at the end of the study. **A retrospective assessment of these predicted harms will be due by 19 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	We use in vitro experiments (i.e. cultured cells in a dish) extensively to investigate the development of an enlarged heart muscle and an irregular heart beating. Whenever possible, we also use available data generated by other groups or available in public research databases to answer our research questions. However, non-animal approaches such as cell culture in a dish are of limited use for our research, since the gene regulation found in cultured cells drifts substantially from that in the original tissue from which the cells were derived. Post-mortem tissue samples from animals are essential for our studies to understand normal tissue-specific gene regulation, such as that operating in the heart. For our experiments investigating how, where and when genes are expressed during cardiac stress, animal samples are crucial for similar reasons. Tissue biology is profoundly influenced by its environment within the body and the interaction between different types of cells, and in vitro methods in a dish are unable to faithfully mimic this complex interplay. **A retrospective assessment of replacement will be due by 19 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	Animal numbers are minimised by designing husbandry strategies to produce experimental animals as efficiently as possible, and by the use of statistical analyses to determine the minimum number of animals required without compromising the scientific aims of the experiments. We also use pilot experiments with small numbers of animals to validate experimental conditions before conducting larger studies, and ensure we maximise the utility of results by careful reporting of experimental conditions and conclusions. We collect multiple samples from experimental and control animals to form a tissue bank for future ex vivo experiments. If possible, we use tissue from this collection rather than use new animals. Careful banking of tissues also allows us to use samples from the same animal in multiple studies. **A retrospective assessment of reduction will be due by by 19 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 mouse is the experimental animal of choice for this project. The availability of extensive genetic resources in mice, such as many well-characterised and readily available genetic alterations, are crucial for our studies of cardiovascular gene regulation. There is reliable technology to genetically manipulate mice and an extensive inventory of readily available genetically modified mice. This resource allows us to assess changes in gene regulation (e.g. in the heart) caused by altering specific components of gene expression control. Our choice of whole-body and organ-specific models of cardiovascular stress is based on the experimental needs to introduce perturbations in tissue function, and to use organ-specific models of clinical relevance (such as models of an enlarged heart, a condition which often associates with heart failure in humans). Appropriate health monitoring protocols, such as increased close monitoring, are implemented for mice expected to have adverse effect, for example caused by a genetic modification which results in impaired tissue function or by surgical interventions. When appropriate, aseptic surgery and suitable pain killers (i.e. anaesthetic and analgesic regimes) are used to minimise suffering. Animals are closely monitored so we can intervene before or at the onset of signs of disease, or are killed by a humane method at the end of the study. **A retrospective assessment of refinement will be due by by 19 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?

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Project	**33. Education in pharmacology**	
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
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
	X	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 primary objectives of this education in pharmacology project are to: 1. To use *in vivo* models to demonstrate the actions of catecholamine transmitters, their receptors, and cardiac drugs upon the heart and blood pressure. 2. To use *in vivo* models to demonstrate the actions of acetylcholine and its receptors upon the heart, blood pressure and other physiological parameters. 3. To use *in vivo* manipulations combined with *in vitro* models to demonstrate the effects of anaphylaxis and allergic responses in response to antigen exposure, and how these effects may be prevented by anti-histamine and other chemical mediators.	

	4. To use *in vivo* models to demonstrate the effects of natural mediators and drugs upon bronchial smooth muscle contraction and airways function in a model for human respiratory disease. 5. To monitor the success of the course, specifically how well the above objectives are met and more generally the value of this education to successful students in their future employment. **A retrospective assessment of these aims will be due by 12 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 education and training of medical and biomedical science undergraduate and postgraduate students are firmly based in the fundamental sciences. The ability to observe, collect, analyse and interpret experimental data is essential to the successful completion of their education. The power of using whole animals in experiments is illustrated by the integrated physiological response elicited by discreet events such as drug administration. For example, a drug administered for one purpose can often have other important effects in non-target tissues, which are not possible to observe in non-sentient species. Experiments using animals also allow students to develop an understanding of the strengths and weaknesses of biological experimentation. This allows students to appreciate the limitations of pharmacological research and development, and the validity of drug discovery. Students also observe first-hand the variability inherent in biological measurements obtained from live animals in experimental settings. Students develop an acute awareness of the ethical issues of experiments on animals and the tenet of the 3 R's. Prior to practicals students attend lectures covering ethics, animal welfare, and experimental design. In practical classes students engage in advanced discussions of the ethics of using animals for teaching and research purposes, and

	are invited to express their views on alternative teaching approaches such as computer simulations and videos. These discussions encourage a mature appreciation of animal experimentation, including the need to avoid unnecessary use of animals due to ill-considered, poorly designed or conducted experiments. Through participating in practicals involving living animals, students will develop unique understandings and experiences which are key for future careers in medicine and biomedical research. The pharmaceutical industry, one of the UK's major industries, is unable to recruit sufficient individuals with in vivo experience. Pharmaceutical companies universally support the development of individuals who appreciate and have skills in in vivo experimentation, and provide funding support via UK and EC research councils as well as the British Pharmacological and Physiological Societies.
What species and approximate numbers of animals do you expect to use over what period of time?	Principally rats and guinea-pigs, but a small number (6% of total) of ferrets will be used. Over the 5-year period up to 695 animals may be used, which equates to less than one animal for every student taught in this 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 vast majority of the practicals are performed using deeply anaesthetised animals that do not recover from anaesthesia. Suffering is limited to transient discomfort associated with the induction of terminal general anaesthesia, perhaps similar to that experienced by patients undergoing a surgical procedure. In a small number of other practicals, animals (10% of total) receive one or two injections of a non-toxic substance and are then killed a few weeks later for post-mortem tissue analysis. **A retrospective assessment of these predicted harms will be due by 12 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	The practical classes form a small but important part of a fully integrated course which uses a range of teaching methods including experiments using tissues in vitro or the students themselves,

non- animal alternatives	but also video recordings and simulations, lectures, seminars and tutorials. Whilst simulations or videos are adequate to illustrate basic principles, a full understanding of the nature of biomedical research and its attendant sources of error and variability, requires first hand exposure to experiments including use of living animals. Comparisons of physiology teaching and learning outcomes using computer simulations versus live animal demonstrations consistently emphasise the differences between these approaches, and their relative strengths. Students cannot gain a full appreciation of the nature of any branch of science from textbooks and lectures alone, and science education at all levels is supported by experimental demonstration of fundamental principles. In the case of pharmacology practicals, students apply the pharmacological and physiological concepts they learn in lectures and seminars to appreciate the complex, inter-related systems responsible, for example in the control of blood pressure. Moreover with a live animal, neither demonstrator nor student knows precisely what will happen next. This uncertainty teaches a respect for experimental observation and illustrates that scientific knowledge is not preordained but comes from measurement, analysis and hypothesis testing. **A retrospective assessment of replacement will be due by 12 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	We are conscious of the need to use the minimum number of animals in our practicals. A main means to achieve this is for students to work in groups. The size of each group is determined from teaching experience, student feedback, and also the nature of the practical. The number of animals used represents the best compromise between the minimum animal use and the greatest benefit to the students. In some cases, students observe an experienced individual demonstrate complex pharmacological and physiological principles in a challenging experimental model, and at the same time have the opportunity to discuss data with the

	demonstrator, as it emerges. In other cases, group sizes are smaller so that students get the opportunity for hands-on experience and the independence of running the experiment and thinking more for themselves. It is common practice to use unwanted tissue from one practical in another practical, and also for unwanted tissue to be used by researchers. An ethical review process monitors the practicals with the aim to offer advice on minimising the number of animals used, identifying refinements to procedures, and maximising the benefits. Feedback is also relayed from various teaching committees, scientists, clinicians, vets, and the students themselves. **A retrospective assessment of reduction will be due by by 12 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 species have been selected as they best model major human health problems including cardiovascular and respiratory disease, and allergy, which are not easily modelled in vitro or non-sentient species. Rats, guinea pigs and ferrets are used to demonstrate cardiovascular and respiratory effects of drugs because they are able to tolerate large physiological changes. This allows testing of multiple manipulations thereby markedly increasing the value of the experiment. Guinea pigs are used to model allergy mechanisms because they generate a highly efficient antibody response. This represents a reduction and refinement because mice or rats would require multiple exposures over a considerable period, and even then it would not be guaranteed. All surgical procedures are performed under non-recovery general anaesthesia, and animals will not experience pain beyond the transient discomfort associated with the induction of terminal anaesthesia. Surgical procedures, and anaesthesia induction and maintenance, are carried out by skilled demonstrators who stay with the animal for the duration of the

	experiment. Students are not involved in setting up the experiments. **A retrospective assessment of refinement will be due by by 12 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	**34. Effects of tumour-stromal interactions on tumour progression and therapeutic responses**	
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)	Breast cancer is one of the leading causes of cancer death in women. Although most cancer in the breast (primary tumours) can be resected surgically, it often spreads to different parts of the body, such as the bone and lung (metastatic tumours) and this is responsible for the high mortality of the disease. Although metastatic tumours are primarily treated with chemotherapy (administration of anti-cancer drugs), overall survival for patients with metastatic tumours has not changed much for more than twenty years. This indicates that current medicines are	

	ineffective for treatment of metastatic disease, and that development of new therapies is essential to effectively cure the disease. It is well known that tumours consist not just of malignant cancer cells but also a number of non-malignant cells called stromal cells that include various types of immune cells. Our previous research has indicated that a unique type of immune cell called tumour-associated macrophages provide essential support for cancer cells to establish metastatic tumours. In contrast, other types of immune cells called cytotoxic lymphocytes can kill certain types of cancer cells. However, studies from our and other labs have suggested that the anti-tumour abilities of cytotoxic lymphocytes are suppressed by tumour-associated macrophages in the tumours. Furthermore, several studies including ours have suggested that tumour-associated macrophages can suppress efficacy of chemotherapies. This data suggests that interactions of tumour-associated macrophages with cancer cells and cytotoxic lymphocytes play important roles not only in the metastasis formation but also in therapy resistance. However, the molecular mechanisms behind these cell-to-cell interactions are largely unknown. This project aims to define key molecules that regulate the macrophage mediated cell-to-cell interactions and to investigate whether blockade of these molecules can prevent development of metastatic tumours and their resistance to current therapies. **A retrospective assessment of these aims will be due by 19 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 will enable us to reveal the role of interactions between cancer cells and stromal cells (e.g., macrophages and cytotoxic lymphocytes) in tumour progression and metastasis. This project will also allow us to identify key stromal factors that affect cancer cell behaviour, anti-tumour immune responses, and efficiency of current therapies. Such information will lead to novel therapies aimed at preventing tumour metastasis by inhibiting these stromal factors. Data of tumour promoting functions of stromal cells will also provide important information for cancer researchers to develop novel prognostic markers to predict disease outcomes, as well as diagnostic markers to follow disease progression and to select optimal therapy for this deadly disease.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use around 58,000 mice 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?	Animals will develop benign to malignant tumours by tumour injection or genetic alterations, which may cause discomfort. Clinical signs such as tumour growth will be closely monitored and strict humane endpoints implemented. Most animals will also be administered substances that might modify tumour growth or development of metastasis. Some animals will be irradiated and injected with bone marrow cells before tumour development. Because the bone marrow transplanted mice are susceptible to infection, they will be kept under clean conditions. On rare occasions, we will cut the skin, remove the tumour, and close the wound under anaesthesia. To minimize infection risk and pain by surgery, we will give pain relief and antibiotics when appropriate to the animals after the surgery. To reveal tumour cell behaviour in the complex environment, we need to image the tumours in mice. To obtain clear images, we will insert a small window over the tumours developed under the skin or in the mammary gland, which will cause momentary pain and possibly induce post surgical inflammation in very rare cases. We will control the pain by general anaesthesia and pain relief, and minimize a risk of infection by good surgical and aseptic techniques. The implantation site is monitored for signs of inflammation and

	infection, and antibiotics will be given if necessary. To analyse tumour cell behaviour in the lung, we will insert a small window into the pulmonary cavity. This procedure is performed under terminal anaesthesia with close monitoring of body temperature. Therefore, animals will not feel any pain or discomfort. All animals used in this lung imaging experiment will be euthanized by overdose of anaesthesia immediately after imaging. **A retrospective assessment of these predicted harms will be due by 19 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	We have established and have been using several *in vitro* assays that mimic a certain aspect of interactions between cancer and stromal cells. However, these models are over-simplified and do not recapitulate the complex tumour microenvironment where multiple factors and cell types are interacting. In order to understand such a complex tumour environment, and to identify potential therapeutic targets, we need to integrate our *in vitro* assays and animal experiments modelling human diseases. **A retrospective assessment of replacement will be due by 19 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	To minimize the number of mice used to generate significant results, group sizes are calculated by examining previous data from our laboratory and published studies. Samples generated from previous experiments are archived and stored appropriately so that experiments are not repeated unnecessarily. To find therapeutic targets, we need various

	genetically altered (GA) mouse strains, which requires complicated breeding. To minimize the number of animals involved we have identified the most efficient breeding strategies to generate mice with the correct genotype. We will utilize elaborated experimental techniques such as bone marrow transplantation from GA animals to wild type recipients and real-time detection of internal tumours, which greatly reduces the number of mice to be used for experiments. **A retrospective assessment of reduction will be due by by 19 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 can use only mice since our biological reagents are developed in mice. In addition, well-characterized morphology and genetics of this species enables reliable models that mimic tumour progression in humans that are essential to develop more effective therapeutic strategies. We have developed many of these tumour models and therefore we have a vast knowledge of the clinical signs they will show, which allows us to avoid severe health problems in the animals during the experiments. Furthermore, we continue to monitor technical advances and to innovate novel techniques in an attempt to reduce the impact of experimental cancer on the animals. If suitable models occur, we will immediately adopt them into our research strategy. We will ensure that all animals receive the highest standard of care, and animal suffering is kept to a minimum by close monitoring of tumour development.Animals exhibiting severe clinical signs will be humanely euthanized. We will follow “redacted” Guidelines for surgical/post-surgical care including appropriate anaesthesia/pain relief protocols at all times. In collaboration with veterinary staff, we will use several support measures to refine our models, such as heat pads, fluid supplementation and more palatable food.

	A retrospective assessment of refinement will be due by by 19 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	**35. Enteroendocrine signalling in 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)	After food consumption the gut sends signals to the rest of the body preparing it for the arrival of new nutrients. Some of these signals have already been developed for the treatment of high blood sugar with the added benefit of patients often losing weight. The same signals have also found to be strongly elevated after surgical rearrangement of the gut, a method to treat very overweight patients. However, some patients experience complications following the surgery, e.g. they feel very sick after meals, which again has been linked to signals from the gut. We aim to understand how the gut controls these signals in health, disease and after surgery. Which	

	cells in the gut send these signals and what do they respond to? Which cells in the body receive the signals and how do they control the whole body response? **A retrospective assessment of these aims will be due by 30 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)?	Once we identify which signals (alone or in combination) result in weight loss and better control of blood sugar, we hope to be able to modify these signals to help patients that store too much fat and fail to control their blood sugar levels adequately. We aim to be able to modify the signals, increasing them in patients that do not have strong enough signals after a meal and thus help them to keep better blood sugar control and lose weight, whilst decreasing or blocking the signals that make some people sick after surgery, thereby improving their post-surgical quality of live.
What species and approximate numbers of animals do you expect to use over what period of time?	The cells that send the signals from the gut are very rare. We will use mice, as we have made mice in which these cells are manipulated to make a coloured label. We have made other mice that label the cells that respond to the signals. This allows us to characterise when and how the signals are sent. We will use ~15000 mice 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?	Most of the ~15000 mice will experience minor or mild severity. We use special mice that have been manipulated to express coloured markers either in the cells that produce gut born signals in response to a meal or in the cells that respond to these signals. As the label is not inherited by all mice, we will use the label-negative mice as negative controls in experiments wherever possible or these mice will be culled at a young age. Even of the label-inheriting mice many will be organ donors for experiments taking place after they have been culled. ~5000 mice will undergo procedures like fattening by feeding of a fatty diet and/or will be injected with drugs to trigger signals from the gut or to alter the responses of the signal receiving cells. The mice behaviour (e.g. how much and what is a mouse eating after one of the above experiences? Is the mouse changing its energy usage?) will be investigated and mice might be bled repeatedly to measure and identify different signals from the gut. Overall this should only cause minor discomfort of short duration to the animals. ~1500 mice will undergo surgery, to either manipulate cells within their brain, with the aim to identify how the gut signals are integrated to affect feeding behaviour, or to surgically rearrange the gut in a similar way used for the treatment of very overweight people. Adverse effects will be minimised by careful post-surgical care and animals will be culled should they show signs of prolonged discomfort. **A retrospective assessment of these predicted harms will be due by 30 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	We work with cultured cells wherever possible. The cross-talk between gut cells and other cells in the body and the complex circuits regulating feeding behaviour can, however, not be studied in vitro. Similarly, changes in response to surgical rearrangements of the gut can only be studied after the procedure has been performed. Whenever possible, samples from several tissues (e.g. fat tissue) will be taken and stored from animals having undergone these surgical procedures to enable future studies. **A retrospective assessment of replacement will be due by 30 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	Mice will be bred at the lowest numbers possible to keep the different strains with labelled signal producing and receiving cells alive. Harvested tissues will be shared between different group members where possible, and in vitro cultures that can be used over prolonged time frames will be established whenever possible. Pilot studies will be performed to assess variability and time courses of effects, to optimise and minimise the final group sizes for metabolic assessments of live mice. All experiments will be planned, analysed and reported in line with best practice guidelines. **A retrospective assessment of reduction will be due by by 30 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.	Under previously licenced work we have established that mouse and human signals from the gut share many similarities, justifying the use of this animal model. We constantly review the literature whilst planning our experiments and acclimatize animals to new experiences (e.g. environment changes, food changes) before the actual data is collected to minimise stress effects. Whilst we are not using a defined score sheet to assess animals after surgical manipulation, we monitor behaviour and weight progression intensively in all operated animals and for example have in the past been able to spot complications after gut-rearranging surgery early, thus minimising potential suffering. Our laboratory also has and continues to work on the improvement of the analysis of samples after they have been taken from the animal; this includes the development of better methods to measure the signals from the gut in blood, for example enabling simultaneous detection of several signals in small volumes; we adopt and develop new life tissue preparations for the characterisation of the cells that produce or respond to the gut signals, for example brain tissue slices and gut tissue preparations kept alive for several hours for scientific characterisation, with the aim to minimise additive procedural impact on live animals. **A retrospective assessment of refinement will be due by by 30 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	**36. Evaluation of rodenticides in the laboratory and field**	
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
	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)	This Project Licence will authorise work to monitor, regulate and develop rodent control methods, to monitor the environmental impact of chemicals used to control rodents, and to investigate the development of physiological resistance to the anticoagulant rodenticides.	
Retrospective assessment	**Retrospective assessment** Published: 15 March 2024 **Is there a plan for this work to continue under another licence?** No	

	Did the project achieve its aims and if not, why not? During the project we were able to assess the toxicity and palatability of registered and novel rodenticides on both house mice and Norway rats. This allowed for the continued production of commercially available products and the development of novel ones. This work was not done by the University but industry partners. However, fewer tests were conducted than expected due to the covid pandemic. Rodenticide resistance analysis was highly successful and this added to knowledge of resistance in the UK for both house mice and Norway rats. This work was shared with the Rodenticide Resistance Action Committee along with the work of other laboratories.
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)?	Rodents can be a major pest to humans, because they consume our food, transmit diseases, damage our property through gnawing, and they can be a major conservation problem, particularly for many endemic island bird species. The development of the anticoagulant rodenticides revolutionised rodent control. However, resistance to rodenticides has been developing for many years and is now a major problem for achieving effective control in the UK, Germany, France and elsewhere. The research authorised by this Project Licence will be used to: • extend the useful life of anticoagulant rodenticides by reducing the environmental impact of these chemicals • investigate resistance to anticoagulant rodenticides and develop resistance management strategies • develop more effective rodenticide formulations • develop novel rodenticide active ingredients The output from this research is currently being used by the UK Regulatory Authorities (Health and Safety Executive) and the European Commission, and has benefited developing countries, where the rodent problem has life threatening impacts on subsistence crop production, and where the potential impact against species of conservation concern is far greater. In the future we aim to continue these partnerships to provide up to date scientific data for the correct management of rodents.

What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 880 Norway rats and 880 house mice will be used over the five year period. Most of these animals will be purpose bred for these studies. It may be necessary to conduct some studies on rats and mice trapped from wild populations where resistance is widespread.
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 a rodenticide to be considered efficacious there are many tests that can be done, the first of these is a Blood Clotting Response (BCR) test. This is used to determine if an anticoagulant has an effect on the clotting time. Animals are given a dose of anticoagulant via oral gavage. 24 hours later under terminal anaesthesia a blood sample is taken to measure the blood clotting time. Animals on this procedure experience a mild severity limit. Once an active ingredient is proven to have the desired effect a Standard no choice mortality test can be conducted to determine how the active ingredient will be accepted by the rodent. Animals are given the bait in a no choice feeding test to measure the amount of bait eaten when no other food stuff is present. Animals on this test can experience a severe severity limit due to possible symptoms of toxicity such as laboured breathing, hunched posture, anaemia and swollen joints. Animals that show clinical signs will be humanely dispatched. If a product shows positive signs during a no choice test then it is generally placed in a choice test. Animals are given a choice of poison bait and non-toxic bait. This test can be used to determine palatability and efficacy. For tests of palatability the test ends on a mild severity limit as animals are not allowed to progress onto an observation period where they could develop symptoms. For efficacy tests, a severity limit of severe is required as animals can develop symptoms of toxicity. Once it is clear that a humane endpoint is reached then animals are humanely dispatched.
Retrospective assessment	**Retrospective assessment** Published: 15 March 2024 **What harms were caused to the animals, how severe were those harms and how many animals were affected?**

	30 house mice were tested under protocol 3 which was a standard choice mortality test. This test was listed as severe as animals could experience haemorrhaging, swollen joints, laboured breathing or sudden death. Of the 30 tested only 5 were found dead, 23 were euthanised at a humane endpoint, 19 moderate and 3 severe and 1 did not experience any signs of toxicity. All mice were singly housed so experienced a mild severity limit. 30 Norway rats were tested under protocol 3, a standard choice mortality test. Of the 30 rats tested 5 were found dead and 24 experienced a humane endpoint, 11 moderate, 14 severe, and 1 did not show any signs of toxicity. 10 Norway rats were tested under protocol 1, standard anticoagulant bait choice test, no animals showed any signs of toxicity as they were euthanised before any symptoms were presented so experienced a mild limit due to being singly housed. 3 Norway rats were tested under protocol 5, standard acute mortality test. This was considered a severe protocol due to the fact that animals could experience sudden death, laboured breathing and swollen joints for anticoagulant rodenticides. For non-anticoagulants animals were given a score between 1 and 5 depending on the cumulative score of symptoms. These symptoms could include salivation, incoordination, muscle tremors, self-mutilation and convulsions. Of the 3 rats tested one was euthanised at a moderate endpoint and two at a severe endpoint although no animals were found dead.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	For regulatory studies, there is no alternative to using live animals, as detailed Government guidelines (from the UK, Europe, the US and elsewhere) specify the animal species and group size required for data submission. For the investigation of physiological resistance, the majority of investigations will now be molecular tests conducted on tissue samples, and will not involve regulated procedures. However, there will still be a requirement to conduct tests on live animals, to further validate

	the molecular methodologies.
Retrospective assessment	**Retrospective assessment** Published: 15 March 2024 **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?** Replacement for efficacy testing of rodenticides and DNA analysis is not possible. Testing on live animals is a requirement for regulatory authorities across Europe, in the US and elsewhere when determining if a rodenticide is efficacious and palatable.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Efficient experimental designs will be used to minimise the number of animals used. Where appropriate, advice from a professional statistician will be sought to ensure the number of animals required is minimised. Further, when necessary, pilot studies will be used in the design of a main study, to ensure the best information possible is used in its statistical design, ultimately reducing the number of animals required. For the anticoagulant rodenticides, humane end points have been identified that are implemented routinely in order to reduce unnecessary suffering. Animals showing clinical signs of rodenticide toxicity will be humanely killed. We use in vitro methods to investigate resistance to rodenticides wherever possible. This has resulted in a marked reduction in the number of tests conducted on live animals.
Retrospective assessment	**Retrospective assessment** Published: 15 March 2024 **How did you minimise the number of animals used on your project and is there anything others can learn from your experience?** Where testing of rodenticides was conducted the lowest number of animals that allowed for the highest statistical power were used. This was a maximum of 10 animals per test. When novel rodenticides were being tested only 3

	animals were used as this allowed for any differences between individuals to be shown without subjecting further animals to unknown symptoms. The number of animals used in any test was kept to a minimum.
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 Norway rat and House mouse are two globally important rodent pest species, and are the main target species for rodenticides and rodent control. The requirements to conduct tests which give an indication of mortality and the use of the proposed animal models are specified by the Regulatory Authorities in Europe, the USA, Australia and elsewhere. These studies aim to develop new, humane rodenticides which are shown to be efficacious. As a consequence of this some tests require a measure of mortality. Humane endpoints have been developed over the years so that animals showing clinical signs as a result of the studies will be humanely dispatched. However, some animals die suddenly without showing any clinical signs prior to death. Experienced technicians continually monitor symptoms that develop in the test animals, so that end points can be refined to minimise suffering. For resistance testing new in vitro methods that don't use animals are used for many tests and these minimise the need to use live animals.
Retrospective assessment	**Retrospective assessment** Published: 15 March 2024 **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?** As live animal testing of rodenticides is a regulatory requirement for the commercial sale of new and existing rodenticides it would be difficult to refine these tests. However, the use of the grimace scale when identifying clinical signs of toxicity should be further employed. It was used in our experiments to identify any early signs of toxicity as a method to help monitor animals. An in-depth study to determine the association between clinical signs of toxicity and the grimace scale would be required to use it for determining humane endpoints.

	Despite the difficulties involved, cage experiments could be refined to increase the amount of enrichment particularly for singly housed rats. It was not done for our experiments due to needing to weigh all uneaten food not remaining in the food bowls. To reduce harm to the animals involved in experiments early endpoints were determined to reduce the numbers of animals experiencing symptoms that were considered painful

Project	**37. Evaluation of therapeutic strategies for epilepsy**	
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)	Epilepsy is estimated to affect about 60 million people worldwide with more than half a million patient reported in the UK. Epilepsy is characterized by seizures that can occur as early as infancy and often consists of multiple daily attacks. The treatment options currently available for epilepsy are often unsatisfactory due to suboptimal efficacy, side effects, and frequent pharmaco-resistance. Amongst all epilepsy types, frontal and temporal lobe epilepsy represent a particularly significant unmet need as more than 30% of patients are refractory to all currently available strongly suggesting that better therapies are needed for	

	this disease. The overall aim of the project is to gain a better understanding of the mechanisms regulating seizure generation and pharmacoresistance to identify new therapies for this disease
	Retrospective assessment Published: 10 November 2022 **Is there a plan for this work to continue under another licence?** No **Did the project achieve its aims and if not, why not?** A series of preclinical models were developed, including an *ex vivo* rat brain slice activity model and *in vivo* models of epilepsy to successfully provide a proof of principle for a novel target in the brain to reduce neuronal excitability. These studies provided evidence that modulating this target could have clinical benefit for treating refractory focal epilepsy. The developed models could also have been used to evaluate the ability of novel test compounds for decreasing neuronal brain hyperactivity and reversing seizures *in vivo*. An *ex vivo* rat brain slice activity model and two *in vivo* models of epilepsy were used to evaluate a novel target in a series of studies. The overexpression of this novel target in the brain was proven to reduce neuronal hyperexcitability induced by two different protocols in an *ex vivo* rat brain slice activity model. In an *in vivo* rat model of neuronal hyperexcitability, overexpression of this target in the brain also had a significant effect in decreasing neuronal excitability and protected the animals from developing severe seizures. Furthermore, overexpression of this target also resulted in the significant reduction of recurring spontaneous seizures in a chronic *in vivo* mouse model of focal epilepsy.

	These data provided proof of principle that directly modulating levels of this target in the brain could have clinical benefit in reducing neuronal hyperexcitability and treating refractory focal epilepsy. No further objectives were met since no further work has been conducted under this project licence due to an alteration in research strategy
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 main goal of this project is to gain a better understanding of the mechanisms involved in the generation of seizures and the development of pharmacoresistance. A better understanding of those particular aspect of the pathology will allow the development of therapies with better efficacy and significantly less undesirable side-effects therefore providing real improvement in the quality of life of epilepsy patients.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice and rats (5200 and 5500 respectively). These are the maximum numbers to be used 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?	Mice and rat used in this project are expected to reliably recapitulate the pathophysiology of the human disease. Chronic epilepsy will be induced in rodents and will lead to the spontaneous recurrence of epileptic seizures which will be carefully and regularly monitored in terms of severity and frequency to avoid any long lasting harm. Any side effects are likely to involve body weight loss and excessive seizure severity leading to deterioration in clinical signs. Any animals exhibiting such signs will be removed humanely from the study.
	Retrospective assessment Published: 10 November 2022 **What harms were caused to the animals, how severe were those harms and how many animals were affected?** The chronic model of focal epilepsy in mice is comprised of two phases, the induction phase and the chronic phase. For this model, the principle harms arose during the induction

	phase following the administration of a chemoconvulsant into the brain. These animals developed prolonged seizure activity immediately following the administration of the chemoconvulsant and were monitored closely over the initial 4 hours post-surgery. The development of the initial prolonged seizure activity was an integral part of the model and dictated the development of spontaneous seizures in the animals that occurred three weeks later, during the chronic phase of the model. These spontaneous seizures were a key feature of the disease in this mouse model. During the administration of the chemoconvulsant into the brain, mice were also implanted with cranial screws which in turn were connected to a headmount to enable brain activity recordings and with a cannula to enable the administration of a genetic construct that overexpressed the target of interest (this was administered during the chronic phase). All these procedures were performed under general anaesthesia with the appropriate pain management and using aseptic methods. Animals were also monitored closely both during and following the surgery. From the thirty five mice used in this protocol, nine animals were terminated during the induction phase and one animal was terminated due to excessive seizures during the chronic phase. In addition, a further twenty two rats were used in a rat model of neuronal hyperexcitability. This model involved implanting a stimulating electrode (into the brain) and cranial screws which were in turn connected to a headmount to enable brain activity recordings. Animals were also injected in the brain with a genetic construct that overexpressed the target of interest. All these procedures were performed under general anaesthesia with the appropriate pain management and using aseptic methods. Animals were also monitored closely both during and following the surgery. Once the animals had recovered from the surgery, one of the principal harms with the rat model was the use of low electrical stimulation to the brain to evoke seizure activity via the implanted stimulating electrode. With our experimental protocols, rats received up to 12

	stimulations/day. During the conduct of these rat studies no animals had to be prematurely killed using a Schedule 1 method as a result of this process. To summarise, thirty five mice were used under this protocol and they were all returned as "severe" and a total of twenty two rats were used under this protocol and they were all retuned as "severe".
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The animal models and techniques used on this license are widely used by experts in the field of epilepsy research and have been thoroughly described in the literature and have been shown to recapitulate the electrophysiological and behavioural characteristic of the human disease. The use of these models will prove highly relevant in establishing clear relationships between drug exposure and efficacy, identify and characterise biomarkers and therefore facilitate the selection of the candidate molecules with a high likelihood of becoming successful drugs. Cell and ex vivo assays can give a good indication of the potential ability of a compound to modulate neuronal activity but they cannot reliably predict in vivo efficacy on complex neurological processes such as seizure generations. In vivo models are therefore an absolute necessity to relate in vitro data to efficacy on key symptoms, tested in an intact mammalian system, in order to predict a potential clinical benefit. In addition, the PK/PD relationship, driven by distribution, metabolism and elimination, cannot be accurately modelled in vitro. Finally, proven in vivo efficacy data is a prerequisite of the regulatory bodies that have the authority to approve or reject a new drug application.
	Retrospective assessment Published: 10 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? No suitable opportunities were identified during the duration of this project licence.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Protocols covered by this project licence application are designed to use the minimum number of animals possible. Tolerability studies are performed with small groups of animals in order to establish the maximum tolerated dose and suitability of a dosing regimen prior to larger efficacy studies. Only then, can the larger and more complex *in vivo* efficacy studies commence in the knowledge that the animals are likely to tolerate the compound. Minimum group sizes for efficacy studies will be calculated using power analysis will incorporate any previous experience of qualified personnel and consultation with a statistician. The use of techniques that allow repetitive recordings of neuronal activity will enable longitudinal studies that are more statistically powerful while avoiding unnecessary termination of the animal therefore decreasing the number of animals necessary for each particular study.
	Retrospective assessment Published: 10 November 2022 **How did you minimise the number of animals used on your project and is there anything others can learn from your experience?** The overexpression of the novel target of interest in the brain was initially demonstrated to reduce neuronal hyperexcitability in an *ex vivo* rat brain slice activity model before being evaluated *in vivo.* Repeated longitudinal measures of brain activity were made with both the *in vivo* rat and mouse

	models of hyperexcitability and focal epilepsy respectively until the endpoint was reached. This reduced the overall number of animals that had to be used for these studies.
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.	Both mice and rats will be used in this project. Rats will be used generally to study efficacy and PK/PD relationships and identify biomarkers as they allow for more measures/samples to be taken from a single animal while mice will be used to genetically induce recurring seizures and specifically interrogate relevant mechanistic pathway. Finally, studies in rodents deliver robust, reproducible data and so it is often unnecessary to evaluate efficacy of new compounds in higher species. The project uses techniques that can also be used in patients during clinical trials such as detection of epileptic seizures *via* EEG. These techniques will not only provide efficacy data on the key symptoms of the disease but also provide key information on the mechanism of action of the compound being tested that will be directly translatable to the clinical situation. Work is closely monitored by the NACWO and named veterinarian to ensure maintenance of high standards of animal welfare. Clinical signs are assessed daily and body weight is recorded at least once a week. At the first signs of abnormal behaviour, the frequency of monitoring shall be increased and any change in condition documented. In the unlikely event that an animal experiences an unacceptable degree of discomfort/distress, that animal will be immediately killed by a Schedule 1 method. In addition to tolerability studies, pilot studies may be conducted in a small number of animals in order to refine the parameters and methodology for ensuing studies. These are intended to define the risk/benefit ratio of each procedure to generate statistically significant data whilst causing the least adverse effects.Also acute models which allow better control of the induced seizure severity will be favoured to reduce the number of animal presenting chronic epileptic seizures.
	Retrospective assessment Published: 10 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? In terms of pathology and disease severity, the rat model is a less severe model of disease than the chronic mouse model. Initiation of studies with the chronic mouse model of epilepsy was dictated by the outcome of a study in the rat model of neuronal hyperexcitability. The chronic mouse epilepsy model was only utilised when a statistically significant outcome was achieved using the rat model. Immediately post-surgery, for the chronic mouse epilepsy model, animals were allowed to recover on a heating mat and under inhalational anaesthesia (for at least one hour) to reduce the level of brain activity during the induction phase to aid recovery and reduce mortality. Post-surgery, mice were monitored closely, particularly over the first 24-48 hours. The condition of the animals generally improved overnight. If animals continued to deteriorate (e.g. body weight loss, decline in clinical condition) they would be killed using a Schedule 1 method. Brain activity recording sessions with all animals were made as infrequently as possible to minimise distress to the animals

Project	**38. Fish health and immune function in farmed 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
	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 overarching aim of this project is to improve the basic knowledge of the fish immune system. The fish immune system is impacted at multiple levels, including nutrition, developmental stage and environment, with many unknown factors contributing to positive health outcomes. This is especially relevant for the aquaculture industry during development of new sustainable diets and diets designed for health management. The immune system in fish changes during development. During early life the fish's immune system is immature and only when fish reach a certain size can they be vaccinated. New knowledge of how the	

	immune system develops is of great importance to improve the wellbeing of the fish and improve production of fish as a human food source. **A retrospective assessment of these aims will be due by 22 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 key benefits include: Basic knowledge of the fish immune system and how this enables the fish to respond correctly to infection. This will give new information on the evolution of the immune system and help understand immune responses in higher vertebrates. Understanding disease responses and maintaining healthy stocks is central to aquaculture that provides healthy proteins and oils for human consumption. Globally aquaculture is the fastest growing food production sector and in the UK is worth >£1 billion. To ensure high welfare standards, research into fish health is essential. Part of health management is the use of vaccines for protection against key pathogens. There are still many diseases requiring new vaccines, research is required to design and test newly developed vaccines, and also how the protection can be assessed with minimal numbers of animals. The relationship between nutrition and health is complex, but in all animals it is known that feeds impact health. The nutrition / health relationship is important as more sustainable diets (based on non fish sources) are continually being developed. This research will help explain the health benefits / impacts of different nutritional regimes and design of diets in the future. Finally during development capacity of fish to defend themselves from pathogens varies, especially during early life stages and also in Atlantic salmon when they migrate from freshwater to saltwater. In salmon when the freshwater stage (parr) changes to a salt water tolerant salmon (smolt) many hormones influence the animals' physiology and the immune system is suppressed. This

	project will address how the immune system is regulated at sensitive periods to improve fish welfare and survival following transfer to seawater in wild and farmed fish.
What species and approximate numbers of animals do you expect to use over what period of time?	Atlantic salmon and rainbow trout are the major aquaculture species in the UK and will be used in this research. The numbers used will be the minimum to ensure robust statistical analysis. For example, for a feeding trial a minimum of approximately 30 fish are required per tank to ensure natural behaviour. Triplicate tanks per diet to remove "tank effect", hence a four diets trial would require 360 individuals. It would be estimated that 4-5 feeding trials would be performed per year (up to 1800 individuals per year). During vaccination immune response will be examined at times post vaccine to understand the process of protection. The number of fish for vaccine trials would be up to up to 1000 fish per year. For developmental studies replicate tanks would be kept, with minimum of 30 fish per tank and fish examined for immune response during key stages (500 fish per year).
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?	During feeding trials fish will be monitored for growth and tissue harvested the end of the trial (mild). Diet trials are not anticipated to lead to harm, but fish are monitored for food consumption as taste may vary from control diet. On occasions a short term immune responses will be initiated by substance that activates an immune response, these molecules would be given at natural levels via injection (mild). These are not expected to give adverse reactions in the fish. Vaccine trials may include adjuvant to improve immune stimulation, this may result in local effects (palpable lumps) although these do not cause discomfort they would be monitored to ensure no ulceration occurs (mild). Expose the fish to live pathogens (bacterial and viral pathogens) may allow disease to induce pathology. It is anticipated less than 20% of fish would be used in such studies (severe). During pathogen trials fish will be routinely monitored, fish showing signs of disease or abnormal behaviour will be humanely killed by an approved method with less than 10% of those infected dying, as found from

	experience. At the end of all trials fish will be humanely killed and tissues sampled as needed. **A retrospective assessment of these predicted harms will be due by 22 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	It is necessary to perform this research animals, as the immune system is influenced by diet, stage and environment, hence it is not possible to have non animal models for all experiments. There are few fish immune cell lines and although cell lines can be used many of the experiments proposed cannot be done in vitro. **A retrospective assessment of replacement will be due by 22 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 minimum number of animals will always be used to achieve robust statistical analysis. The number will vary depending on the experiment being performed but experimental design will be in consultation with a departmental statistician, power analysis will be used to determine number of animals. The data gathered will be analysed by parametric and non-parametric statistical tests. One exception is experiments involving exposure to pathogens, which require a more specialised statistical analysis taking into account that each individual contributes to the risk of death. **A retrospective assessment of reduction will be due by by 22 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 work will be carried out using farmed strains of salmon and trout which are used to living in a manmade environment. We have excellent aquarium facilities and highly trained staff at looking after the fish which are checked on a daily basis, to ensure their wellbeing. Automatic alarms warn of any problems with the water parameters, so we can get immediate help/remedies, with contingency plans in place as a precaution. In some cases for example following a feeding trial cells can be taken from fish and used in cell culture to examine immune responses, without needing to infect a whole fish. Such refinements are continually being improved and it is expected during the life of this project more aspects of the fish immune system can be assessed used cell culture methods. **A retrospective assessment of refinement will be due by by 22 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	**39. Function and dysfunction of the basal ganglia and their partner brain circuits**	
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
		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 basic research project is to provide fundamental new knowledge about how parts of the brain called the basal ganglia work in health and disease. Understanding more about how the basal ganglia work is important because these brain circuits control many of our daily behaviours. When nerve cells in the basal ganglia do not work properly, like in Parkinson's disease, behaviour is greatly affected. In this project, we will focus our efforts on achieving four objectives: (1) To map the molecular building blocks and connections of these nerve cells; (2) To define the electrical	

	and chemical signalling of these nerve cells; (3) To clarify how these nerve cells influence behaviour; and (4) To establish how the properties of these nerve cells are altered in models of brain disease. **A retrospective assessment of these aims will be due by 11 August 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 benefits of the project will include a better understanding of how the brain is put together and how the brain works to control behaviour, for better or worse. The project will also deliver new insights into what goes wrong in human brain diseases like Parkinson's, which should in turn be useful for designing new and better treatments for these diseases. The main outputs of this work will include new data (results) that address our four objectives listed above. These outputs will primarily benefit the large community of researchers working to understand the brain in health and disease. Our work could also be used to guide the future use of animals in research, including the design of more refined or relevant animal models of disease. We anticipate that the primary benefits of this work will be achieved within 5 years.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use about 8000 mice and 1800 rats over the 5 years. This number of animals is required because we will have to breed special animals that have had their genes altered.

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 have their genes altered, for example, to copy the genetic changes that are important for some human brain diseases. We do not expect any of the genetic alterations to impact greatly on the animals' wellbeing. Some animals will be aged, because age is a big risk factor in some human brain diseases. Old animals can become unwell, just like old people can become unwell. Some animals will be anaesthetised and have surgery to inject substances into their brains: some substances will mark nerve cells so we that can monitor and manipulate them; other substances will damage cells so that we can model disease processes. Some animals will be anesthetised and have surgery to place or implant devices in their brains, so that we can monitor and manipulate animals' nerve cells and behaviour. Animals that have had surgery are expected to recover quickly and will be given painkillers and post-operative care, just like people recovering in hospital. We will motivate the behaviour of some animals by carefully controlling their access to food or fluids. These animals will be hungry or thirsty for short periods, but we do not expect this to impact greatly on their wellbeing. Some animals will have their heads temporarily fixed in place while they are awake, so that we can monitor and manipulate animals' nerve cells and behaviour. These head-fixed animals might initially experience frustration or stress for short periods, but we do not expect this to impact greatly on their wellbeing. Animals will be killed by a humane method and, typically, tissues taken for analysis after death. **A retrospective assessment of these predicted harms will be due by 11 August 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 working to understand how nerve cells of the basal ganglia work in health and disease, and how they control behaviour. Non-protected animals, like flies and worms, do not have basal ganglia. We will use computer modelling to

	assist our investigations, but computer models cannot properly replicate the complexity of the brain. Cells grown in dishes do not work like cells in the real brain. It is also not possible to gain the deep understanding we need from studies in humans. For all of these reasons, we have to use protected animals to achieve our scientific objectives. **A retrospective assessment of replacement will be due by 11 August 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 use of the minimum numbers of animals by; (1) careful monitoring of animal breeding; (2) using scientific approaches that allow us to obtain the maximum amount of data from each animal; and (3) using good experimental design, including appropriate statistical analyses. **A retrospective assessment of reduction will be due by by 11 August 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 use mice and rats to study the basal ganglia in health and disease because the core features of these brain circuits are similar in all mammals. This means that the new scientific information we generate using mice and rats should be applicable to humans. To help understand what goes wrong in disease (e.g. Parkinson's), we will choose models that best capture particular aspects of the disease. For example, to investigate the influence of Lewy bodies, we will inject animals with proteins that cause the formation of Lewy bodies. To mimic other aspects of disease, for example, the loss of a chemical messenger called dopamine that underlies most of the symptoms of Parkinson's, we will use established genetic models or toxins

	that cause these dopamine-producing nerve cells to malfunction or die. To help understand how the brain controls behaviour, we will monitor and manipulate nerve cells in the brains of animals that are freely moving or that have their heads fixed. Animals will be gradually habituated to head fixation to minimise any frustration or stress. When motivating the behaviour of animals by controlling their access to food or fluids, we will use a gradual (staged) process and give the animals breaks with additional or free access. We will minimise welfare costs to the animals by carrying out this work to the highest standards in animal husbandry, including care during and after surgical and other procedures. Pain relief will be given as needed, according to a regime recommended by a vet. All animals will be used in a timely manner. All animals will be carefully assessed to minimise the impact of potential adverse effects; refinement measures and controls specific to each procedure will be used, as will humane end-points. **A retrospective assessment of refinement will be due by by 11 August 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	**40. Functional improvement post nerve 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)	The objectives are: 1) to develop novel biomaterial (i.e. hydrogels)-based combinational treatment strategies to repair damages following injury to the central or peripheral nervous systems (CNS and PNS), as no effective treatments are currently available to regrow injured nerves in human patients; 2) to develop novel treatments for neuropathic (nerve) pain (NP) following injury to the CNS and PNS as currently there are no effective treatments available. **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)?	The UK has about 50,000 spinal cord injury (SCI) patients and about 1000 new SCI patients per year. There is no cure for SCI. The socioeconomic importance of research in this area is immense. The project will help identify novel regenerative strategies that have huge translational potential. The project will also utilise behavioural testing for pain in rodents that mimics those used for estimating pain intensity and the impact/burden of pain in humans, thus considerably increasing clinical validity and translational potential. The ultimate goal is to help SCI patients improve their movement and sensory functions. The possibility of developing new treatments for SCI permits the quality of life to be improved and reduces a financial burden from the individual and society. NP following SCI has significant impact on daily functioning and rehabilitation, and often leads to depression and in extreme cases, suicide. SCI patients consider eliminating it as important as improving other dysfunctions. However, there is no effective treatment to relieve this pain. By using clinically relevant animal modelling and testing of NP, the project will help identify effective, safe and long-term analgesic compounds for NP after SCI with potential to be translated to the clinic to benefit SCI patients.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use approximately 180 rats per year 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?	Depending on the individual study, operations conducted in animals may result in neurological deficits, for example, motor disability of the hindlimbs. When we need to collect embryos, we will use the most appropriate non-recovery terminal anaesthesia regime to minimise animal suffering during the collection procedure. In some cases, animals may have unilateral or bilateral hindlimb paralysis, sensation loss in the affected hindlimbs, development of NP at injury level and below the injury level, and bladder dysfunction, symptoms typical in SCI patients. However, SCI animals will be given full post-operative support, including the use of soft bedding and manual bladder emptying. In our proposed work related to SCI, about 1/3 of the animals will receive SCI, and the rest would be control animals. In other cases, animals may have paralysis of one hindlimb, loss of sensation and NP development of the affected hindlimb following peripheral nerve injury (PNI). In our proposed work related to PNI, about 1/3 of the animals will receive PNI, and the rest will be control animals. The extent to which the animals will be allowed to develop impairment of movement or other symptoms following the surgery will be clearly defined and, if the clinical signs shown by the animals reach unacceptable levels, they will be killed to prevent unnecessary suffering. At the end of each study, animals will be humanly killed and the tissues analysed for example to assess whether the treatments we test have regenerative effects. **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	CNS/PNS trauma triggers a complex cascade of events, both in the affected organ and in other organs around the body. There is no alternative that would entirely replace the use of living animals for studying the complex response of the injured CNS/PNS. Most literature in neurotrauma research has been produced in injury models in rats and mice, as they have a nervous system, which is similar to humans. However, cell culture systems that mimic certain aspects of SCI and PNI will be used to identify the best combinational strategies for nerve regeneration, and similarly be used to pre-screen novel analgesic agents for NP following neurotrauma, before testing *in vivo*. NP can only be assessed by examining the integrated response of the CNS/PNS to injury, which can only be properly examined in intact organisms that have a nervous system comparable to that of humans. However, we will use alternative *in vitro*(cells) and *ex vivo*(tissue from animals) techniques (histology, gene expression) where possible to limit the use of live animals. We will continually monitor the literature, appropriate site (e.g. NC3Rs) etc for alternatives to using animals. **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	Animal numbers will be minimised by careful experimental design and appropriate statistical analysis to minimise animal use and thus apply the principles of reduction and refinement in animal use. We will use cell culture systems as pre-screen tools for identifying the best nerve regeneration strategies and novel potential analgesic agents, therefore reducing animals used. Anatomical data will be correlated with behavioural measures in the same animal, leading to a reduction in the use of animals. **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.	Rats will be used in preference to other species because they are: • the “standard” neuroscience animal, allowing comparison to data from other neurological diseases. • the evolutionary lowest species to display behaviours, which are considered analogous to those of human pain and for which suitable models are available. The measurement of complex behaviours, such as those associated with NP, are technically easier in rats than mice, and are not aversive and mimic natural behaviours. We will use models which, although complex, we have personal and successful experience of using. These are well established and extensively used in the literature to study regenerative strategies and NP following injury to the CNS/PNS. All surgeries will be carried out aseptically under general anaesthesia. We will apply appropriate and robust peri-operative pain relief and post-operative care. After injury we will closely follow the recovery of the animals. We have accumulated a huge amount of experience of using refined post-operative care procedures to monitoring and improving the lives of the rats, and these include adequate and robust post-operative analgesia, manual emptying bladders, the use of antibiotics to treat urinary infections, the use of appropriate nutritional support to reduce weight loss, and appropriate hydration to compensate for the loss of blood during surgery. **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?

Project	**41. Gene delivery to small animals**	
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)	We are developing gene therapy to treat, or even cure, incurable and often untreatable fatal childhood and adult genetic diseases, including haemophilia A and B (diseases of blood clotting), Gauchers Disease (a disease of liver, spleen, lung, and the brain) and Dravet syndrome (an inherited epilepsy). Gene therapy is a medicine where, instead of delivering a drug, genetic material is injected to give the patient's body the instructions to make a protein that they were born unable to make. We first develop the gene therapy in cells, in a tissue culture dish. Ideally, these cells are obtained in small numbers from patients, made into stem	

	cells, and grown up into huge numbers for us to use. Once we know that our gene therapy medicine can work in human cells we need to verify that it can cure a disease in a complex organism. Mice are perfect for this for several reasons. One reason is that mouse models of many human genetic diseases exist, so when we test our gene therapy on such mice, we have a good indication as to whether it might work in humans. Another reason is that the regulatory authorities insist upon medicines being shown to work in animals before they can be tried in humans; these authorities are increasingly willing to accept data from mouse studies and, for gene therapy, often do not insist upon larger animal models. A second part of our project is to use the tools of gene delivery to reduce the number of animals used in biomedical research. We are able to deliver the gene which allows fireflies to glow, in order to make mice which emit light in proportion to certain signals in their cells. This means that it is possible, without killing the animal or taking blood, to monitor the processes occurring in its cells using a powerful photon-multiplying camera. **A retrospective assessment of these aims will be due by 19 August 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)?	With our preclinical gene therapy research we hope to provide cures for incurable genetic diseases of humans. With our light-emitting gene delivery we hope to reduce the number and improve the welfare of animals used in biomedical research.
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 8000 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?	Many of our protocols cover mice which develop similar genetic diseases as the human diseases they model, but over a much shorter time span. Mice with haemophilia may develop spontaneous bleeds. Mice with genetic thrombosis may develop spontaneous blood clots. Those with thalassemia may develop anaemia. Those with metabolic disease may fail to gain weight and their fur will be sparse. Mice with genetic neurodegenerative disease are, without gene therapy, likely to develop paralysis In the worst case scenarios, they develop rapid onset paralysis. Those with genetic neurotransmitter diseases may become hyperactive, then show signs of Parkinson's disease, such as tremor and slow movement. Those with genetic lipid disease may gain large amounts of weight. These are all in a moderate severity category. Mice with genetic epilepsy are likely to develop spontaneous, unpredictable seizures, from which they may not recover; this protocol is categorised as severe. We have four non-genetic protocols, in which we hope to cure mice using gene therapy. We expect, only rarely, to see clinical symptoms (such as immobility, fur standing on end) in mice undergoing chemical lung disease or induction of sepsis. Those in which we induce preterm labour are likely to give birth 24 or 48 hours earlier than expected (20 days) and pups may be cannibalised more frequently. Mice in which intrauterine fibrosis is induced are unlikely to show clinical symptoms.. Any mouse showing these signs, or any other signs of ill-health, is euthanized and tissues are collected so we can learn more about the disease. **A retrospective assessment of these predicted harms will be due by 19 August 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	Most of the diseases we study affect many tissues and organs, including the brain. We are developing gene therapy medicines to treat or cure these human diseases. We always perform most of our development work in cells

	in tissue culture. However there is a limit to the amount of information these can provide For us to be allowed to proceed to clinical trial, we must demonstrate that we can cure a sufficiently complex animal model of this disease. Mice are the simplest model with this level of complexity **A retrospective assessment of replacement will be due by 19 August 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 performing mouse work, we calculate the minimum number of mice required to be sure that we will get an answer. We perform pilot experiments for any new technology. By controlling other variables (age and strain of animals, environment) we can minimise the experimental variability which allows us to use the minimum number of animals. We share the tissue collected from the same group of animals with several groups of researchers, where possible, to avoid duplication. **A retrospective assessment of reduction will be due by by 19 August 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 have chosen to use mouse models of disease which have the least amount of inter-individual variability. This allows us to identify illness more easily as it tends to follow the same pattern. Therefore, we are able to euthanize the animals before they suffer. For our biosensor work, we have developed a technology whereby we can measure biological processes in freely-moving animals, in a non-invasive way, just by measuring light emission. For other work, where possible, we are using cameras to monitor activity which means that they remain undisturbed. All mice are monitored, by both REDACTED

	staff and by our own team members. For mice where we anticipate disease symptoms, they are monitored daily and weighed as per protocol. Where possible, we genotype mice by collecting a spot of blood, rather than taking an ear biopsy. After surgery, animals are allowed to recover in a warm chamber. Analgesics are used before and after surgery. Viscotears are used to prevent drying of the eyes. Inhalation anaesthesia is used as this minimises the duration of anaesthesia and recovery time. Mice are provided with environmental enrichment, including tubes and wooden blocks. **A retrospective assessment of refinement will be due by by 19 August 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	**42. Gene function in the formation and maintenance of neuronal connections**	
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 understand the molecular mechanisms that regulate the formation, function and maintenance of contacts between nerve cells or neurons called synapses in health and disease. Our experiments will be conducted in mice. Our objectives are: 1) To examine the mechanisms by which naturally occurring proteins—specifically a class of proteins called Wnts—regulate the behaviour of neurons during the formation of neuronal connections. We have demonstrated that Wnts (a name derived from the fly gene Wingless and the mammalian genes Int), which are released by	

	cells, stimulate the formation of synapses between nerve cells in the central and peripheral nervous system in vertebrates. However, the mechanisms are not well understood. 2) To analyse the contribution of proteins or factors, including a class called Wnts, in the maintenance of synapses in normal and diseased conditions in the adult and ageing brain. Here we will test the hypothesis that these naturally-produced proteins protect neurons against the toxic effects of molecules such as Amyloid-ß, a key factor implicated in the pathogenesis of Alzheimer's disease. **A retrospective assessment of these aims will be due by 12 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)?	Our research will elucidate the role of secreted molecules on the function of diverse brain cells (neuron or glia) and will determine the mechanisms by which these signals modulate brain connectivity. The identification of molecules that stimulate the formation and maintenance of neuronal connections (contacts between neurons called synapses) is an emerging approach for the treatment/prevention of neurological diseases including Parkinson's, Alzheimer's and Huntington's diseases where synapses are compromised. Our previous studies have demonstrated that a specific class of secreted proteins, called Wnts, promote the formation of synapses between neurons and also between motor-neurons and muscle cells. We have also showed that Wnt proteins are decreased in neurodegenerative conditions such as Alzheimer's disease (AD). Importantly, we found that boosting Wnt signalling might protect synapses in AD. These findings provide new insights into the cause and possible treatment of neurodegenerative conditions such as AD and Parkinson's disease that leads to the degeneration of neurons. More recently, we found that Amyloid-ß, a toxic protein that contributes to the development of AD, decreases the function of Wnt proteins. Importantly, if we

	promote Wnt function, synapses become protected against Amyloid-ß. These findings suggest that boosting the function of Wnts might protect synapses from degeneration in AD. Our proposed studies will shed new light into the molecular mechanisms that control the maintenance of synapses in the brain and how synapses are lost in AD. Our studies will lead to the identification of potential molecular targets to boost Wnt signalling for the prevention and treatment of neurodegenerative diseases such as AD. Our proposed studies will shed new light into the molecular mechanisms that control the maintenance of synapses in the brain and how synapses are lost in AD. Our studies will lead to the identification of potential molecular targets to boost Wnt signalling for the prevention and treatment of neurodegenerative diseases such as AD.
What species and approximate numbers of animals do you expect to use over what period of time?	We plan to use up to 10,000 mice over a period of 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 our protocols involve procedures of moderate severity, according to the Home Office regulations. Moderate procedures may result in short-term moderate discomfort or long-lasting mild pain. Breeding protocols of genetically altered mice (moderate): Some mutant mice exhibit mild limb deformities that might affect their ability to move freely. Access to food and water will be provided. Maintenance of aged mice protocol (moderate): We might observe adverse effects associated with normal ageing or the development of symptoms associated with AD. Mice that undergo surgery will be quiet and move less for a day or two after surgery. After the procedure, animals will be given pain relief. Animals might lose some weight, but will typically regain their weight within two to three days. If signs of infection are evident, vet consultation may be sought and the animal will be treated. If the animal does not respond to treatment within 24 hours, the animal will be humanely killed. **A retrospective assessment of these predicted harms will be due by 12 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	We are studying the role of secreted factors such as Wnts in the formation of neuronal circuits during development and in the integrity of neuronal circuits in the adult and ageing brain. To date, there is no alternative to the use of animals as a source of nerve cells. Therefore, the use of nerve cells from animals is crucial for our research program. In addition, we will perform experiments in live animals to determine the impact of signalling molecules or compounds on the function of neuronal circuits that affect for example learning and memory. **A retrospective assessment of replacement will be due by 12 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	To ensure the use of a minimum number of animals in our in vivo analyses, we will first perform experiments in isolated nerve cells. Therefore, the data generated from cultured neurons will be used to plan experiments in live animals. This has been taken into consideration when determining the numbers of animals in different protocols. We will use statistical methods to determine the minimum number of animals required to obtain meaningful results Animals will be shared with different members of the research team to maximise their use. Animal use will also be minimised by importing lines of genetically modified animals generated elsewhere. When transgenic animals are not needed, we will reduce the size of the colony to a minimum. If we believe that we will not use a line of animals, we will consider freezing embryos to obtain a colony at a later time. We have dedicated personnel to carry out a good

	and effective breeding program to avoid the generation or maintenance of an unnecessary number of animals. **A retrospective assessment of reduction will be due by by 12 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.	To understand human development and disease we must use closely-related animal models where diverse functions are similar as observed between rodents and humans. Mice are the only mammalian model organism currently available where specific gene mutations that are associated with human diseases can be easily generated. This will allow us to carefully dissect the function of genes in live animals to obtain meaningful answers that relate to the human condition. One of the main objectives of our research program is to elucidate the mechanisms that trigger the loss of nerve connections between nerve cells in the brain. Our studies have led to the discovery that deficits in specific signals that released by nerve cells contribute to deficits in neuronal connections in Alzheimer's disease. We have therefore chosen mouse lines that carrying mutations in these signals or are models of Alzheimer's disease. As some of the mouse models we use are modelling neurodegenerative conditions such as Alzheimer's dieses, they might develop a harmful phenotype. We have taken a number of measures to reduce harm to the animals by carefully categorizing the lines based on their severity. This allows us to implement specific monitoring programs to evaluate distress, pain or other adverse effects. When employing surgical procedures, every care will be made to reduce pain and distress. Anaesthetics will be used during sterile

	procedures and analgesia provided peri- and post-operatively. If adverse effects appear, we will seek advice from the vetNVS. Procedures to reduce harm include regular monitoring of their general appearance (check if animals exhibit ungroomed appearance, loss of coat condition, decreased activity and movement around the cage), weighting monitoring when necessary, keeping animals warm after surgery, provide food pellets on the floor of the cage if they experience difficulties in locomotor activity, the use of good surgical and aseptic techniques, use of analgesia after surgery when necessary. **A retrospective assessment of refinement will be due by by 12 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	**43. Generation of positive antisera to infectious horse 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)		Basic research
	X	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 sufficient antisera positive to Glanders to fulfil the UK statutory testing requirement for the foreseeable future. To generate antisera and cells to validate UK reference laboratories serological tests in light of the diversity in the Equine Infectious Anaemia Virus. **A retrospective assessment of these aims will be due by 23 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	Producing this material will allow the continued trade of horses and other Equidae in the UK and should it enter the	

this project (how science could be advanced or humans or animals could benefit from the project)?	country contribute to its eradication. In our attempts to source Glanders antiserum, to avoid the use of additional animals, we have established there is world shortage. Thus if sufficient material is generated it will be sold to other national authorities who have the same requirement for this testing. The benefits would start as soon as the material is used and would last as long as stocks of the antisera do. The production of the EIAV antisera and immune cells is necessary for validating existing and future diagnostic tests to detect the disease in the face of variation of the virus. The achievement and maintenance of disease freedom from Glanders and EIAV has substantial benefits for the health of all the equidae in the country and in case of Glanders potentially human health as this disease is zoonotic. Benefits also include the international movement of horses and ponies as well as trade, economic and also personal freedom benefits.
What species and approximate numbers of animals do you expect to use over what period of time?	11 horses over a period of up to 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?	This is a moderate severity licence with only two techniques being used. The injection of small amounts of non-infectious material under the skin to generate an antibody response. There may be moderate and painful swellings at the injection site which will improve within a few days. Prophylactic medication is given to avoid these effects. Should they occur, the NVS will be informed immediately and, on their advice, the horse will be observed closely, treated or, in cases of severe reactions/abscessation, euthanased. Small blood samples to monitor antibody response and larger ones to harvest antibodies. Possibly a small amount of bruising around sampling site but this should be minimal due to experience of licencees doing the technique. At the end of the study, the animals will be assessed to determine if they can be re-homed. If they cannot be re-homed, they will be euthanased and the maximum volume of blood and tissues will be taken after death. **A retrospective assessment of these predicted harms will be due by 23 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	It is not yet possible to produce polyclonal antisera in vitro as the complexity of the horse immune system is required, particularly as the reagents are used in the diagnosis of disease in animals and high specificity is required. The use of non-animal alternatives will be reconsidered as the study progresses via exploring new scientific data, engaging with companies developing in vitro methods, consulting 3Rs centres and in-house expertise. **A retrospective assessment of replacement will be due by 23 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	11 horses will be used in total 3 for each of the 3 EIAV antigens. The additional horse is to allow for greater biological variation between individuals on immune response produced. For Glanders, two horses will be used test, again to cope with potential immune response variations but also providing a greater volume of material. Using two or 3 at a time will also give the animals a companion **A retrospective assessment of reduction will be due by by 23 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.	These antisera need to be raised in horses, as they are being used in tests for horse diseases. Raising in other species would lead to reductions in sensitivity and specificity due to genetic differences in their immune systems. The use of dead antigen and adjuvant avoids using infectious agents which would cause disease and is the most refined method known. The use of adjuvant for Glanders antiserum production can be reduced to boosters in case the horses do not

	seroconvert easily (to be determined by small test bleeds around 5 days after vaccination/booster). Prophylactic medication will also be administered at the time of inoculations to minimise swellings and discomfort in the injection site. We have a lot of experience in raising antisera in other species and have techniques to minimise any adverse reactions to injections or blood samples that are needed. **A retrospective assessment of refinement will be due by by 23 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	**44. Gene regulation of cardiovascular 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)	This project aims to identify molecules and mechanisms that play important roles in blood vessel formation and the development of cardiovascular diseases, and to develop approaches such as gene or cell therapy that can effectively target these molecules to achieve a therapeutic effect in human cardiovascular disease. Cardiovascular disease is one of the major causes of death in the developed world and is rapidly increasing in developing countries. However, the mechanisms that cause, or protect against this disease are poorly defined, and there is a continued need for new therapeutic approaches. The work planned under this project licence will lead to the identification of molecules	

	and mechanisms with important roles in heart disease, disease-related angiogenesis and cardiometabolism thereby potentially discover new therapies or therapeutic targets. **A retrospective assessment of these aims will be due by 22 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)?	This work will improve understanding of mechanisms and the key molecules involved that maintain cardiovascular health. VEGF and Neuropilins-linked signalling pathways, which are the focus of this application, are already known to be important for human cardiovascular health and in human diseases such atherosclerosis. Since many of these mechanisms and molecules are conserved between vertebrate species, the work proposed here will have direct relevance for analogous process and disease states in humans. This work will therefore advance knowledge and understanding of important processes underlying human health and disease. Furthermore, by identifying key novel molecules in these processes we will be able to identify novel targets for the development of therapeutic drugs, which may lead to the development of novel therapies for heart disease and vascular disease.
What species and approximate numbers of animals do you expect to use over what period of time?	We anticipate to use no more than 5,000 mice and 10,000 zebrafish over the course of the 5 year 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?	Our protocols are based on well-established procedures that have already gone through a considerable amount of refinement. Most animals will not undergo procedures that will inflict harm. Instead these animals will be used for phenotyping the effects of mutations using minimally invasive imaging or analysis. Some animals will undergo procedures that include minor damage to the lining of a small region of a single artery or ligation of an artery in the mouse that will restrict blood flow to the hindlimb, or in the zebrafish, injury to a small region of the heart or in complete resection of the caudal fin. Based on our experience, adverse effects are anticipated to be very limited in all our protocols and where they do occur to be very brief in duration. Adverse effects that may occur in rodents include lethargy, hunched posture, loss of appetite, weight loss, and in fish, difficulty breathing, abnormal colouration, abnormal swimming, feeding or schooling behaviour. All our protocols, have a severity level of mild or moderate. All animals will be humanely killed at the end of the relevant protocol, and/or when signs of discomfort or pain are manifested. All animals undergoing surgical procedures are expected to recover quickly and will be given appropriate painkillers and post-operative care. At the end of a procedure, animals will be killed by a humane method and tissues taken for analysis after death. All animals will be humanely killed at the end of the relevant protocol, and/or when signs of discomfort or pain are manifested. All animals undergoing surgical procedures are expected to recover quickly and will be given appropriate painkillers and post-operative care. At the end of a procedure, animals will be killed by a humane method and tissues taken for analysis after death. **A retrospective assessment of these predicted harms will be due by 22 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	While cell culture models have been helpful and we continue to use them extensively, there are no computer, tissue or cell culture models that successfully mimic human cardiovascular disease or angiogenesis. Two major reasons for this are: these diseases develop in complex multi-tissue environments in living animals, which cannot be mimicked by non-animal models; they occur over long time periods which make it difficult to perform similar studies in non-animal models. **A retrospective assessment of replacement will be due by 22 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	Where necessary, pilot studies involving small numbers of animals will be performed to establish the proof-of-concept, and only if these small studies are encouraging, will we proceed to larger studies. Since protocols are already well-established in the chosen species, the minimum numbers of animals needed can be determined more accurately, and unnecessary pilot work can be avoided. Studies will be performed only using animal numbers sufficient to produce statistically robust results. **A retrospective assessment of reduction will be due by by 22 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.	Animal species were chosen mainly because protocols were established in those species, avoiding unnecessary pilot work. Small rodents (mice) were chosen, as these are the simplest appropriate mammalian organisms. The choice of mouse and fish is determined by the unique ability to genetically alter these species. Use of zebrafish allows us to perform studies wherever possible in simpler vertebrate organisms. **A retrospective assessment of refinement will be due by by 22 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	**45. Gene Therapies for Neuromuscular Degenerative 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)	The overall objective of this project is to test pre-clinical gene therapy protocols for inherited muscle-wasting diseases prior to clinical trials. **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?	

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 will study different gene delivery systems for use in gene therapies for neuromuscular and cardiovascular diseases to restore or compensate for damaged gene function. This is important because, as yet, there are no restorative therapies for disabling and lethal conditions like Duchenne muscular dystrophy (DMD), which affects 1:3500 boys. The major potential benefits will be the development of (i) efficient gene delivery systems applicable to a number of lethal diseases, (ii) gene replacement and repair strategies for DMD. We believe that this project will progress into clinical trials and lead to the development of therapies for DMD and other muscular diseases.
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 8,800 mice will be used over the course of this 5-year programme of 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?	Animals will only be used in our experiments if they are in excellent health. They will be housed in conditions of very high supervision and welfare. The mice under study here will undergo treatment regimes to correct genetic defects they carry, and occasional surgical procedures. Regular checks will ensure that any rarely expected adverse effects are rapidly discovered and managed. Effective pain relief and anaesthetics will be used rigorously to minimise the severity of the procedures to moderate. The animals will be used under Schedule 1. **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?
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 the research programme is to develop human gene therapies for muscular dystrophy and muscular atrophy. Towards this goal a range of gene therapy reagents will be designed and routes of administration evaluated for effectiveness and safety. Extensive development studies will be performed in vitro on cells. Having

	examined the appropriate websites. There is as yet no alternative available for evaluating physiological responses to gene therapies in the context of cardiovascular and neuromuscular diseases (www.frame-uk.demon.co.uk, www.nc3rs.org.uk). These animal experiments are essential steps in translating new gene therapies into human clinical trials. We have considered the use of animals ethically, but as we are not aware of an alternative way of direct gene expression in vitro that would enable this proposed study to be carried out successfully and in a practical manner, we believe their use to be justified in this instance. **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?
2. Reduction Explain how you will assure the use of minimum numbers of animals	Before embarking on regulated procedures, all gene therapy reagents will be extensively analysed and developed in vitro in cells to ensure that only the most effective test therapies are taken forward to animal experimentation. Group sizes will be predicted from prior knowledge of the scale and variability of measured parameters to ensure adequate statistical power. Expert advice on experimental design, group sizes, statistical power calculations and relevant statistical tests has been sought. **A retrospective assessment of reduction will be due by 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?
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	The primary purpose of the research programme is to develop human gene therapies for muscular dystrophy and muscular atrophy. A range of animal models exists for some of these conditions (mouse, rat, rabbit, dog), however, mice are a widely accepted logistically favoured experimental model for pre-clinical and therapeutic studies. The following clinically-relevant mouse models of

minimise welfare costs (harms) to the animals.	human disease will be used in these studies: **MDX Mouse:** The mdx mouse is a naturally occurring animal model of human DMD. This mouse model is the least severe, most cost-effective and most extensively studied mammalian model of DMD. Mdx mice exhibit no profound behavioural phenotypes, but do exhibit biochemical, histological, mild activity, and muscle electrophysiological changes. The *mdx* mouse serves as a very appropriate animal model for testing a range of gene transfer strategies. **OPMD Mouse:** Oculopharyngeal muscular disease is caused by a mutation a protein involved in RNA metabolism. The OPMD mouse is a model of this disease. Up to the age of 6 months, OPMD mice exhibit no profound behavioural phenotype, but do exhibit some muscle atrophy, reduced body weight and biochemical changes. Beyond 6 months of age the OPMD mouse exhibits progressive muscle atrophy and weakness leading to severe motor dysfunction by 12 months. This is the only animal model available of OPMD. **Myostatin (MSTN)-/- mouse:** The MSTN-/- mouse is a null GAA which expresses no myostatin hormone from skeletal muscle, leading to increased muscle bulk and decreased fat deposition. However, the MSTN-/- mouse does not exhibit any obvious pathophysiology and reduced life-span and provides an excellent model for studying regulation of muscle growth. **FSHD Mouse:** The iDUX4pA mouse is the only model up to date exhibiting remarkable and important similarity to FSHD-associated phenotypes (i.e. disease-relevant low level and stochastic DUX4 expression, muscle wasting, muscle weakness, skin hyperkeratosis, alopecia, high frequency hearing deficit). This model will facilitate investigations into the mechanisms of FSHD as well as pharmacological therapies for the disease. The measures we will take to minimise welfare costs to the animals include implementation of NC3Rs ensuring numbers used are minimized by careful project design and the procedures are carefully refined to ensure severity is as mild as possible. Animals will only be used in our experiments if they are in excellent health. They will be housed in conditions of very high supervision and welfare. They will be supervised by skilled and

	caring technicians on a daily basis and supervised regularly by an independent veterinary surgeon. The animals will be housed communally and in enriched environments to maximise social / welfare/ rehabilitation considerations. **A retrospective assessment of refinement will be due by by 16 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	**46. Genetic mechanisms of metabolism and metabolic 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 project aims to understand the mechanistic basis of genetic risk for type 2 diabetes, obesity and metabolic disease. There are 5 main objectives in this project: 1) identifying and characterising genes underlying loci mapped in human genome wide association studies for type 2 diabetes and associated metabolic disease traits, 2) characterising candidate type 2 diabetes genes identified by other studies in mouse and human, 3) investigating environmental factors that alter the risk of developing diabetes such as obesity and the effect on offspring of maternal overnutrition, 4)	

	testing potential therapeutic compounds selected from targets that we identify in order to generate preclinical data for further therapeutic development and 5) testing implantable cellular devices and adipose tissue transplants as a way to study mechanism in adipose tissue and to alter whole body metabolism as proof of concept for a therapeutic approach to treating obesity.
Retrospective Assessment	**Retrospective assessment** Published: 15 September 2022 **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 original project aims of this project were to understand the mechanistic basis of genetic risk for type 2 diabetes, obesity and metabolic disease. We have particularly focused on adiposity and fat distribution genes alongside studies on islet and other tissue physiology. GWA loci of interest were the WARS2/TBX15 Waist-Hip-Ratio locus, ARL15 gene T2D and adiposity locus, RREB1 gene T2D locus (adipose and beta cell signals) and the PAM gene locus (T2D beta cell function). Functional characterisation of human GWAS candidate genes for type 2 diabetes. 1) In the WARS2/TBX15 WHRadjBMI locus we have carried out further work on both genes. We have shown that one candidate SNP in the WARS2 gene does not affect RNA stability indicating that differential regulation of WARS2 alleles is likely at the transcriptional level pointing towards enhancers in the region (published in BBA gene regulatory mechanisms (2020)). Further we have a manuscript under review at the International Journal of Obesity describing the effect of high- and matched low-fat diets on a hypomorphic allele of Wars2 showing that the diet does not rescue the low adiposity phenotype and further showing that the effect is partially mediated by reduced food intake likely due to elevated GDF-15 hormone.

	2) For the TBX15 gene we have extensively characterised the effects of two coding polymorphisms linked to WHRadjBMI and have data showing body composition phenotypes of interest. Further work is underway by another laboratory to understand the transcriptional network controlled by this gene in adipose tissue. 3) Work on the ARL15 gene has shown that the protein is localised in Golgi and redistributed on adipose differentiation and that this process is dependent on palmitoylation of the protein (Published in Open Biology 2021). Work on a mouse knockout model which shows a homozygous cleft palate phenotype and mild differences in body composition in the heterozygous state is being written up. 4) For the Kcnj11 gene we have examined the common human E23K variant in a mouse model and shown that it hastens diabetes by impairing insulin secretion (Published in Diabetes 20212). 5) Our work on the FTO locus which is associated with BMI in humans has been completed with a paper describing how an intron 1 variant affects a transcriptional programme, relevant whole-body traits and alters adipose metabolism (Published in Science Advances (2021)). 6) We contributed data to a publication describing a regulatory variant in the ADCY5 gene locus that conferred increased pleiotropic risk for hyperglycaemia and altered bone mineral density (Published in Cell Metabolism (2021)). 7) Collaborative work with a US University on the RREB1 gene is being prepared for publication. We show in a mouse model effects on adiposity and adipocyte cell size and translate these findings into humans. We plan to submit this work to Cell Metabolism in the first instance as well as depositing in bioRxiv. Identification of selected novel genes and pathways for diabetes and obesity. Investigation of environmental factors that affect

	adiposity and metabolism. 1) In a diet study we have shown genetic and histological signature in mouse pericardial tissue (Published in Nutrients (202)) and in a further maternal and offspring diet study showed platelet hyperactivation (Published in Sci. Rep. (2021)). 2) In a collaborative study with another laboratory we have developed an American lifestyle-induced obesity syndrome diet in the mouse which recapitulates NAFLD and MASH (Published in Am. J. Physiol. Gastrointest Liver Physiol (2020)). 3) We have described a mouse Pcsk1 mutant model showing obesity and diabetes recapitulating many of the features of mutations in this gene in humans, opening the way for therapeutic studies (Published in Mammalian Genome (2020)). 4) We participated in a study of the Abcc5 gene in collaboration with another laboratory and showed that this gene expressed in enteroendocrine cells have lower fat mass and increased plasma GLP-1 (Published in Obesity (2019). 5) We have facilitated studies of other genes with various collaborators including the role of Brd4 mutation in Nephrocalcinosis (Published in J. Bone Miner Res (1019)), Mylk3 gene in cardiomyopathy (Published in Life Sci Alliance (2020)) and the CRH gene in steroid -induced osteoporosis and bone nanostructure (Published in Bone 2020)). Testing of potential therapeutic compounds against identified targets in the mouse to generate preclinical data. In collaboration with another laboratory we have shown therapeutic effects of a calcilytic in an autosomal dominant hypocalcemia type 1 mouse model (Published in JMBR Plus (2020)). Testing of implantable cellular devices and adipose tissue transplant to modulate metabolism and investigate mechanism.

	Our initial work developing and characterizing 3D spheroid adipose cultures (published in Biofabrication (2019)) has now progressed to using primary mouse derived cells to form organoids. Some final experiments are underway to characterize organoids from mice carrying a heterozygous knockout of the RREB1 gene. A collaborative laboratory will continue working on the project will focus on the analysis of human primary cells in this system and the original plans for cellular devices and transplant. In total 17 peer reviewed publications have been generated from this programme of work with more to follow.
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 short-term benefits of this project are: 1) the mechanistic understanding of selected diabetes risk gene loci of relevance to human disease, 2) insights into the biology of fat and fat distribution and how this affects the risk of developing metabolic disease and, 3) new preclinical data that promotes further research in biotechnology and pharmaceutical companies treatment discovery programmes. The longer term aims are: 1) better understanding of diabetes disease processes in humans leading to new gene and pathway targets for development, 2) identification of new drugs and compounds for therapeutic benefit that can be developed for ultimate patient benefit in terms of reducing morbidity and mortality, 3) developing implantable devices for patient treatment.
What species and approximate numbers of animals do you expect to use over what period of time?	Over 5 years we expect to use approximately 60,000 mice, mainly adults.

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?	designed to collect urine and faeces samples, breath samples, measure activity and food intake, measure energy expenditure. These cages may also alter environmental conditions to give a cold challenge or to maintain individuals at thermoneutrality (the temperature at which energy expenditure is not required to stay warm), the latter being closer to human environmental conditions. A range of imaging techniques may be applied to mice such as ultrasound to image the heart, echoMRI (magnetic field) to measure fat and lean mass, DEXA (x-ray) to additionally measure bone parameters and diagnostic X-Rays. Mice may also undergo behavioural tests which involves specialised caging or equipment which is novel and therefore a potentially stressful environment. A selection of mice may have devices implanted surgically under an appropriate level of anaesthetic. Implantable devices may be used to track the identity of an animal, measure body temperature and blood glucose in real time or may be part of mechanistic studies of adipose tissue aimed at developing therapeutic approaches in the long-term. Mice may also be exposed to altered diets to promote obesity and fatty liver disease as part of specific gene studies. As part of developing new drug treatments mice may be fed or injected with characterised or novel drugs and their affects assessed, drugs may also be used in mechanistic studies to affect specific proteins.

Retrospective Assessment	**Retrospective assessment** Published: 15 September 2022 **What harms were caused to the animals, how severe were those harms and how many animals were affected?** This programme used 8,911 mice, retrospectively assessed at the following severity categories: Subthreshold: 4,540 Mild: 2,624 Moderate: 1,705 Severe: 10 Non-recovery: 32 Notable is the large number of mice with a subthreshold or mild severity. These represent the large breeding effort required in modelling complex genetic disorders. The local AWERB considered these numbers to be appropriate for the significant outputs from this programme. The 10 animals that reached a severe category fall largely into two groups: 1) Mice found dead where the cause being due to the GA could not be ruled out; 2) Mice failing to thrive and breaching the moderate weight-loss limit of 15%. 9 were homozygotes from the same stock in a pilot cohort. The majority of mice having been phenotyped for metabolic conditions will have been subjected to blood collection under a local anaesthetic, tolerance tests requiring an oral or injectable challenge and blood collection, challenged with high fat diet, measurement of body composition using EchoMRI (magnetic field imaging) or subjected to calorimetry measurements in specialist caging. These tests are mild/moderate severity but have been repeated at several time-points in most of the animals recorded in the moderate category.

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Since diabetes and obesity is a condition that involves multiple organs that all communicate with each other we need to use an intact organism for our experiments. The mouse has well conserved physiology, including multiorgan control of glucose homeostasis, with human. Some studies could be carried out in fish or lower organisms such as the fly given the conservation of these fundamental pathways but these organisms lack the appropriate whole-body mammalian physiological context for modelling human disease. However, we use some techniques that use tissues from animals
	rather than a living mouse in order to study particular questions. We are also involved in developing cell culture systems that may better represent the organ systems we are interested in.
Retrospective Assessment	**Retrospective assessment** Published: 15 September 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?** Replacement We considered many in vitro and ex vivo systems during this work. One of the most promising is our work on spheroids. We have established a 3D adipose spheroid culture system which we are developing as an organoid. The preparation and handling of the spheroids is automated allowing high throughput applications. This system will shortly be applied to human cells and could lead to some replacement of animals as tissue level analysis may be possible.

2. Reduction Explain how you will assure the use of minimum numbers of animals	Experiments are planned in detail and where necessary a statistician is consulted in the design and plans for statistical analysis of data. Power calculations are carried out using data from previous experiments to ensure we use the appropriate number of mice in order to obtain robust and reproducible data. Randomisation and blinding are used to reduce confounding factors and statistical tools applied to take account of litter effects as appropriate. We maximise the efficiency of breeding to ensure that excess mice are not generated.
Retrospective Assessment	**Retrospective assessment** Published: 15 September 2022 **How did you minimise the number of animals used on your project and is there anything others can learn from your experience?** Reduction Cohort sizes have all been estimated using power calculations (GraphPad StatMate and Gpower*2) using data from previous studies for each of the tests we regularly carry out and by estimating the biological differences we wish to detect in the experiment. Experimental design was informed, considered and reviewed by a project team and in discussion with a senior statistician. We routinely test both male and females as sex is a significant factor in metabolic traits. Breeding in each colony used was tightly controlled using dynamic breeding systems. Breeding strategies/numbers for each experimental cohort were individually calculated using breeding statistics based on the fecundity and reproductive data from each individual colony. This has proven very valuable in ensuring the correct numbers for experimental significance but avoiding breeding too many mice. Genetically altered lines were cryopreserved when the experimentation was complete to ensure the availability of the lines in the future and a reducing breeding numbers.

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 appropriate physiology, for example in its regulation of blood glucose, and is well characterised and conserved in terms of its genes. Similar tests can be applied and interpreted in the context of human physiology. Its core biology is conserved with some differences in for example patterns of ion-channel expression in beta cells. Mice are subject to welfare assessment that identifies animals that require intervention and limits harms. We have specialised caging that is able to collect multiple types of data at the same time, instead of separately. We are also evaluating in-cage monitoring to track general and feeding behaviour without the need to isolate mice in specialised caging. This allows early identification of problems and generates valuable data whilst minimising welfare costs. In drug treatment protocols we will as appropriate use implantable osmotic minipumps so that a mouse does not have to be repeatedly dosed by for example injection. When surgery is used to implant devices, aseptic technique will minimise the risk of infection. We continue to refine protocols based on experience to minimise welfare cost.

Retrospective Assessment	**Retrospective assessment** Published: 15 September 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?** Refinement We have use isolated islets where appropriate. Isolated islets allow us to investigate insulin secretion dynamics in vitro without the necessity to use a live animal. This has refined some of our testing. We have also employed the use of pilot cohorts for characterising new mutations. This has involved the very controlled breeding of a small number of animals which have been observed very carefully for any welfare issues. These small cohorts have also allowed us to produce some preliminary phenotype assessment can help inform power calculations.

Project	**47. Genetics of Rhythms, Sleep and Behaviour**	
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
		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)	important factors in determining the health of brain, liver and cardiac functions. Challenges to modern life including shift work, travel across time zones and a 24-hour culture can challenge our biological systems and have significant impact on mood, on metabolic function and on the immune system. Sleep disturbances in turn can have a significant impact on cognitive function. Consequently genes important in the regulation of rhythms and sleep have been put forward as critical factors that underlie many of the body's homeostatic mechanisms and have been shown to have a significant impact on psychiatric conditions including mood disorders and affective disorders. **A retrospective assessment of these aims**	

	will be due by 23 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)?	In understanding how genes function in different regions of the brain, we hope to provide some important insights into the genetic and neurological basis of disease. Furthermore, we hope to identify factors that underlie sleep disturbances, particularly where they apply to shift-work, jet-lag and seasonal changes in well-being. Understanding these processes can have a significant impact on quality of life, particularly in an ageing society. Finally, the increased understanding of genes, circuits and mechanisms may be used to identify targets that may be used for therapeutic interventions.
What species and approximate numbers of animals do you expect to use over what period of time?	We use the mouse in all of our studies. Over the course of 5 years, we plan to breed approximately 50,500 mice. However, the majority of these numbers will be used to generate the correct combinations of different gene alterations for further study. Less than 20% of these will be used in further experimental 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?	The majority of work to be carried out in this project will be on mouse mutants with subtle behavioural abnormalities. In cases where abnormalities are more evident, we will strive to modify the testing regime to minimise welfare issues. Because we investigate new genes and mechanisms in mutants, we occasionally see unexpected phenotypes in some animals and this can include seizures and sudden death. The majority of procedures used in this study are mild, non-invasive procedures and consist of moving animals from one apparatus (chamber) to another or on animal observation. Some of tests involve mild, temporary and reversible periods of discomfort such as exposure to bright light, constant darkness, restricted access to food. We keep these periods to an absolute minimum and remove any animals from our study that show signs of discomfort. Restricted access to food may induce anxiety in some animals. In a small

	number of cases we use surgical techniques to inject agents directly into brain regions or implant recording devices and record brain activity over a number of days or weeks. In some cases, injections will enable us to activate transgenes or knock out genes in specific brain cells or regions. These region-restricted effects may also be associated with unexpected phenotypes. The procedure is not expected to result in prolonged pain or discomfort but we will closely monitor animals and, where any is evident, we will remove animals from the study. The level of severity for these studies is expected to be moderate. At the end of these studies, the mice will be humanely killed to collect tissue for pathological studies and this will provide us with valuable information that can be used to study molecular mechanisms that might be responsible for behavioural changes. **A retrospective assessment of these predicted harms will be due by 23 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	We use a number of approaches to study brain function and this includes the use of *in vitro* work and work using cells in a dish. Use of these techniques has lowered the numbers of live animals used in our work. However, the complexity of the brain is such that, in some cases, we need to explore the function of genes in the context of circuits and whole tissues. Although technologies have developed incredibly in the past decade, they are not quite sophisticated enough yet to explore specific functional circuits and cells as they exist in the whole organism. Furthermore, the elaboration of the mammalian brain, particularly in the context of complex behaviours, is such that the use of non-protected animal alternatives in many of these circumstances will not give us the appropriate information.

	A retrospective assessment of replacement will be due by 23 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	For breeding purposes, we pay close attention to the number of animals required for each of our studies and plan the appropriate number of matings in advance. To employ this, we use conventional statistical methodology. We also hold regular meetings with animal facility staff to maintain efficient colony management. In experimental testing we ensure that appropriate numbers are being used using statistical calculations and by comparing planned experiments with studies carried out previously. Wherever possible, we use the same animals in multiple tests with the assurance that there are no additive adverse effects in animal cohorts. Wherever possible, randomisation and blinding are used to reduce factors that lead to bias and increased variability. **A retrospective assessment of reduction will be due by by 23 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 have chosen the mouse for these studies as it is the lowest mammalian species that we can use to examine the full complement of parameters that are measurable in behavioural and physiological changes associated with sleep and cognition. Genetic tools and technologies for mouse work are second to none while our understanding of brain and behaviour in the mouse strains being studied is well developed meaning that our hypotheses regarding genes and behaviour can be tested robustly. Many of the protocols being used in the study have been developed or modified by us with the distinct priority being minimising

	welfare costs to animals. For example, we use non-invasive methods to inform our studies and never progress to using invasive tests until a sound scientific basis has been established using a combination of *in silico* studies, *in vitro* investigations and non-invasive animal testing. Some of our refinements include uninterrupted automated measurement of motor function and cognition in standard caging, non-invasive monitoring of sleep and circadian rhythms in standard caging, remote cage monitoring and use of remote EEG monitoring. **A retrospective assessment of refinement will be due by by 23 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	**48. Helicobacter pylori infection and protection against multiple sclerosis**	
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)	Multiple Sclerosis (MS) is the leading cause of adult disability, and the UK is a high prevalence area, with approximately 100,000 people affected, and an estimated cost of £12,000 to £60,000 per MS patient per year. This represents a considerable amount of human suffering and a significant burden to healthcare, and there is an urgent need for greater understanding of why the disease occurs and who is at risk, as well as the development of new therapeutic agents. We and others have found that MS patients are significantly less likely to be infected with the stomach-colonising bacterium *Helicobacter pylori.* Several studies have also shown reduced disability amongst MS patients who have this chronic infection. Despite the evidence of a protective association, there is very	

	little mechanistic data to explain how this occurs. Under a previous project licence, we were the first to demonstrate that *H. pylori* could protect mice against the experimental autoimmune encephalomyelitis (EAE) mouse model for MS. Our results indicated that the mechanism is via suppression of the autoimmune response and we have identified candidate *H. pylori* proteins with suppressive activity. This project aims to: 1. Determine how *H. pylori* infection inhibits the development of MS and EAE, by characterising its effects the immune response, and the effects of different *H. pylori* mutant strains. 2. Determine whether a previously eradicated infection is protective. 3. Deliver bacterial components and assess their efficacy in suppressing or preventing EAE, and as putative therapeutic agents for MS. 4. Develop and evaluate more refined models for EAE and multiple sclerosis. **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)?	The results of these studies will enable us to: Understand who may be at risk of developing MS in the future. Understand why some MS patients respond differently to certain therapies, and therefore optimize their treatment. Develop new therapeutic agents for MS, based on bacterial components or protective immune mechanisms induced by the infection.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use up to 900 C57BL/6 mice over 5 years. We will conduct approximately 3 experiments per year. The experimental groups, each comprised of 4 groups of 15 mice, will be sub-divided amongst 2-3 blocks in a randomised block design.
In the context of what you	The animals will develop EAE, and therefore

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?	experience increasing degrees of motor paralysis which typically involves the hind limbs and may affect the forelimbs. The likely level of severity is severe. At the end of the experiments the animals will be killed and tissues collected for analysis of the immune response in the spleen and CNS. **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	A large proportion of our studies will involve characterising data from MS patients, and performing experiments *in vitro* to investigate the immunomodulatory activity of *H. pylori* and its components. We will only use animals where it is not possible to gather mechanistic data via other means. In order to study how the gastric mucosal infection influences the development of MS, we require intact gastrointestinal, immune and nervous systems. Such complex interactions cannot be reproduced *in vitro.* **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	Experiments will be carried out using randomised block designs. EAE clinical scores are categorical variables and therefore non-parametric analyses are more appropriate for the statistical analyses. Power calculations have been performed based on previous data. For experiments comparing the differences between mice infected with bacterial mutants, the effects on EAE are likely to be more subtle than observed when comparing infected and uninfected mice. This has been accounted for in the power calculations. The randomised block design will test treatments in batches of 6-10 mice per group, building up to a maximum of 20 per group. This will enable us

	to examine variation between independent experiments (blocks), while keeping group sizes to the lowest numbers required to achieve statistically significant differences. **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.	As per institutional guidelines, we will use refined animal handling techniques (https://www.nc3rs.org.uk/how-to-pick-up-a-mouse). Mice will be used because they are the lowest susceptible species for EAE. EAE in the C57BL/6 mouse is clinically and pathologically similar to MS. A transgenic mouse optic neuritis model is available, where there is no induction of paralytic disease. However, it is not yet clear whether the immunological mechanisms driving demyelination, neurodegeneration and focal brain lesions are the same as in the established EAE model. In a TCR-MOG transgenic mouse EAE model, 4% of mice spontaneously develop EAE, and EAE can also be induced using lower doses of MOG peptide and adjuvant. This could reduce the risk of adverse effects at injection sites, but provide less consistency in EAE. The C57BL/6 mouse model for *H. pylori* is the best-characterised and most widely used. Both models have been used in Nottingham for over 10 years, and thus there have been many opportunities for refinements. 99.5% of *H. pylori*-infected mice in our experiments have experienced mild severity, with just 1 mouse reaching moderate severity in the past 4 years. The number and content of EAE immunisations have been optimised so that disease severity is reduced. To ensure that EAE mice have easier access to food and do not suffer from dehydration, we provide shallow dishes of sloppy mash diet and water bottles with extended nozzles. A highly reproducible clinical EAE scoring system has been developed, involving monitoring and weighing at least daily, and this ensures that mice are killed before they experience severe paralysis. Additionally, we aim to use refined less severe endpoints in mice with EAE following careful monitoring and observation. We also plan to develop

	other mouse strains for EAE, which develop disease without the need for large doses of immunogen and adjuvant. Since the programme of work aims to investigate treatments that reduce disease severity, the proportion of mice experiencing severe disease will be less than 30%. **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?

49. Host and Bacterial interactions in mycobacterial infections

Project duration

5 years 0 months

Project purpose

- Basic research
- Translational or applied research with one of the following aims:
  - Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants.
- Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the aims mentioned in purpose (b)

Key words

tuberculosis, macrophage, granuloma

Retrospective assessment

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

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Contains severe procedures

Objectives and benefits

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

What's the aim of this project?

TB continues to be a major disease throughout the world and more information is required as to how the causative agent, *M. tuberculosis*, develops during infection. In order to address this, we will examine the relevance of mouse and mycobacterial genetics/ proteins on bacterial growth. Genes will be deleted from mycobacteria to assess the impact on growth and also the effect of the absence/presence of mouse proteins/genes will be examined.

A retrospective assessment of these aims will be due by 06 January 2025

The PPL holder will be required to disclose:

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

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

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

M. tuberculosis continues to infect and kill millions of people each year. This is the leading cause of deaths in HIV co-infected individuals and is further complicated by the occurrence of multi- drug resistant and extensively drug resistant strains. There is an urgent need for a new vaccine and new anti-tuberculosis drugs. Animal model studies of tuberculosis can provide substantial advances in these areas. This project aims to further knowledge regarding the effect of mouse (host) and mycobacterial genes/proteins in development of infection. The long term aim is for this knowledge to be used for developing new strategies to combat TB.

Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

Mice, 5000/year

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?

The project will use a mouse aerosol TB infection model to examine the effect of deleting mycobacterial genes involved in bacterial metabolism and/or host (mouse) genes on growth of mycobacteria and development of disease. Genes considered important for mycobacterial growth will be deleted and those found to grow less in culture, when compared to the original strain, will be examined in the mouse model using wild type mice. Other studies will use mice which have had specific genes deleted or inserted to compare these to the control mouse strain. The genes altered will be related to proteins considered important in cells that regulate the response to mycobacteria. These mice do not have any abnormalities due to these changes. In addition, well characterised compounds that can alter the host response to infection will be tested in the mouse model. The gene changes and modulators are most likely to be beneficial to the mouse as these are expected to decrease the amounts of bacteria during infection. Mice exposed to a low dose aerosol infection can survive for more than 1 year without showing signs of discomfort. Some of our studies may run for approximately 6 months.

The majority of mice in these studies will be wild type mice, relatively resistant to TB

infection, undergoing a standard aerosol infection that results in no change in condition of the mice over the duration of the study. Some studies using this model will require mice to be administered modulators. These modulators are not expected to cause increased severity; indeed these may decrease the amounts of bacteria in treated mice. Multiple vaccinations may be required as some compounds may only be active for short periods of time. Throughout the studies mice will be monitored for any signs of physical or behavioural changes and weighed to ensure they are not becoming severely affected by the procedures. Where more sensitive strains of mice are used these will undergo shorter infection programs and routine monitoring to ensure they are not severely affected. There are some instances where we may need to assess more virulent strains of mycobacteria and under these circumstances mice used in these studies may experience more severe symptoms. As soon as there is an indication of this severity, from their physical and behavioural readouts, these mice will be humanely killed. All animals will be humanely killed at the end of a specific set of procedures. Animals exhibiting any unexpected harmful abnormal phenotypes will be killed and advice will be promptly sought from the NVS and local Home Office Inspector.

A retrospective assessment of these predicted harms will be due by 06 January 2025

The PPL holder will be required to disclose:

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

Replacement

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

Most of the exploratory work will be performed in vitro with isolated cells from either mice or human samples (e.g. blood). We will generally be examining compounds that already have been tested by others but not necessarily in TB studies. Initially, the substances of interest that we believe could result in an improved response against TB will be examined using cell culture systems. Information from these studies will guide our mouse infection studies. However, the response by the body to TB infection is very complex and requires various cell types to come together and function at different times during the infection. So ultimately to be able to perform detailed immunology and pathology investigations we need to use animals e.g. the effectiveness of vaccines and antimicrobials cannot be carried out without the use of animals. Also, although compounds that eventually will be tested in the mouse model will already have been screened for suitability using cell culture tests. These compounds may be less effective in mice than in cell culture so we cannot replace the animal tests; where the real conditions of an infection are present.

To achieve the objectives of this project, we propose to use the laboratory mouse as the model organism. This model is the best-characterised model for TB studies with many components of the infection being similar to that of the human infection. Aspects of the mouse model that make this accessible are: techniques for producing genetically modified mice are well established; mice have a relatively short generation time and their biology has been extensively studied. There is also a range of reagents available for mice specifically that make studies in this model much more accessible than other species. To our knowledge, no other species of lesser sentience can fulfil the requirements of this

project to the same extent as the mouse.

A retrospective assessment of replacement will be due by 06 January 2025

The PPL holder will be required to disclose:

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

Reduction

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

Only samples that have undergone prior screens in culture to meet the criteria for progressing to testing in the mouse are used. Colonies are managed to produce enough mice for each study with minimal excess. The numbers of mice used in our studies are based on the PREPARE guidelines where the sample size is defined by performing a power calculation. This calculates the number of mice required to give a significant result - where the result is unlikely to be due to chance. Based on this calculation and the results of the extensive data already generated from our studies we require at least 5 mice per group for each study point. Where possible to reduce numbers, we use the same sample for multiple types of tests and samples may be stored for reassessment or made available for other studies if applicable. Also using new imaging technology to study each infected mouse will ultimately decrease the numbers required for studies.

A retrospective assessment of reduction will be due by by 06 January 2025

The PPL holder will be required to disclose:

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

Refinement

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 the exploratory work will be performed in vitro with isolated cells from either mice or human samples. Mice are the least sentient species available for studying TB with the best set of reagents available for analysing their response to TB. We have chosen the mouse aerosol infection model to study TB disease as this model is the best characterised model for TB infection studies with many features shared with human infection. The mouse immune system is well defined and the technology available for assessing this is highly developed. Additionally, the use of aerosol exposure, to produce an infection, makes this a physiologically relevant model. This route is also less invasive than others in use e.g. intratracheal. We respect maximum volumes indicated for each route of administration and the experiments are only performed by highly trained professionals. The low dose aerosol is well characterised so we are aware of how mice should respond. In these studies, the growth of the organism in the mouse model can lead to disease so body weight, level of activity and general appearance will be closely monitored to minimise

harm. If any alterations are made to the protocol then this is refined to ensure changes do not lead to increased suffering. Initial infection studies can guide us to reduce timescales and numbers for future studies. Certainly, the use of new imaging technology will help us gain more information as to how the disease progresses and will ultimately aid in modifying the system and decreasing the numbers required for studies.

A retrospective assessment of refinement will be due by by 06 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	**50. Identification and characterisation of haematopoietic stem and progenitor cells and their niches**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 Years 0 Months	
	X	Basic research
		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 stem cells reside in the bone marrow of the adult and supply all cells of the blood system throughout life. We aim to gain an understanding of the signalling processes in the embryo that lead to the production of the first and subsequent blood stem cells. We have developed novel methods to grow cells that allow us to study, for the first time, the development of blood stem cells in the lab from their precursors. We now aim to use a variety of approaches to investigate which genes are significant for blood stem cell development, including genes in the surrounding cells that might	

	play a role in blood development. We are also advancing methods for development of blood stem cells from embryonic stem cells in the lab. **A retrospective assessment of these aims will be due by 30 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)?	This research will further our understanding of blood stem cell biology and the blood system, which is of fundamental scientific and medical importance. It aims to gain a detailed understanding of the cell types, developmental mechanisms and genes involved in the formation and regulation of blood stem cells and determine how the mature blood system arises and is regulated. There is an unmet need to supply blood stem cells to patient groups who have limited availability of donors. Therefore, many laboratories around the world are seeking methods to generate these cells in the laboratory. The knowledge and resources generated in this research programme will help in generating methods to produce blood stem cells or mature blood cells, for example from human embryonic stem cells or reprogrammed “stem cells”, which will have therapeutic uses in treating blood disorders or leukaemia. Establishing robust methods for producing and expanding these cells in the laboratory is a major goal in the field, as it could ultimately help address the shortages of mature blood cells and blood stem cells.
What species and approximate numbers of animals do you expect to use over what period of time?	65,000 mice 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?	Experimental material such as cells and tissues are obtained mainly from mice, including those genetically altered, in order to study genes and cell types involved in blood stem cell emergence and their regulation. We expect few adverse effects in the breeding colony, which is the main source of study material. The only reliable method to detect functional blood stem cells is to transplant them and follow their contribution to the recipient’s blood system. The use of mice to

	detect functional blood stem cells is therefore unavoidable. This transplantation procedure has a moderate severity and the recipient animals are carefully monitored and are humanely culled at the end-point, which is typically after 16 weeks, or sooner if they display signs of sickness where recovery is not expected. **A retrospective assessment of these predicted harms will be due by 30 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 development of blood stem cells in mouse models is well characterized and coupled with the power of transgenic technologies, there is no better system to investigate genes important in determining blood stem cells in mammals. The long-term repopulation assay is also the only means of detecting the presence of functional blood stem cells and no alternatives are available. **A retrospective assessment of replacement will be due by 30 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	Animals are bred according to current established guidelines in order to achieve a colony size that is sufficient to meet our research needs and to maximise efficiency. We have considerable experience in blood stem cell transplantation and, coupled with robust statistical methods, we calculate the number of animals needed to ensure that meaningful data sets are obtained. We combine studies where possible, so that controls do not need to be repeated. This ensures that an appropriate number of animals are used. Embryonic material is needed to study the origins of blood stem cells in the body. Improving methods of growing the cells of interest in the laboratory, which is one of the goals of this

	research, will further reduce the amount of embryonic material required for studies. We routinely use currently available methods to grow cells in the laboratory and study them in this way wherever possible, so that fewer animals are used. If our aims of extending knowledge we gain from the embryo studies can be used to inform design of methods to produce blood stem cells from other sources, such as embryonic stem cells or "reprogrammed" stem cells, we will ultimately reduce the requirement for mice. **A retrospective assessment of reduction will be due by by 30 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.	Mice are the most accessible mammals for studies on stem cells due to the ease and isolation of stem cells. We have a state-of-the-art animal facility and all researchers are fully trained in the procedures they perform in their experiments and will follow recommendations given by the animal facility staff to further refine techniques. The research involves creating genetically altered animals to study the role of specific genes in the blood system. This helps in identification of specific genetic mechanisms and cell types involved in blood development. Animals are bred according to current guidelines to minimise numbers. If substances are administered, only approved and appropriate routes and doses are used. Newly published refinements are routinely monitored, tested and implemented wherever possible. In the transplantation experiments, mice are irradiated to reduce their own blood stem cell numbers before test cells are transplanted. The irradiation dose is given in two parts in order to minimize stress and animals are maintained on antibiotics afterwards to prevent infection. Health status is closely monitored throughout. **A retrospective assessment of refinement will be due by by 30 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	**51. Immune Suppression in 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)	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)	Pancreatic cancer does not respond well to standard chemotherapy; less than 5% of patients survive more than five years. New treatments aim to activate the immune system to fight pancreatic cancer. However, these treatments have worked in only a minority of patients. One reason for this limited success is that the immune system in patients with pancreatic cancer does not work properly. This may be due to high levels of stress hormones that dampen the immune system. The causes of high levels of stress hormones in patients with pancreatic cancer are not well understood. However, stress hormones may be increased in response to weight loss, inadequate sleep and surgical procedures. This project will investigate (i) why stress	

	hormones are increased in pancreatic cancer (ii) how stress hormones affect cancer development and (iii) how stress hormones may be reduced to improve immune function and treatment. **A retrospective assessment of these aims will be due by 23 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 project will help us to identify why stress hormones become elevated in patients with pancreatic cancer. This will enable us to improve immune function and treatment for these patients.
What species and approximate numbers of animals do you expect to use over what period of time?	The project will utilise mouse models of pancreatic cancer. We have previously demonstrated that these mouse models are effective in providing high quality data of relevance to human pancreatic cancer. Over a period of 5 years we expect to utilise a maximum of 2000 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?	Mice that develop pancreatic cancers will be used to study how cancer affects the level of stress hormones and the immune system. Mice with pancreatic cancers may develop weight loss, reduced appetite and fluid build up in the abdomen (similar to humans with pancreatic cancer). In all cases, mice will be closely monitored and, if any of these adverse characteristics develop, they will be addressed immediately to minimise any suffering. To investigate the underlying causes of elevated stress hormones, mice may undergo short periods of food restriction (similar to humans with pancreatic cancer who lose their appetite), dietary modification, or changes in their normal day and sleep cycle. To investigate the impact of elevated stress hormones on tumour development and treatment, mice may undergo imaging and low stress blood sampling from a tail vein. To evaluate the impact of stress hormones on the immune system, mice will undergo treatments designed to activate the

	immune system, including vaccination. Treatments to affect stress hormones may be administered, for example as an injection under the skin or as a continuous infusion by implanting a small pellet under the skin. The impact of age and sex on these effects will be evaluated by enrolling both male and female mice, and mice that have been aged naturally up to 24 months (equivalent to a human age of 70 years). At the end of the planned procedures, mice will be killed humanely by competent and trained persons. The expected level of severity of the overall project is moderate. In all cases maximum effort will be undertaken to ensure that this level is adhered to. **A retrospective assessment of these predicted harms will be due by 23 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	Animal studies are required to effectively study the interaction of an individual's immune system, metabolism and cancer. This cannot be reproduced without animal models. However, our animal work will be complemented by research using cultured cells. **A retrospective assessment of replacement will be due by 23 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 will always use the least number of animals required to generate high quality and statistically relevant data. We will maximise the amount of information to be gained from each study by conducting multiple analyses on each sample, when possible. **A retrospective assessment of reduction will**

	be due by by 23 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 will use animal models of pancreatic cancer that most reliably display the same clinical characteristics as patients with cancer. This will help us to conduct precise experiments that provide reproducible data that is relevant to human cancer. Our studies will always aim to adopt protocols that minimise harm to the animals, including low stress techniques and close monitoring of animal wellbeing. **A retrospective assessment of refinement will be due by by 23 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	**52. Immunological interventions against pathogenic bacteria**	
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)	Infections with bacteria are a current and increasing threat to human health. The purpose of this application is to study two infections in particular- tuberculosis (caused by *Mycobacterium tuberculosis)* and melioidosis (caused by *Burkholderia pseudomallei).* Tuberculosis is one of the leading causes of death by infection worldwide, combining with HIV to kill over 1 million people each year. In contrast, melioidosis is a major cause of severe, lethal infection in SE Asia and Northern Australia, with increasing reports of infections in other tropical countries such as India. It can also be wrongly diagnosed as tuberculosis, resulting in patients receiving the wrong antibiotics and not responding to treatment. In both diseases, infection kills if untreated, antibiotics are available but treatment takes 4-6 months to be	

	effective (and often fails) and there is no available vaccine to prevent the majority of human cases. Furthermore, both infections target the lung as a site of exposure and tissue damage and both have the ability to live within cells of our immune system. The objectives of this application are to use models of infection with these bacteria in mice, which are known to mimic many of the clinical features of the human disease, to i) understand more about how our immune systems respond to these organisms and ii) build new therapies which activate the immune system to kill the bacteria, and better vaccines in order to prevent infection.
Retrospective Assessment	**Retrospective assessment** Published: 21 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?** **Describe to what extent the programme of work has been carried out** Work under this PPL was undertaken in three main themes of experimentation: 1) Development of a mycobacterial growth inhibition assay and its use in establishing a mouse model of co-infection with Cytomegalovirus and M. tuberculosis 2) Use of mouse models of infection with M. tuberculosis to evaluate the actions of anti-tuberculosis drugs with potential to also boost host immune defenses. 3) Investigation of new vaccines against Burkholderia pseudomallei, the bacterium which causes human melioidosis. This programme of work was drastically curtailed due to the impact of the COVID-19 pandemic. 992 mice were reported as used in Annual Returns out of a possible total allowance of 8700 (for the duration of the license). All work has now stopped. It

	will not be continued under a further license. Describe if, and to what extent, the objectives of the work have been achieved? Aims and objectives of the PPL: We will study murine models of infection with pathogenic bacteria of importance to human health, in particular M. tuberculosis (MTB) and Burkholderia pseudomallei, in order to: 1. Identify the molecular basis of virulence and the nature of protective immunity. We established a Mycobacteria Growth Inhibition Assay to measure the ability of host immune defences to kill M. tuberculosis. We then used this assay in developing mouse models of coinfection with Cytomegalovirus and M. tuberculosis, in order to better understand the possible biological basis of strong epidemiological evidence linking CMV infection with TB disease in humans. We successfully established a co-infection model, and the growth of M. tuberculosis when animals were also infected with CMV was found to be different to M. tuberculosis infection alone. This implies that coinfection with CMV has the potential to alter the host immune control of M. tuberculosis. Biological samples taken from mice after Schedule 1 killing are now undergoing detailed in vitro investigation for changes in immune cell function and host gene expression changes. Information we will obtain from these samples will tell us how this virus might interfere with the protective immune response which can occur in people infected with M. tuberculosis and whether this might explain why people in tropical countries with high exposure to the virus seem to be more susceptible to tuberculosis. 2. identify and evaluate novel host-directed therapies for improving resistance and treatment. We evaluated high and low dose challenge models of infection with M. tuberculosis in order to study the actions of anti-tuberculosis drugs with the potential to also boost host immunity. Both models were successfully set up and the performance of two drugs was tested against standard laboratory M. tuberculosis strains and also bacteria taken more recently from infected humans (clinical strains). The effectiveness of anti-TB drugs in the chronic (low

	dose) infection and treatment model showed differences between different clinical strains of M. tuberculosis. This makes it important that future drug studies include a range of MTB strains in order to mimic more closely the possible diversity in efficacy that could occur in human populations in different countries. We also started development of a new method to measure how drug treatment alters M. tuberculosis gene expression and metabolism in mouse tissue. After Schedule 1 killing of infected animals we obtained biological samples needed to develop this method, the samples were frozen and are now undergoing in vitro analysis. Information we will obtain from these samples will tell us how existing and new candidate drugs against M. tuberculosis interfere with the bacteria's metabolism and function in the infected tissues. This will help guide other investigators in development of more effective and faster acting antibiotics for treatment of tuberculosis in order to combat drug resistance and to shorten the current 6-month routine course of treatment. 3. identify novel antigenic components of the bacteria and methods to promote their immunogenicity, in order to develop new vaccine candidates. We used our existing models of acute infection with Burkholderia pseudomallei to study the potential protective properties of new vaccine candidates including those derived from fragments of the bacteria cell wall (Outer Membrane Vesicles). No protection was observed in the candidates selected for evaluation. This suggests that these bacteria components or the way in which they were presented to the immune system were not suitable for vaccine development. Other methods of selection will need to be investigated by others before an effective vaccine can be obtained.
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 that will be generated from this study are: • Identification of genes which determine the virulence of M. tuberculosis and B. pseudomallei in vivo. These are a necessary prerequisite for the generation of rationally attenuated live vaccines as well as providing targets for immune intervention. • A better understanding of how the immune response controls these infections. The information obtained here will be directly applicable to other intracellular organisms. • Development of non-specific immune

	stimulating medicines (so called Host Directed Therapy) which can be used alone or together with antibiotics to reduce mortality. This is particularly important in fighting the growing worldwide problem of antibiotic resistant bacteria. • Generation of novel vaccines against tuberculosis and melioidosis. • Further development of non invasive imaging to allowing us to reduce the numbers of animals needed in these experiments in the future.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice, adult , 8700 in total 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?	The animal models to be used are designed to mimic the infections that M. tuberculosis, Burkholderia pseudomallei cause in humans. For many experiments, (such as using bacteria with deletions in key virulence genes, or wild type bacteria given to previously vaccinated mice) infection is likely to cause either no obvious clinical signs or signs of moderate severity, such as minor but not extensive weight loss, transient ruffling of fur or huddling. However, in some experiments, control animals in which wild type bacteria or untreated mice are used, the infection will proceed as it does in humans to extensive weight loss and severe infection with the potential for death. In these cases, mice will be culled when reaching a defined humane endpoint which allows us to assess the true protective efficacy of our interventions with the least amount of distress to the animals. At the termination of each experiment all animals will be humanely killed.
Retrospective Assessment	**Retrospective assessment** Published: 21 November 2023 **What harms were caused to the animals, how severe were those harms and how many animals were affected?** Describe the actual harms that have been caused to the animals (number, species, severity) • CMV-M. tuberculosis co-infection: Adult mice (301 in total) were used: Transient minor weight loss was observed without any other clinical signs of infection in all animals. • TB drug studies: Adult mice were used. Most

	animals (419 of 455 =92.1%) experienced no more than transient discomfort from the injection or drug administration procedures, 32 of 455 (=7%) were observed to have moderate clinical signs of infection with slight weight loss and minor piloerection, while 4 of 455 (0.9%) showed severe clinical signs with more prolonged and extensive weight loss, piloerection and grimace. These mice (4) were humanely culled prior to reaching the approved humane endpoints of the PPL. • B. pseudomallei vaccine studies: Some animals (6 of 221 =2.7%) experienced no more than transient discomfort from the vaccination and infection, 187 of 221 (=84.6%) experienced moderate signs of weight loss and piloerection, while 28 of 221 (=12.7%) showed severe clinical signs with more extensive weight loss and piloerection and were humanely culled prior to reaching the approved humane endpoints of the PPL. In addition, 8 mice were reported in Annual Returns as Schedule 1 and 7 mice were reported in Annual Returns as Non-recovered. Overall, of all mice used under this PPL, 73.2% were classified as Actual Severity in annual returns as Mild, 22% as Moderate and 3.2% as Severe.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The purpose of this research plan is to study the interaction of bacteria with the host immune system in order to identify fundamental mechanisms in host resistance and to develop new treatments and prevention strategies. Where possible, new compounds will be tested first in cell culture for killing of the bacteria, to reduce the total number of animals used. However, such studies do not mimic the complexity of immune responses in vivo including immune cell recruitment, activation and the effect of local tissue specific environments. This requires vaccination/ treatment of live animals, followed by challenge with Mycobacteria or Burkholderia spp. to gain a more predictive estimate of their efficacy in humans in vivo.
Retrospective Assessment	**Retrospective assessment** Published: 21 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? Replacement To the best of our knowledge, no non-animal alternatives became available to address these research questions after the project licence was granted.
2. Reduction Explain how you will assure the use of minimum numbers of animals	All experiments are set up to minimise bias by randomly allocating animals to each treatment group, ensuring that housing and all subsequent treatments are done in random order and that where possible researchers are not aware of the treatment to which individual groups have been allocated. We have access to statisticians to discuss new or refined experimental designs.The number of mice used for these experiments will be the minimum number needed to provide sufficient cells/tissues for assays of immune function and to achieve adequate statistical power. The number of animals will be reduced by our development and usage of non invasive imaging technologies. In all cases, sample sizes will be continuously monitored and adjusted in the light of analysis of the data as it becomes available. In each experiment, it will always be necessary to have a contemporaneous control group. However, analysis of multiple gene knockouts, vaccination strategies, drug doses or other variables will be routinely performed during the same experiment so that control animals can be shared, thereby reducing the number of animals required.
Retrospective Assessment	**Retrospective assessment** Published: 21 November 2023 **How did you minimise the number of animals used on your project and is there anything others can learn from your experience?** Reduction CMV- M. tuberculosis coinfection: • The MGIA is a single assay which measures the combination of many individual host immune responses in order to kill bacteria in vitro. It was developed as a simplified model of in vivo infection of mice with M. tuberculosis. The number of mice

	required for the assay is reduced significantly in the MGIA as one mouse lung can provide up to ten assay inputs, and one mouse spleen can provide up to 30 inputs. When performing in vivo infection, one mouse is equal to one input. • We utilised the ex vivo lung mycobacterial growth inhibition assay (MGIA) in a preliminary mouse experiment to identify an appropriate time frame between mCMV infection and M. tuberculosis infection to model human CMV and M. tuberculosis co-infection. Use of this assay allowed us to reduce the number of mice we needed to set up the model of co-infection and reduce the number of mice we infected with M. tuberculosis for the project. • In the in vivo infection study, the number of mice required within the study was reduced significantly by obtaining multiple tissue from one mouse and, where required, dividing tissue into sections for use across multiple downstream assays. For example, from one mouse we isolated the lung, spleen, salivary glands and lymph nodes. The spleen was divided into two to quantify M. tuberculosis burden and isolate immune cells for flow cytometric analysis. The lung was divided to provide tissue for pathological analysis, quantification of M. tuberculosis and mCMV burden, isolation of immune cells for flow cytometric analysis and RNA extraction. TB drug studies: We used our own previous experience in TB infection rates, published data and power calculations to ensure we were not using more animals than necessary to achieve the aims of the project. B. pseudomallei vaccine studies: We used our own previous experience in B. pseudomallei infection rates, published data and power calculations to ensure we were not using more animals than necessary to achieve the aims of the project.
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)	The models chosen have been selected to cause least overall harm whilst still achieving the objectives. Mice are widely accepted as models for human infectious diseases, and this is particularly true for both MTB and Bps infections. Other larger animals such as hamsters, guinea pigs, rabbits etc can be used to model infection with these bacteria but the immune systems in these species are less amenable to study due to the lack of defined genetic

to the animals.	knockout strains and immune reagents. The key bacteria strains we will use are derived from human cases of disease and the infection routes we will use mimic the natural route of exposure of humans living in endemic areas. Our goal is to mimic the human disease as closely as possible in order to better understand the nature of immunity and also to provide the most realistic setting for evaluation of new drugs and vaccines in the murine models. Use of non invasive imaging throughout the project will reduce the total number of animals used.
Retrospective Assessment	**Retrospective assessment** Published: 21 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?** Refinement Single use 27-guage needles were used for procedures requiring injection, and all animals were weighed and administered the appropriate volume of anaesthesia for their weight. Heat mats were used in the isolators during recovery of the animals from anaesthesia. Where appropriate, disposable, flexible gavage needles were used for all oral gavage procedures as a refinement for use of stainless-steel needles (TB drug studies only). Tunnel/cup handling of mice was also used routinely.

Project	**53. Immunology and immunopathology of malaria**	
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)	Malaria is a disease that kills approximately 400,000 children a year in sub-Saharan Africa alone. We still do not know how the immune response is regulated in a malaria infection, nor the key components involved in protective immunity, immunological memory and immunopathology. Rodent malaria parasites in laboratory mice are very good models to dissect these mechanisms, as they have many of the features of human malaria. The severity of malaria is determined by interactions between molecules of the malaria parasite with the host. Therefore, it is important to identify the parasite molecules causing virulence	

	and the nature of impact they have on the host. *P. falciparum* malaria in children is often present as a co-infection with other pathogens, and also can be associated with a B-cell cancer, Burkitts lymphoma. The exact mechanisms of the interaction of the malaria parasite and co-infecting viruses with B-cells to influence development of lymphoma are not known. We will use the knowledge of the B-cell response to investigate how malaria interacts to induce lymphoma development. The *Plasmodium* components responsible for stimulating host responses are not fully understood. It is thought the large multigene families of the parasite are involved in these processes. We aim to elucidate the role of a large multigene family called pir in our mouse model. This gene family is shared by all species of *Plasmodium*, and thus our rodent model will provide useful information on their role in human malaria. **A retrospective assessment of these aims will be due by 25 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)?	The potential benefits that will derive from this project include a detailed knowledge of the protective and pathological immune responses induced by infection with the malaria parasite, and the conditions necessary to induce long-lasting immunity. Furthermore, we will generate important information on how malaria impacts on the development of a lymphoma, as a model for understanding the relationship between Burkitt's Lymphoma and malaria in children. This knowledge can be harnessed to develop effective vaccines or interventions. Understanding which parasite components are responsible for a virulent infection and how they act in the host gives us information that is transferable to humans, and will allow us to design effective interventions to reduce mortality and severity of malaria in humans.

What species and approximate numbers of animals do you expect to use over what period of time?	Mouse. For the research projects of 12-15 researchers approximately 17,650 mice will be used over 5 years. Approximately 30% of these are in the breeding programme and will not be subject to any 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?	The majority of mice (60-70%) infected with *Plasmodium chabaudi* will not exceed the moderate limit. Most mice recover from moderate symptoms within 48hrs; however, if mice are still displaying a clinical score of 3 or more for 48 hours they will be culled by a Schedule 1 method or exsanguinated under terminal anaesthesia immediately. Clinical scores are determined as follows: (1 point for each of the following signs displayed by a mouse): Marked staring coat, anaemia, hypothermia, hunched posture where the animal adopts normal posture when provoked, subdued behaviour even when provoked, reduced peer interaction, shivering, moderate respiratory signs (altered or noisy respiration, increased respiratory rate at rest). In the minority of experiments where we need to establish the link between morbidity and mortality in order to gain better end point and prognostic criteria, and to uncover the causes of pathology or the mechanism(s) of immunopathology, symptoms induced by the infection may reach severe (and the mice could potentially die). In these cases if mice display a clinical score of 4 or higher for 48 hours they will be culled by a Schedule 1 method or exsanguinated under terminal anaesthesia immediately. Clinical scores are determined as follows: (1 point for each of the following signs displayed by a mouse): marked staring coat, anaemia, hypothermia, persistently hunched posture, lack of appetite, inactivity, unresponsive behaviour to extraneous activity or provocation, clinical signs of suffering like persistently laboured respiration (dyspnoea), or persistent diarrhoea. If a single sign is shown animals will be monitored more closely. Monitoring during this time will be done in conjunction with animal care staff to ensure that the most appropriate action will be taken. At the end of an experiment mice will be killed by a Schedule 1 method or exsanguinated under terminal anaesthesia. **A retrospective assessment of these predicted harms will be due by 25 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	For breeding and maintenance of the vector mosquitoes, females require blood meals for egg production. We have replaced mice as the source of blood with commercially sourced horse blood. It is not possible to culture any stage of rodent parasites *in vitro*. The complex immunological interaction between the malaria parasite and its host cannot be reproduced in cultures or in animal of lower sentience. **A retrospective assessment of replacement will be due by 25 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 will collect as much evidence as possible from current literature, and through the analysis of available human malaria data. This will precede and guide the generation of relevant transgenic mouse models. The breeding of transgenic animals will be reduced through collaborative access to strains. We will avoid overbreeding, and lines under sporadic use will be maintained at low levels, and frozen whenever practicable, and/or maintained in collaboration with other licences to minimise redundant breeding. For each experiment, we will seek advice from the statisticians employed in my laboratory and/or in the Institute. We will design experiments using agreed guidelines (PREPARE) to obtain significant findings with the minimum number of animals. We will perform pilot experiments in which a small number of animals per group are used for comparisons. Depending on the results obtained from pilot studies we will then proceed to perform larger cohort studies to determine if the observed difference is statistically significant. Furthermore, we will use, whenever possible,

	modified bone marrow cells for the reconstitution of the immune system in host animals, which permits the increase of sample measurements together with the reduction of the breeding of transgenic animals. This approach also allows bypassing complex genetic crosses aiming to identify intrinsic versus extrinsic phenotypes. **A retrospective assessment of reduction will be due by by 25 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.	The mouse is one of the model organisms that most closely resembles humans, and many of its genes are functionally conserved. We have extensive knowledge of the mouse immune system and have reagents to define immune responses. In addition, there are many genetically altered mice available that allow us to ask whether defined components of the immune response are necessary for control of the parasite or for inducing pathology. Knowledge of the mechanisms leading to malaria disease and immunity can only be dissected in animal models. We have refined our model to use mosquito transmission as far as is possible, which most closely resembles the human infection. This has substantially reduced the severity of the blood- stage infection, and results in lower parasitaemias, less anaemia, smaller drops in temperature and little to no loss in body weight in most inbred strains of mice. These all fall within the “moderate” range. Therefore, most procedures in this protocol are now designated “moderate”. However, in immunodeficient, genetically altered or mutant mice, and particularly in splenectomised mice, infections are likely to be more virulent. We will monitor as follows to minimise suffering: If animals display a marked staring coat and anaemia plus additional signs of persistently hunched posture, anaemia, shivering, lack of appetite, inactivity, unresponsive behaviour to the environment, or provocation, and/or clinical signs of suffering like persistently laboured respiration, or persistent diarrhoea for a period of 48 hours they will be

	culled using a schedule 1 procedure. If a single sign is shown animals will be monitored more closely. If 4 or more signs are present for 48 hours, animals will be culled by a Schedule 1 method or exsanguinated under terminal anaesthesia immediately. Monitoring during this time will be done in conjunction with animal care staff to ensure that the most appropriate action will be taken. We expect that from this proposed study we will define better predictive markers of malarial disease and virulence, which will be immediately incorporated into our experimental work. **A retrospective assessment of refinement will be due by by 25 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	**54. Impact of early life adversity on brain function and health**	
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 understand the impact of stress experienced by mother's during pregnancy or in early life on the long-term health and well-being of the offspring and the mechanisms involved. We will focus on understanding the changes in the mother and placenta that occur in response to stress and how these effects are relayed to the fetuses. We will also assess the mechanisms in the brain that underpin altered stress responsivity, emotionality, cognition, social behaviours and metabolic function in the offspring of stressed mothers. Furthermore we will investigate whether any of these adverse effects can be prevented or reversed by targeted interventions based on knowledge garnered. **A retrospective assessment of these aims will be due by 19 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)?	Maternal stress exposure during pregnancy or adversity experienced in early life can result in affected individuals being more susceptible to a range of adulthood diseases (e.g. mood disorders such as anxiety and depression and heart disease, type 2 diabetes) and other adverse effects (e.g. impaired learning and memory, social behaviours) which have negative impacts on health and quality of life. This project will advance our scientific understanding of the fundamental mechanisms through which this occurs and reveal approaches through which we can prevent or reverse these effects. In the future this may lead to new approaches to develop drugs or treatment strategies to improve, prevent or reverse the adverse lifelong effects of stress exposure in pregnancy can exert on the offspring.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use approximately 1900 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?	As the primary objective is to investigate the effects of stress exposure in pregnancy/early life, the animal's experience of stress is unavoidable, however the animals will typically be exposed to mild stressors and exposure on any one day will be brief. Stress exposure may cause some weight loss but stress exposure will be stopped if weight reduces by more than 15%. Measures (e.g. handling, familiarisation/acclimatisation) will be taken to exclude any extraneous stress caused by other procedures. Where possible drugs are administered by the least invasive route e.g. in drinking water. To minimise animal suffering, all surgical procedures will be carried out by experienced investigators under deep anaesthesia and sterile conditions (to avoid infection) adhering to best practice and in consultation with experienced veterinarians to minimize post-operative pain. Surgical procedures are necessary

	for various reasons e.g. to insert a catheter (flexible tube) in a vein for blood sampling/drug administration so as to avoid repeated restraint/needle sticks or to insert a cannula (stainless steel tube) into the brain for drug administration or collection of extracellular fluid. On other occasions we need to remove the source of a certain steroid hormones to properly understand how these contribute to a particular phenomenon or behaviour, so we surgical remove the gland chiefly responsible for production of the hormone. Surgery typically takes 15-60 min, depending on the procedure and procedures used here are of moderate severity. Where feasible and where more than 1 surgery is necessary, procedures are combined to avoid anaesthetising animals on multiple occasions. Adverse effects of surgery are very rare but may include pain, infection or sutures (stitches) failure. However, these are mitigated by administration of pain-killers (before and after surgery), sterile operating conditions and adherence to best practice. Moreover, all animals used in experiments are checked regularly by experienced animal technicians, a veterinarian and the personal licensees, thus any adverse effects (which are expected to be rare) will be quickly discovered and be immediately treated. Any animal showing signs of distress, pain or suffering to an extent exceeding moderate severity (e.g. poor coat condition/lack of self-grooming, pale eyes, hunched posture, piloerection, has stopped eating/drinking, >15% weight loss, limited movement/cage investigation) will be humanely killed. All animals will be humanely killed at the end of the procedures and post-mortem tissue will be collected for further analysis. **A retrospective assessment of these predicted harms will be due by 19 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	A major element of this project involves studying the long term effects of stress exposure during development (i.e. during pregnancy or early life) and this cannot be done without living animals. Similarly, where assessing the effects of stress on future behaviours, there is no reasonable alternative but to use living animals. **A retrospective assessment of replacement will**

	be due by 19 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 minimise the number of animals used by making multiple measurements in individual animals where possible (e.g. behavioural assessment combined with blood collection before and after a manipulation), thus maximising data collection and reducing future use of animals. However, the number of animals used for each study needs to be sufficient to make meaningful conclusions from the data collected and for reliable statistical analyses. These numbers are decided based on our previous experience with similar study designs and also on calculations used to determine optimum group size to detect a biologically relevant effect. **A retrospective assessment of reduction will be due by by 19 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.	Both the prenatal stress and early life stress models were developed in rats. Rats display robust and reproducible behaviours and extensive physiological and behavioural data is available for this species. The size of the rat facilitates the investigative techniques needed to address the objectives of the project, some of which are extremely difficult in smaller species e.g. mice. Any refinement that minimises stress is beneficial to the animal and the science, as extraneous stress may influence the results. Thus, for behavioural studies, we familiarise rats with the test arena and the environment is kept constant and quiet, essential for reliable measures. Remote digital recording of behaviour minimises animal disturbance. We continually monitor animal welfare and review whether our next planned step in a study is justified by the preceding data. Surgical procedures are performed with appropriate anaesthetic, analgesic and under sterile conditions, and in our experience

	are tolerated well by the rats. Implanted cannula permit blood sample collection/drug administration and avoids disturbing the rats and the stress and discomfort associated with repeated restraint and venepuncture/venesection. **A retrospective assessment of refinement will be due by by 19 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	**55. In vivo studies of pathways and cells involved in detecting infection, damage 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 fundamental objective is to understand how the immune system "decides" how to react to antigen challenge. The detailed objectives can be summarised as: 1) Which signals and pathways activate dendritic cells (DC) and how are they integrated? 2) Do all signals and pathways lead to the generation of "effector" DC with similar properties? 3) How do different DC subsets develop, what are their properties and	

	do they respond to distinct activation signals? 4) What are the consequences of differential DC activation and DC heterogeneity for adaptive immunity and tolerance? 5) How can DC activation be manipulated to control the adaptive immune system? **A retrospective assessment of these aims will be due by 05 August 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)?	Cell-mediated immunity holds promise in diseases such as cancer, HIV infection and malaria where antibody-based immunotherapies have failed to deliver clinical benefit. Cell-mediated immunity to cancer is, effectively, a T cell response. The success of cancer immunotherapy, therefore, depends on our ability to prime T cells specific for tumour antigens and to steer their differentiation into effector cells capable of tumour destruction. Priming and directing T cell responses is the principal function of dendritic cells (DC), the major class of antigen-presenting cells (APC) in the body. Despite appearing as a basic research programme, this work has the potential to lead to design of better vaccines and immunotherapies for both infectious disease and cancer and to the development of immune deactivation strategies for autoimmune disease.
What species and approximate numbers of animals do you expect to use over what period of time?	Over the course of a five-year study we anticipate that we will require up to 75,000 mice and 50 rats to undertake a project of this scope.
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 vast majority of animals bred and used under this Licence are expected to have a lifetime experience equivalent to that of their wild-type background strains. Genetically altered mice are also expected to develop the same range and incidence of known strain-specific health conditions as wild-type individuals. For example, wild-type C57BL/6 · mice have reported incidences of ophthalmic abnormalities such as microphthalmia of between 4.4%-10% and are prone to hydrocephalus and dermatitis. We expect most

	of the genetically altered strains used in this study to exhibit similar pre-weaning losses and display rates of adult mortality similar those of equivalent wild- type mice. We will monitor continuously for any significant increases in these rates. However, approximately 25% of mice used under this Licence will be those that may present phenotypes with the potential exceed the mild severity classification. These will include such genetically altered mice as those with immunodeficiencies, a predisposition for autoimmunity or for tumour development, and those wild-type mice where such conditions are induced experimentally. Throughout this Project Licence the FELASA and NCRI guidelines, will be used to define severity categories objectively. Any individual mouse will typically undergo only a very limited number of the optional steps available and it is not anticipated that cumulative adverse effects will result from any combination of such steps. However, as it is not possible to fully predict the nature or severity of all potential adverse reactions for all types of mice undergoing novel combinations of procedures there will be careful monitoring for possible side effects. For animals exhibiting any unexpected clinical signs, such as piloerection and an intermittent hunched posture for 24hrs the humane endpoint will be deemed to have been reached and the animal will be culled, otherwise at the end of any protocol all animals will be humanely killed. **A retrospective assessment of these predicted harms will be due by 05 August 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	Research into the cellular and molecular interactions that determine the outcome of antigen challenge requires an intact immune system. Therefore, by definition, such research cannot be carried out in vitro. The development and function of the immune system involves many different cell types interacting in a

	dynamic environment. For example. the progression of an infection within a whole organism involves changes of antigen expression and presentation that evolve with both time and spacial distribution. Similarly, cancer development and spread involves a plethora of interactions between cancer cells and their surrounding cells, governed by multiple signals originating from both their immediate neighbours and from distant tissues. These factors combined with the involvement of multiple host cell-types and the clonal expansion and migration of effector cells mean such research cannot be carried out in tissueculture alone and can only be addressed by the use of animals. The mouse is one of the model organisms that most closely resemble humans. The human and mouse genomes are approximately the same size, and display an identical number of genes, which are functionally conserved. Further, mice have genes not represented in other animal model organisms (e.g. nematode worms and fruit flies) such as those involved in the adaptive immunity. Mice can be genetically altered, there is extensive literature concerning the topics of our investigation, and our own studies can be enhanced by combination with many complementary models developed by others in the field **A retrospective assessment of replacement will be due by 05 August 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 collect as much evidence as possible from current literature, and through the analysis of available data. We will also perform studies in vitro using established cancer cell lines and mouse primary .non-transformed cells. These studies will precede and guide the generation of relevant transgenic mouse models. We will minimise the number of animals by mostly using inbred mouse strains, and by housing them under identical conditions to limit variability. We will avoid overbreeding, and

	lines under sporadic use will be maintained at low levels, and frozen whenever practicable, and/or maintained in collaboration with other licences to minimise redundant breeding. The proposed experimental designs and methods of analysis will be discussed with members of the laboratory, and those of our collaborators, and we will seek additional advice from the statisticians employed by our REDACTED. We will perform pilot experiments for comparing genotypes using small numbers of animals per group. If some effects are worth investigating further we may perform larger cohort studies to determine if the observed difference is statistically significant. The size of the cohort will depend on the observations made from the pilot studies, and will be determined using power calculations. We aim to use the minimum number of mice per group that will be informative. **A retrospective assessment of reduction will be due by by 05 August 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 use of genetically identical mice in the field of immunology not only reduces experimental variability but also eliminates immunological incompatibility when cell transfers are carried out between various knockout, transgenic and wild-type strains of mice. Without such a defined genetic background nearly all of these experiments would be impossible. In addition, we use specific genetically-modified animals to understand the molecular events and steps involved in the immune activation process or as a way to direct immune responses against defined model antigens, thereby making analysis and quantitation of immunological effects easier. The categories of genetically modified mice necessary for achieving the objectives of the project include; i) Strains expressing transgenes which play a role in, or with expression targeted to cells involved in, immune function and regulation. ii) Strains with the absence of, or

	modifications to, genes involved in both the innate and adaptive immune system, examples include pattern recognition receptors, components of signalling pathways, lymphocyte surface markers and receptors. iii) Strains expressing DNA recombinases and/or reporter genes of such recombination. iv) Strains expressing oncogenic transgenes that increase the incidence of spontaneous tumours. v) Strains developing (or with an increased tendency to develop) spontaneous autoimmune or inflammatory conditions due *to* transgenic modification or mutation. vi) Crosses of such strains. Whenever possible we will generate transgenic mice in which mutations are induced specifically at certain times or places where mice should not display a phenotype until the mutation in the candidate gene is induced. Where the immune status of the animals might compromise health, they will be maintained in isolators or IVCs (individually ventilated cages) under barrier environment, to avoid infections. In our experiments we will set clear humane endpoints and will for each and every experiment, as part of good laboratory practice, write an experimental protocol, which will include details of possible adverse effects. These experimental protocols will be provided to all the staff involved in the experiment. In addition, when considering which route of administration of substances to employ, we will strive to use the least invasive route whilst maintaining direct control of dose. The choice of route to administer a substance or cells will be such as to achieve "best practice", i.e. to minimize or avoid adverse effects, reduce the number of animals used, and maximize the quality and applicability of results. For that reason we propose in this project licence a variety of routes of administration of substances and cells to achieve the scientific objectives. Although in the majority of cases we will primarily use standard routes of administration such as intravenous or intraperitoneal injections, the active concentration, volume, stability, and toxicity of a particular substance or cells may require administration through a non-standard route such as injection adjacent to or directly into a tumour. For all procedures coded (AB) or (AC), general or local anaesthesia as appropriate will be induced

	and maintained using agents and routes of administration suitable for the species and the nature and duration of the procedure. **A retrospective assessment of refinement will be due by by 05 August 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	**56. Increasing our understanding of the pathogenesis of 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 common clinical syndrome associated with high rates of morbidity and mortality. Risk factors include aging, ischaemic heart disease, high blood pressure, metabolic syndrome, diabetes, obesity and kidney disease, but the mechanisms leading to impaired cardiac performance are not fully understood. The over-arching aim of our studies is to broadly investigate the role of modifications of specific proteins by reactive molecules (known as oxidants) that can form in cells and contribute to the development of heart failure. We anticipate that the enhanced understanding of heart failure	

	that our studies should bring, will potentially allow new targets for drug development to be identified. **A retrospective assessment of these aims will be due by 22 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)?	Although heart failure is increasing in prevalence, there is not only a lack of effective therapies, but there also a significant deficit in our understanding of the mechanisms by which it develops. Our studies are aimed at defining the role of specific modifications of proteins in the development of heart failure. By undertaking such work, we enhance the likelihood of identifying novel drug targets which may ultimately result in new therapies to combat heart failure.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to generate through breeding about 7500 mice suitable for our experimental studies, which relate to heart failure. Approximately 1000 rats over 5 years will be used during our heart studies on heart failure.
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?	Animals will be used in the context of providing clinically-relevant models of heart failure that are of direct relevance to the human condition. When breeding genetically modified mice for use in these studies, we do not anticipate any significant adverse impact on their well-being, as past work with related mice did not identify any such issues. Some experiments will involve natural aging or obesity with assessment of cardiac function by heart ultrasound (a mild procedure), followed by humane culling at the end of the investigation. Some experiments involve surgical procedures such as removal of a kidney or placing a ligature around the major blood vessel that comes out of the heart (the aorta), which are severe procedures as they can induce heart failure. However, these mice will always be closely monitored for undue stress, which if detected will result in humane culling. Ultimately, at the end of all the experimental protocols, the animals will be humanely euthanised and their tissues collected

	for comprehensive analyses to maximise the information we collect from using them. **A retrospective assessment of these predicted harms will be due by 22 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	Unfortunately, cell models of hypertension, heart failure and sepsis are limited in their ability to provide information that is useful in terms of translational therapies. Although cell and other experimental models can provide crucially important information, and indeed we plan to use such complementary models as part of these proposed studies (which will help limit animal use), it is critically important to substantiate any findings in the setting of a more relevant model to the human disease condition. Small animals, especially mice, are widely used in the study of hypertension, sepsis or heart failure and we aim to use these validated approaches - which we also have a lot of experience of using over many years. Studying heart failure, especially the latter stages, is very difficult in test tubes or isolated cell systems – whereas the animal models we use are well-accepted, used worldwide and provide hugely valuable information relevant to humans. It is commonly accepted by biomedical experts that animal models are therefore required to fully understand the pathogenesis of complex human diseases, as well as to test novel potential therapeutic strategies that research such as ours may unveil. The mouse models we plan to use are undoubtedly directly relevant to the human heart failure condition, a syndrome that accounts for a high level of deaths and for which there is currently significant unmet clinical need in terms of effective, life-saving therapies. **A retrospective assessment of replacement will be due by 22 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 have over 20 of years of experience in animal research, having received significant training relating to *in vivo* physiological and pharmacological methodologies involving long-term care of rodents in various disparate models of cardiovascular disease. This will serve us well when performing the research outlined here, including in the context of minimizing use of animals without compromising our ability to deliver robust outcomes. Our studies with animals are only initiated following comprehensive *in vitro* and *ex vivo* investigations that have robustly implicated specific proteins targets as likely to be important in the pathogenesis of heart failure. These comprehensive initial studies avoid unnecessary investigations of targets proteins or pathways in mice that have only been weakly implicated in the progression of heart failure, therefore avoiding the use of animals as a screening tool or first line of enquiry. Even when we utilise animals, our studies are almost always complemented with investigations using an array of ancillary approaches involving purified proteins, cell lines or cells and tissues isolated from humans or the animals being investigated. So, whilst it is not possible to carry out our work without using animals, whenever possible we try to avoid or minimise using live animals. The data obtained from tissues collected during terminal procedures increases data yield and so maximises our understanding from each subject, with samples being collected without any additional stress to the animals. These comprehensive analyses of tissues from animals at the end of experiments not only increases the volume of data but can also provide information that often leads to a reduction of animals in subsequent studies. The number of animals used will also be reduced by banking and sharing the samples collected between specialist investigators. Thus, again data and scientific understanding is maximised whilst numbers are minimised as far as practicable. Dynamic blood pressure measurement and repeated non-invasive imaging enables longitudinal studies, and in some cases animals to serve as their own controls, which again reduces the number of animals used. The use of statistical power analysis, which is

	typically performed using assumptions based on our previous studies with the same experimental approaches, allows us to estimate with some confidence the minimum number of animals likely required to obtain robust endpoints without compromising our studies by under-powering them as a result of insufficient experimental group sizes. **A retrospective assessment of reduction will be due by by 22 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.	Most of our studies will be carried out using mice, which are the least sentient small mammal model commonly available. They are relatively easy to maintain and cost effective with a short inter-generation time. Data can therefore be obtained in a timelier manner than if a larger species were used. Well-developed and clinically-relevant mouse models of heart failure have been developed and are routinely used in many laboratories, including ours, worldwide. The use of transgenic animals can significantly aid studies of precise pathways, for example by knocking-in or -out specific genes. This allows the impact of a particular protein to be examined more specifically than can be typically achieved if pharmacological tools alone are used. Bespoke transgenics have and will be specifically generated to aid us in our goal of better understanding the pathogenesis of heart failure, representing a refinement of our overall research strategy involving animals. Over the past decades we have continually refined our in vivo techniques and protocols, improving our ability to collect high quality, novel data whilst compromising animal welfare as little as possible. We have refined our study approach by employing several, complementary clinically-relevant methods to assess responses to treatment interventions. Thus, we are able to obtain much broader and detailed insight, and so confidence in any conclusions we arrive at. Further refinement results from use of longitudinal, non-invasive measures of cardiac function, which allow robust outcomes to be identified with fewer animals than single endpoint

	approaches. Longitudinal physiological in vivo experiments will commonly be followed by end-terminal procedures, as well as complementary ex vivo or in vitro analyses of harvested tissues. This approach maximises the data yield from each experimental subject, without causing any additional distress to the animals. Specific actions will be taken to reduce pain and suffering of the animals. We have substantial hands-on experience of the surgical techniques to be used, and they will be performed by experienced licensees following good laboratory practice. They will also implement best practices of peri-operative care and consult regularly with veterinary surgeons and experienced technicians to minimize any discomfort or distress. Animals will be inspected daily for any signs of distress, and subjects with significant adverse effects, or whose general health deteriorates will be humanely killed. **A retrospective assessment of refinement will be due by by 22 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	**57. Investigating bone marrow derived stem cell therapies for neurological 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
		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)	Neurological diseases such as multiple sclerosis and the hereditary ataxias, which result in nerve cell damage and loss, represent a major cause of disability. They produce a vast range of symptoms and the functional limitations they pose provide daily challenges to both patients and their families. These conditions thereby impose a considerable social and economic burden on society. Despite increased understandings of the underlying cause, most have no cures, nearly all worsen with time and are either fatal or result in a shorten life expectancy. Current available treatments may slow the progression of nerve cell damage but lack the ability to	

	bring about effective nerve cell repair. Adult stem cell populations display a wide-range of biological properties that may be used to provide an effective treatment for neurological diseases. The aim of the work conducted under this licence is therefore to develop stem cell therapies for people with these conditions. **A retrospective assessment of these aims will be due by 25 August 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 knowledge gained from this research will be applicable across a wide range of neurological diseases, therefore has the potential to benefiting patients suffering from a wide range of clinical conditions. The knowledge gained through these studies will also be of direct value to the wide research community struggling to find cures for degenerative conditions. This project is expected to provide a greater insight into the processes involved in tissue repair following injury. Specifically, this project will help advance knowledge of the molecular and cellular processes that occur in response to nerve cell injury. It will also give insights into the protective and regenerative mechanisms of adult stem cell populations and how these processes may be manipulated to improve nerve cell repair and the functional recovery of patients with neurological disease. In the longer-term, data obtained from work undertaken on this project are likely to play a critical role in developing new reparative drug and cell-based therapies for patients with nerve cell injury. This will include many patients with neurological diseases, for which no treatments are presently available.
What species and approximate numbers of animals do you expect to use over what period of time?	This project will use mice and rats. A large proportion of our experimental work is confined to cell culture models and human tissue specimens. Animals will only be used when no other methods are available to address the research questions being posed. We anticipate using approximately 1300 mice and 100 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?	A large proportion of the animals used in this study will experience no suffering or pain and will only be used to provide tissue for either tissue culture or cell transfer studies. The study will utilize several animal models of neurological diseases. Animals in these studies are expected to develop signs such as poor balance or reduced coordination. In all cases studies will be designed to minimise suffering and clear end points will be used to ensure that such studies are terminated at the earliest point to prevent animals suffering unnecessarily. Most animals used will fall with the severity bands of either sub threshold or mild. A small proportion will undergo small surgical procedures under general anaesthesia. These animals will be provided with post-operative pain control and have an expected severity level of moderate. A small proportion of animals will be used in procedures with a severe severity level, however endpoints will be set to ensure that any animal is killed before reaching this level. At the end of the study, all animals will be killed humanely, and tissues collected for analysis. **A retrospective assessment of these predicted harms will be due by 25 August 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	Extensive experimental work relating to this project, using both cell culture models and human tissue specimens, has been performed to determine proof-of-concept of our scientific approach. A vital preliminary stage in the development of treatments for neurological diseases is to define treatment responses in animal models prior to clinical trials. Our studies are now at a critical stage. We need further validation in living animals to fully elucidate the biological mechanisms, safety and efficacy of our approaches in the whole body, where the complex environment of the adult nervous system is present (in addition to other organs), and where functional recovery of the injured nervous system can be measured. **A retrospective assessment of replacement will be due by 25 August 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 studies are designed with the assistance of a qualified statistician who will regularly review the power calculations used and update them in accordance with data obtained. This ensures that animal numbers are minimised, and that correct analysis is carried out to give the best possible interpretation of the results. Extensive training and supervision of research staff ensure that procedures are carried out with minimal animal suffering and performed in a reproducible way that minimises variation. Tissue samples obtained from a single animal are analysed using several different methods to answer multiple objectives, thereby minimising the number of animals required for the project. All tissues samples are stored in an appropriate manner
	that preserves them for use in future projects and by other research groups. All strategies for the breeding of transgenic mice will be planned to minimise the numbers of animals bred. **A retrospective assessment of reduction will be due by by 25 August 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 be using mice and rats, which have a similar genetic makeup, neuroanatomy and physiology to humans. The use of rodents also allows us to perform behavioural tests to assess neurological function, something that cannot be done using models based on less sentient animals. Mice are the most refined mammalian species in the context of genome manipulation and transgenic modelling. We have chosen the most appropriate animal models of human neurological disorders to successfully accomplish our project objectives. All models used have been validated in previous studies and we believe are the least severe models to investigate our approaches to discover new therapies for patients with neurological disease. High animal welfare standards are consistently maintained within our animal facilities. All work is conducted by trained and experienced staff. Close monitoring will be in place for all animals under our studies. Where any animal show signs of poor health or distress we seek advice from the veterinary surgeon to further minimise any suffering. All surgical procedures are performed under aseptic conditions and appropriate general anaesthesia. This is followed by appropriate peri-operative analgesia and care. **A retrospective assessment of refinement will be due by by 25 August 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	**58. Joint damage and regeneration**	
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 objective of this project is to identify cells and molecules that can limit the destruction or induce the healing of joint tissues (bone, cartilage, menisci, ligaments) within the joints when they have been injured due to arthritis or following trauma. **A retrospective assessment of these aims will be due by 31 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 surface of the bones, at a joint, are covered with an elastic, resistant, and very lubricated tissue called cartilage, which guarantees the frictionless motion of our joints. Cartilage has a limited capacity for repair, and when damaged by arthritis or trauma, often fails to regenerate and this results in irreversible disability because of permanent pain, reduced mobility, and joint swelling. In fact, for this reason, arthritis is the most common cause of disability allowance in the UK and the most frequent cause of disability worldwide. Our long term research in this field has already yielded a potent cell-based product, now available worldwide, to repair relatively small, isolated cartilage defects, and we are now aiming to make these technologies more potent, safer, more affordable, and especially suitable to treat also large and diffuse defects such as those resulting from arthritis. Every success in this field leads to patients regaining their independence, ability to work, be mobile, and to look after themselves and their families.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mostly mice, but for specific experiments we may also use rats. We expect to use approximately 5,000 animals per year.
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?	Using methods in cells in culture we identify cells and molecules that, based on tests performed in the laboratory in test tube, appear to have properties that support the healing of joint tissues. The most promising of these cells and molecules are then tested in animals in models of arthritis or of tissue formation. Some of these molecules also improve pain perception and therefore we also check whether this is just due to the improved integrity of the joints or because they also act on the brain directly. Most protocols are mild to moderate in severity and the animals in general only experience from mild to moderate joint pain. In only one protocol, which we expect to use very rarely, it is expected to observe severe arthritis and the animals are expected to develop joint pain, swelling, and, in rare cases, even ulcerations. At the end of the protocols the animals are killed and their joints are analysed under the microscope and biochemically to confirm whether the treatment had any effects. Pain will also be measured. **A retrospective assessment of these predicted harms will be due by 31 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	We have a wide array of tests that we can perform in the laboratory to test if cells and molecules can form cartilage. These tests efficiently replace most experiments in animals. These experiments drastically reduce the number of conditions and molecules that require animal experimentation. Although these tests help reduce the number of animal experiments, they cannot replace them because repair mechanisms rely on mechanisms that are not limited to the injured tissue itself. For instance, cartilage repair relies on stem cells that reside in bone, the synovial membrane and in blood vessels and from signals that come from nerves and other tissues. This complex interaction cannot be simulated in a test tube and its complexity is still incompletely understood. Hence the need to study it. **A retrospective assessment of replacement will be due by 31 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 have carefully optimized our animal models so that the minimum number of animals is needed to measure differences and treatments. Therefore we will avoid situations in which the effects are going to be too small or too large to measure any differences effectively, and which therefore would otherwise require high numbers of experimental animals. We will perform careful power analysis to adequately power our experiments hence avoiding the need to repeat them. The number of mice to be breed is 10.000 in the mild protocol. That is because the vast majority of these mice have genetic modifications that need a lot of crossing of different strains in order

	to combine them in the right proportion. These mice experience virtually no suffering at all as long as they are in the breeding scheme because the genetic modifications manifest themselves only after we administer an activating drug which we do only when we experiment on them, or because they have one copy of the normal gene or because the effect of the genetic modification is subtle and can only be detected when we put the animals in disease models. We have reserved to breed 5.000 mice with a moderate protocol in those cases in which even in the breeding scheme they display some suffering. In most cases this can only be ascertained once the genetic modifications have been made. In addition, longitudinal monitoring (test the same mice for pain and imaging at different time points) will limit the need to have different batches of mice to be killed at different time points of interest. **A retrospective assessment of reduction will be due by by 31 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.	Different strains of mice develop diseases with different degrees of severity. Therefore, models of disease that are perfect for a certain strain of mice may be too severe or ineffective in others. Since in our laboratory we have expertise with multiple different models that vary in severity, we will be able to match the appropriate model to each strain so to avoid the excessive suffering of a severe model in a susceptible strain. **A retrospective assessment of refinement will be due by by 31 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	**59. Local iron homeostasis in 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 aim of the project is to understand why and how iron deficiency affects the function of the heart, lung, muscle, kidney and placenta. **A retrospective assessment of these aims will be due by 02 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)?	Iron deficiency is the most common nutritional disorder in the world and increases mortality when concomitant with heart or kidney disease. It also reduces exercise capacity and affects fetal growth. Despite this, there are no clear guidelines on the clinical management of iron deficiency in patients, because of a lack of understanding of the link between iron deficiency and disease. Our work is helping grow that understanding, and further provide the basis for the development of new iron-targeting therapies in these disease conditions
What species and approximate numbers of animals do you expect to use over what period of time?	Over a period of 5 years, we will use a maximum of: 8000 mice 1000 rats
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 animals will undergo an intervention to alter their nutritional status or are exposed to a form of challenge in the form of hypoxia (low oxygen concentration). Alteration of nutritional status may be associated with mild to moderate weight loss and, if carried out in younger animals, may slow growth. Manipulation of iron status in pregnant females may be associated with a mild to moderate degree of anaemia and reduced weight gain during pregnancy (of pregnant dams). Prolonged hypoxia exposure is designed to produce the same pathological changes that are seen in humans with cardiovascular disease, and as such this intervention will cause a mild to moderate disease phenotype, which is dependent on the genetic strain used. After dietary modification and hypoxia exposure, animals will undergo a battery of minimally invasive tests to measure heart function (MRI/MRS imaging under anaesthesia), lung function (plethysmography), and skeletal muscle function (treadmill exercise). These tests per se are not associated with any major adverse effects. However, anaesthesia and implantation of a telemetry device (to monitor vital parameters remotely) may be associated with risk of mild to moderate weight loss and infection respectively. A small number of animals (less than 1% of the total animals on this licence)will also be given an acute form of heart disease (myocardial infarction i.e heart attack through surgical means, or acute kidney injury through surgical or through drugs). All animals will be killed at the end of the protocol, either by

	schedule 1 methods or under general anaesthesia for removal of organs. **A retrospective assessment of these predicted harms will be due by 02 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 questions cannot be addressed in cellular systems or in non-mammals because they require intact physiological effects and depend on the complex pathways that exist in tissues and that depend on many cell types. Whenever possible, non-animal alternatives will be used, such as in-vitro cell expreiments. Furthermore, experiments aimed at identifying the disease mechanism will first use cellular and in-silico studies to narrow down the number of candidate pathways that can later be tested in animals. Searches of genome-wide association studies (GWAS) will also be conducted. The project will also adopt any new techniques that enable th scientific questions to be addressed without the use of animals, e.g, organ-on-a chip technology. **A retrospective assessment of replacement will be due by 02 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 to be used are based on statistical power calculations. Most of our readouts use non-invasive techniques. These will be used to enable multiple measurements on the same animal in longitudinal studies, which will reduced the number of animals required to achieve statistical significance. **A retrospective assessment of reduction will be due by by 02 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.	Mice and rats were selected because their bodies' responses to iron and hypoxia mimic closely those seen in humans. These models are the most suitable for addressing the key questions set out in our objectives (i.e dissecting the role of specific pathways at the molecular level), because these models are amenable to genetic manipulation which allows mechanistic studies by using genetically-modified models. To minimise welfare costs to the animals, we have refine our protocols as follows: Treadmill exercise protocol: a nitrile glove and a dark background are used to encourage the rodents to run, instead of previously used electric shock. We will use the "fatigue zone" test to avoid exhaustion, so that any animal spending 5 continous seconds in the fatigue zone is removed from the treadmill and allowed to recover for at least 24 hours. Also, in the hypoxia exposure step; most experiments will include an adaptation period where O2 levels are dropped gradually to allow the animal to adapt to the lower O2 levels. Surgery: General and local anaesthesia, accompanied by appropriate analgesics will be used to minimise pain and suffering. Where new lines of genetically altered animals are used, they will first be bred under a moderate protocol so that their phenotypes is observed closely, and any unexpected harmful effects detected quickly so that the animal is culled humanely. The breeding of such animals will only be transferred to the mild protocol, if they have a mild phenotype. **A retrospective assessment of refinement will be due by by 02 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	**60. Mechanisms of Bone Growth and Development in 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)	This project will study the basic biological control of bone and cartilage development in both health and disease. In particular, it aims to provide an understanding of how genetics and hormones regulate bone growth which is significant for both human and animal development. These fundamental studies have significant implications for the treatment and prevention of human disease, including some increasingly common and significant diseases such as osteoarthritis, osteoporosis, and arterial	

	calcification (hardening of the arteries). In addition there is a less known problem with bone health in individuals with inheritable diseases such as Duchene's muscular dystrophy and metabolic conditions such as chronic kidney disease. Also children who are either on long term steroid treatments, for example for asthma, or experience inflammatory disease such as Crohn's disease also experience bone health problems. In these situations their bone growth can be severely affected and this work will investigate the processes involved with the goal of improving growth in these children. Bone disorders also occur naturally in a range of animal species, such as arthritis in horses, cats and dog; bone degeneration can also occur in livestock, such as bone loss in egg laying hens. Further, companion species, particularly those involved in competitive events (e.g. horse racing), are also affected by trauma and skeletal degeneration, which have financial and more importantly welfare implications. This research programme will increase our understanding of the genes that control bone formation and also help to identify ways to prevent abnormal skeletal formation in animals and man. Our program of work will investigate: ● How switching specific genes on or off affect bone growth and development. ● The effects of chronic inflammation on bone growth and bone mass ● The regulation of energy balance by bone How bone health is affected in human disease e.g. chronic kidney disease and Duchene muscular dystrophy **A retrospective assessment of these aims will be due by 10 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)?	The aim of this project is to determine if the genes/proteins that are responsible for a healthy skeletal development and controlled energy balance represent unique therapeutic targets for the treatment of diseases of great public health concern, e.g., short stature, arthritis, bone loss, and diabetes. Overall, our research aims are to improve the quality of life and mobility of animals and people with bone and joint problems, and associated pathologies.
What species and approximate numbers of animals do you expect to use over what period of time?	Over this duration of this PPL (5 years) we will use a maximum of 11,000 mice; 5000 on mild protocol, 6000 on moderate protocol and 500 on severe protocol. Sample sizes to be used will be based on previous work which was used to estimate the minimum number of rodents required for establishing significant differences between groups.
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 procedures detailed here will have a severity limit of moderate which include animal models of arthritis, inflammatory bowel disease or inflammation. Most mice are assessed as clinically well, however those with inflammatory bowel disease present with weight loss and abnormal stool consistency. A small number of animals will be subjected to severe adverse effects which includes animal models of chronic kidney disease who present with weight loss. In all cases appropriately developed humane early endpoints will endeavour to limit the suffering to the animal. The animal models and protocols to be used here have been developed by us and our colleagues over numerous years to investigate how the skeleton interacts with factors in specific areas of the body and across all tissues as well as environmental factors to form a fully functional organ. All experiments will be performed by appropriately trained technicians and are essential for the success of this project. Animals will be humanely killed at the end of the experiment. **A retrospective assessment of these predicted harms will be due by 10 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	Fundamental *in vitro* (“test-tube”) studies that have used cell and organ culture approaches have been invaluable but are in themselves limiting. As the overall aims of these studies are to mirror what occurs in animals and humans during normal physiological development and chronic disease (e.g. inflammation and arthritis) it is essential to extend these *in vitro* observations to the whole animal. In vitroapproaches have a number of recognised limitations: ● In vitro cell cultures do not represent all in vivo tissues. ● In vitro cell cultures do not represent all in vivo tissues. ● In vitro cell cultures do not represent all in vivo tissues. ● In vitro cell cultures do not represent all in vivo tissues. ● There is a loss of organ interactions between organs; thus indirect effects of agents on bone physiology are not readily detectable in vitro. ● There is a loss of organ interactions between organs; thus indirect effects of agents on bone physiology are not readily detectable in vitro. ● There is a loss of organ interactions between organs; thus indirect effects of agents on bone physiology are not readily detectable in vitro. ● Many tissues in vivo do not change, whereas cells are continuously proliferating. ● Many tissues in vivo do not change, whereas cells are continuously proliferating. ● Many tissues in vivo do not change, whereas cells are continuously proliferating. ● Cell often do not provide a reliable model of in vivo responses to any kind of biological change. This is often because a cell type in culture loses the characteristics it would have in a live animal. ● Cell often do not provide a reliable model of in vivo responses to any kind of biological change. This is often because a cell type in culture loses thecharacteristics it would have in a live animal. ● Cell often do not provide a reliable model of in vivo responses to any kind of

	biological change. This is often because a cell type in culture loses the characteristics it would have in a live animal. Transgenic Mice Removing or increasing the activity of a gene provides information about what that gene normally does in a physiological (whole body) context. This information cannot be obtained from in vitro models where, for example, cell-cell interactions and whole body regulatory pathways are lost. However once information has been obtained from these in vivo models all efforts will be made to minimise the number of mice used by carrying out well designed in vitro experiments using cells from the wild-type and transgenic mice **A retrospective assessment of replacement will be due by 10 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 always aim to reduce the numbers of animals we use. The principals of our experimental design have already been established through our previous work, and that of our close colleagues and collaborators. This therefore allows us to simply undertake the key experiments that will answer our specific questions. Statistical calculations are always used to identify the minimum number of animals that we can use to provide meaningful results. These calculations are based upon our extensive experience in the studies detailed here – for example, we have established in our intestinal inflammation model that a minimum of 6 mice per group is required to secure statistically significant differences. In these studies we will also take repeated measures on the same mice. This and other *in vivo* imaging methods will enable serial data acquisition and remove the need for the humane killing of multiple groups of mice at set time-points. We have many collaborations with other scientists so maximum use is made of animal tissues across many

	related projects. **A retrospective assessment of reduction will be due by by 10 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.	We have chosen to focus on mice. This decision has been made to provide us with the potential to explore the role of specific genes in any biological response we observe, through the use of mutant and transgenic mouse models. We have chosen mice because their basic skeletal biology is very similar to humans and the models to be used here *e.g.* joint loading model, have been developed for, and are widely used in, mice. Furthermore, there is the advantage of available of gene edited mouse models and reagents (antibodies etc). Animal suffering will be limited in our studies by our strict monitoring of severity limits and our use of protocols that do not produce excessive trauma or suffering. Drugs will be administered at non-toxic dosages and if unknown, this will be tested in a carefully graded dose-finding protocol. The alternative strategies which others have used to attain similar end-points frequently involve surgery and our use of surgical approaches will be kept to a minimum. Appropriate pain relief during our protocols will be achieved through appropriate levels of analgesia. **A retrospective assessment of refinement will be due by by 10 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	**61. REDACTED Surgical REDACTED Project**	
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
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
	X	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)	REDACTED, joining together (anastomosing) small blood vessels 0.5 to 2.5mm diameter with the aid of an operating microscope, have transformed reconstructive plastic surgery since the first replantations of severed limbs and digits in the mid 1960s, and the first reported microvascular transfer of tissue from one site in the body to another in a single stage in 1973. These so-called free tissue transfers now allow a relatively reliable method of reconstructing soft tissue and bony defects resulting from trauma, cancer surgery and congenital deformities.	

	The aims of this project are: 1)To provide REDACTED 2)REDACTED 3) REDACTED 4) Address the value of changes in techniques, methods and instrumentation **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)?	REDACTED
What species and approximate numbers of animals do you expect to use over what period of time?	Adult rats, approximately 480 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?	The protocol is for non recovery terminal anaesthesia and with regular monitoring by experienced staff to maintain adequate depth of anaesthesia and fluid replacement no unexpected adverse effects are anticipated **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	REDACTED **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	REDACTED **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.	REDACTED General anaesthesia is used throughout the surgical procedures and is always kept at an adequate level for the animal to remain insentient. The animal is regularly checked by experienced animal care staff who ensure this is the case and administer fluids and anaesthetic top-up's as required. **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	**62. Modelling and therapeutics for 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)	Virus carriers modified to remove all their harmful properties are being used for human clinical trials. Excellent results were obtained in recent human clinical trials using strategies similar to the ones adopted in our team. The same carriers can also be used to make mouse models of human diseases. Our ultimate aim is to develop new treatments for diseases of the brain and the spinal cord (e.g. motor neuron disease, spastic paraplegia). To achieve our ultimate goal our experiments will: 1) develop new models of neurone disease using virus carriers; 2) investigate changes in genes associated with	

	human disease that cause cell (motor neuron) death. This can help identify new treatments; 3) obtain new landmarks of disease progression in our r rodent models in which genes linked to the neurone disease are altered. 4) use virus carrier systems to test new treatements in our newly created animal models. **A retrospective assessment of these aims will be due by 13 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)?	1) The current widely used models have been made using a method called transgenesis, a long process which requires various procedures. This process requires the use of large animal numbers and takes few years to complete. Virus carrier methods would speed up the process and allow reduction of animal use. 2) Animal models will be made when needed. We don't intend to make the models for long term breeding and maintenance. 3) The use of animal models will allow a greater understanding of the molecular and cellular mechanisms by which neurons selectively degenerate and die in in these devastating diseases. 4) Such information will reveal new targets for treatments in which we will test. There are no effective treatments for motor neuron diseases. It is thus important to test new treatments and this involves using cells and animal models of these diseases. By performing such studies, we will be able to determine whether a particular treatment is likely to be useful in humans.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice and rats (mainly mice) Estimated numbers of animals: 12,200 over five 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?	Modelling will be achieved using virus carriers to alter genes in wild type mice. The animals liable to develop neurone disease will be allowed to develop some clinical signs of disease, eg tremor, abnormal walking, partial limb paralysis, but will be killed humanely before the development of complete paralysis of any limb. Our protocols cause the least pain, suffering, distress or lasting harm consistent with achieving our scientific objectives. Some therapeutic studies require the use of protocols of substantial severity. Mice suffering from paralysis we moni-tor them closely and optimize their housing conditions to make them as comfortable as possible. We are actively developing methods to detect very early, subtle markers of disease onset and progression. We hope that these tests will ultimately replace experiments that currently use mice with a significant burden of disease. Modelling protocols are limited to no more than moderate severity. All animals will be killed after completion of each study. **A retrospective assessment of these predicted harms will be due by 13 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 have carefully considered the extent to which these experiments could be replaced by studies involving use of cells in dishes. We test all our vectors in human cells. Only promising virus carriers are then tested in animals. It is important to consider the complexity of the brain and spinal cord and the connections and interactions that cells that innervate muscle make with other cell types in the spinal cord and at the neuromuscular junction (connection between nerve terminal and muscle). It is impossible to replicate this *in vitro*, even using cells collected from embryos (e.g. mouse embryos). We have also considered using zebrafish for our in vivo modelling. However, this was not possible because of the low efficiency of our virus carriers in zerbrafish. **A retrospective assessment of replacement will**

	be due by 13 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 numbers of animals used will be minimised as follows:- i. Our general approach is to test the ideas in *in vitro* systems (cells in dishes) prior to more formal testing in rodents. We plan first to teste the efficiency of our virus carriers in cells in dishes and having observed a positive effect, only then move on to transgenic approaches. ii. By using virus carriers for modelling the disease in rodents we will generate only the number of animals that we need for our studies. This strategy will minimise the numbers of animals by excluding the breeding and keeping large transgenic colonies. iii. Our studies will usually be staged with the aim of obtaining key results on the efficacy of our approach, and in order to perform statistics to determine an appropriate sample size for subsequent investigations. iv. We aim to design experiments that maximise use of animals for data collection. Thus we aim to use the same animals for behavioural/neurological testing, and biochemical and pathological studies where possible. v. We will continuously monitor our experimental results and refine the design of experiments and the number of animals that might be required to provide statistical relevance. Where necessary we will consult biostatisticians REDACTED for confirmation that our approaches use the minimum number of animals necessary. As part of good laboratory practice, we will write a protocol for each experiment including: a statement of the objective(s); a description of the experiment, covering such matters as the experimental treatments, details of the experimental material, and the size of the experiment (number of groups, numbers of animals/group); and an outline of the method of analysis of the results (which may include a sketch of the analysis of variance, an indication of

	the tabular form in which the results will be shown, and some account of the tests of significance to be made and with the treatment differences that are to be estimated). **A retrospective assessment of reduction will be due by by 13 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.	Our protocols cause the least pain, suffering, distress or lasting harm consistent with achieving our scientific objectives and are limited to no more than moderate severity. We wish to use rodents for these experiments as evidence suggests that the genetically altered models (mouse) that have been generated and normal animals are best suited to model the aspects of neurodegenerative disease that we wish to investigate. There are no other suitable vertebrate models that are available to us, which will be suitable for our proposed investigations. Although we have also considered using zebrafish but this option has been dropped because of low efficiency of our virus carriers in this model. Close monitoring will be in place for animals under our studies. We will observe the animals for body weight, morbidity, mortality, injury and intact of food and water supported by close monitoring of body weight. Any animals observed to be in poor health will be identified for further monitoring and possible anticipated study termination. Where any animals show signs of poor health or distress the NACWO and/or NVS will be informed and consulted. We work actively to minimize suffering. We have developed close links with the animal care staff at our facility and we actively involve them in decision-making. We use mice that behave in a very predictable way, allowing us to use fewer. For surgical techniques we will use appropriate anaesthesia and analgesia. **A retrospective assessment of refinement will be due by by 13 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	**63. Models of immunity and autoimmunity**	
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 wish to look at a number of aspects of the immune response in infection and autoimmunity – those diseases caused by attack of the body's own tissues by immune cells, using the mouse as a good model for human immunity. Our questions can be summarised as follows: 1. For a number of clinically important infectious diseases, we want to map which are the critical components of protective immunity. This is both at the level of the microbe – which parts of the microbe (antigens) does the immune response need to attack for most effective, protective immunity? And then, in terms of the actual mechanism of protection: which specific immune cell types (that is, white blood cells)	

	and molecules can be shown to have a true causal role in protective immunity? 2. Where we identify particularly potent microbial antigens, we plan to test them as potential vaccines able to confer protection in models of infection. We are specifically interested in severe bacterial infections. These include a bacterium called Burkholderia – different examples of this cause sepsis in tropical countries and are associated with chronic lung infection in people with lung diseases such as cystic fibrosis. Other bacterial microbes that feature specifically in our models are Pseudomonas (often multi-drug resistant) another cause of chronic. life-threatening, lung infection, and Streptococci. 3. It is clear that the body carries trillions of usually harmless, passenger microbes from the environment, including from the diet. This is referred to as the microbiota. These microbes interact with and shape all aspects of our physiology, with profound impacts on health. We wish to understand more about the precise mechanism of interplay between immunity, whether in infectious or autoimmune disease, and the microbiota. This has profound implications for therapeutic/dietary modulation of disease. 4. For many of the bacterial infections under investigation, the presence of diabetes is a very significant, exacerbating risk factor. The precise reasons for this are unclear. We want to obtain a clearer understanding of the impact of diabetes on normal immunity, particularly in the context of susceptibility to bacterial infection and differences in vaccine efficacy. 5. 'Autoimmune diseases' are those defined as resulting from damage to the body following attack by the immune system itself. Some of our research programmes seek to investigate which cells and molecules mediate this attack, the initial events that allow it to be predicted, and the steps that can be taken to alleviate or treat it. One aspect of this that is currently considered important is impact of the microbiota. Linking with our human studies, we will consider the following examples of autoimmunity: (a) multiple sclerosis, (b) rheumatoid arthritis, (c) lung autoimmunity and (d) the autoimmunity that is observed in

	some cancer patients following a new treatment called 'immune checkpoint inhibition' which enhances anti- tumour immunity by taking the brakes off immune attack, but with sometimes serious collateral damage in unwanted autoimmunity. **A retrospective assessment of these aims will be due by 22 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)?	Our work is advancing rapidly and we hope to make substantive progress towards 'first in man trials' – that is, giving a vaccine to human volunteers to confirm immune stimulation and safety - for vaccines offering protection against some key respiratory infections. We also aim for clinically applicable progress in various aspects of autoimmunity. This includes the role of microbiota species in susceptibility to disease and the potential to generate knowledge of the first signs predicting autoimmune response in cancer patients treated with immune checkpoint inhibitors – the new generation of treatments that work by taking the breaks of tumour immunity.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mice, inbred or genetically altered by inserting or removing specific genes of interest. We estimate that we may use up to 2800 adult mice in these studies over the 5-year period. In order to generate 2800 age and sex matched mice for experimental groups of the exact status needed with respect to altered genes, we will need to breed somewhat larger numbers than this, as well as keeping background stocks of the genetically altered (GA) lines.
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 previous licences doing similar work and techniques we have seen adverse effects extremely rarely. The vast majority of the work is deemed to be within the license categories 'mild' or 'moderate.' Mice may very rarely show an inflammatory response after injection of antigen, the protein of interest that has been given to induce immunity, or become unexpectedly unwell after an injection. Our bacterial inoculation experiments have very precise, limiting humane endpoints before the mice must be culled. As a

	group who work partially on improving understanding of multiple sclerosis, we have provision to do a small number of experiments with the mouse model of MS, termed EAE. This is banded 'severe'. The intention of the protocol is that some of the mice should develop an MS- like paralysis, which typically may range from a limp tail to inability to use the hind legs. Over time we have developed a strict protocol, ensuring that mice are carefully monitored and supported and are maintained in the affected state for the very minimum amount of time compatible with the work. After that, they are culled for extensive analysis of mechanisms underling the disease process. **A retrospective assessment of these predicted harms will be due by 22 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	Much of our research direction is rooted in our attempt to illuminate complex clinical observations made in human patients about multiple immune pathways, cell-types and, indeed, the interactions of the immune system with other systems such as the central nervous system or the hormonal system. Thus, it is inherent in our approach that work is wherever possible done in culture with human cells, but complemented by studies using mouse models. The direct answer to the question "why can this not be achieved without using animals" is thus that a part of our work towards our biomedical research goals necessitates the analysis of interplay between different systems within the body that lead to disease at the level of the whole animal. However, a large part of our work is now achieved by access to *in silico* (meaning, 'by computer') software approaches and, indeed, has been conducted for the purpose of building these artificial intelligence resources. The tools being generated through this approach are already having massive impact on the replacement of animal work on immune mapping by *in silico* prediction. Wherever possible our

	work already makes use of *in silico* predictive algorithms, for example as a means of predicting at the computer which bits of a bug should be tested for ability to induce immunity, rather than having to test all the bits individually in mice. **A retrospective assessment of replacement will be due by 22 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	We only work with inbred mice and design all of our studies for maximised uniformity of treatment groups (matching for age and sex) so as to minimise experimental variability and minimise group size. We take statistical advice on the design of our studies. We have found that, in terms of experiments to understand the specific immune response type of T cells (white blood cells) to antigens, we can achieve statistically significant differences between groups with a sample size of 4-6 mice. We have recently taken steps to reduce numbers for immune studies, using a new mouse strain which 'glows' visibly under a special camera so as to report an immune response non-invasively**.** Thus, immune responses in a single cohort of mice can be followed over time, non-invasively, resulting in substantial reductions in the total number used in any given experiment. **A retrospective assessment of reduction will be due by by 22 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	Our models use inbred, transgenic or knockout mice to investigate immunity in the context either of autoimmune disease or of infection. In both of

regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	these contexts the mouse models can be readily applied in terms of the ability to be bred and housed, with a well-developed arsenal of genomic, molecular and immunological reagents to facilitate analysis to make them a valid model for understanding multi-system interactions in human disease. The details of immune system development and function are sufficiently similar between humans and mice to make the specific pathways and information transferable between the two. We have focused on development of autoimmune disease studies using a model of autoimmunity resulting from removal of the regulatory cells that normally keep it in check. This allows the characterisation of autoimmunity without the need for repeated inflammation-inducing injections. Our experiments aim to minimise the number of days during which any mouse will endure disease symptoms to the absolute minimum that is compatible with analysis of the disease. We describe detailed measures put in place during this period for increased monitoring and support, including special arrangements for feeding. **A retrospective assessment of refinement will be due by by 22 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	**64. Modulating inflammation and immunity**	
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 linings of the gut and lung (epithelium) can be damaged, for instance by drugs (e.g. aspirin), in infections (e.g. food poisoning) and in inflammatory bowel disease. They can then heal, and become healthy again, or damage and disease can continue, potentially leading to cancer. This project sets out to understand how the response of the linings is controlled by messenger molecules used to control the immune response, called fatty acid lipids like prostaglandins. Lipids play critical roles in maintaining healthy function of the linings, and when production of the lipids are suppressed, for example when aspirin is taken, this can lead to damage to the bowel and lung linings. We aim to determine how we could alter the lipid	

	response in various situations, with the ultimate goal of developing new medicines which could alter the lipid response, and enable the damaged linings to return to healthy function. Overall aims include: (1) How do lipids regulate the immune system after infection/immunization? (2) How do lipids control healthy epithelial function and major inflammatory disorders in the lung, skin and gut? (3) How does modulation of lipids contribute to prevention and control of cancer, especially bowel cancer? (4) Do different factors (such as age, diet, microorganisms) interact with the host immune system? Are these interactions involved in controlling the body immune system functions? **A retrospective assessment of these aims will be due by 19 August 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)?	Our research will provide novel understandings of the mechanisms causing inflammatory diseases such as asthma, atopic dermatitis/eczema, irritable bowel disease, bowel cancer, in which the epithelial lining plays an important role. This will help develop strategies to control these diseases and avoid adverse effects of lipid-suppressing drugs such as aspirin, which in turn will improve patient health and quality of life. This will be achieved by focussing on lipid-immune cell interactions and how such interactions are involved during inflammation in the lung, skin and gut. Specifically, this project will provide further understanding of mechanisms by which drugs such as aspirin help treat various inflammatory conditions (e.g. arthritis) but also cause dangerous side-effects in the guts and lungs. On a wider scale, this project will also benefit patients suffering from adverse effects of drugs such as aspirin, as well as clinicians, pharmaceutical

	industries, and the general public, in context of novel strategies for development of new drugs as alternatives aspirin.
What species and approximate numbers of animals do you expect to use over what period of time?	Over the next 5 years, 15000 genetically altered (GA) mice will be bred (Protocol 1) and 8,500 mice will be used in procedures proposed in this project. Both wild type and GA mice will be used in experiments proposed in 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?	The primary adverse effects on the mice in this research programme are the development of tissue inflammation and intestinal tumours. This work will use well-established models for breeding of genetically altered mice, modulation of gene expression, infection/host immunity, lung/skin inflammation will cause mild-to-moderate adverse effects in mice, such as moderate weight loss (up to 20%), transient breathless-ness and lethargy. While most mice will be tolerant, the use of CFA during immunisation may lead to granuloma formation and recurrent or persistent ulceration with accompanying pain. In the lung inflammation models, some mice may lose weight (up to 20%) and condition, and have moderate piloerection, hunched posture and reduced activity. Skin inflammation models (atopic dermatitis and psoriasis) may cause localised inflammation but without systemic symptoms. The initial adverse effects of the models for intestinal inflammation and cancer in the majority of mice will be weight loss (up to 25%), clinical signs such as blood in faeces and diarrhoea, and altered general appearance (piloerection, lethargy). All of the animals will be housed in a modern animal care facility and will be monitored daily for signs of illness due to intestinal inflammation or tumour formation. Thus, the expected adverse effects due to inflammation and tumours will be kept to a minimum, mostly at the mild-to-moderate level of severity (although occasionally severe). At the end of the studies the mice will be humanely killed and dissected to analyse inflammation development and tumour formation and progression. The work of intestinal cancer will be performed in accordance with the principles in the Guidelines for the Welfare and Use of Animals in Cancer Research: British Journal of Cancer (2010) 102, 1555-1577. **A retrospective assessment of these predicted harms will be due by 19 August**

	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	This project involves a range of mouse model systems of pathology including inflammation and cancer, both highly complex processes involving cell-cell and cell-matrix interactions, plus accumulation of multiple genetic abnormalities in a sequential manner. Therefore, it is impossible to reconstruct these processes using *in vitro* assays (studies performed on cells/tissues/other biological material outside the host body). Furthermore, there remains major clinical demand for novel treatment strategies of established epithelial inflammatory diseases (e.g. asthma). **A retrospective assessment of replacement will be due by 19 August 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	A statistical computer programme will be used to determine mouse group sizes for each experiment, and to optimise our experimental design to minimise the number of mice/groups needed to achieve statistical significance. By pooling data across multiple experiments for statistical analysis, we will be able to increase the statistical power and hence, reduce total animal numbers used for experiments. Where possible, we will import existing mouse strains rather than requesting generation of new GA mice. In some circumstances, we may use both *in vitro* studies and small pilot experiments before determining final experimental design. To reduce the variability of data and total number of animals required, we will carry out most (preferably all) experiments using individually ventilated cages to protect the health status of each animal. We will terminate breeding lines

	when experiments on such mice have completed. **A retrospective assessment of reduction will be due by by 19 August 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 use of GA mouse models to represent and re-create human inflammation/cancer are well-established technologies that have had considerable success. Of present, there is already in existence several mutant strains of mice directly relevant to our proposed experiments. We aim to monitor animals using scoring systems to detect early clinical endpoints of tissue inflammation/tumour formation. This approach will be used to determine the early stages of inflammation and tumour formation so that the expected disease severity can be minimised. Strict humane endpoints will be used in all experimental procedures to ensure no unnecessary suffering is endured. **A retrospective assessment of refinement will be due by by 19 August 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	**65. Modulating the ERK1/2 cascade in the heart**	
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 diseases and cancer are leading causes of death and illness. Our research focuses on enzymes in a pathway that regulate proteins called ERK1/2. This pathway protects the heart from damage and causes contractile heart cells to grow, enabling the heart to increase its ability to pump blood around the body in "healthy" situations (e.g. regular exercise) or in disease (e.g. high blood pressure). However, the pathway also increases scar tissue in the heart. Moreover, it causes cancer, and drugs targeting the pathway are used to treat cancer. We aim to determine how the ERK1/2 pathway is regulated in the heart. We also aim to determine how cancer drugs inhibiting the pathway affect healthy or dysfunctional hearts.	

	A retrospective assessment of these aims will be due by 05 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 data from this study will increase understanding of the regulation/role of a key pathway (the ERK1/2 pathway) in the heart, identifying the inputs into the network and establishing how the outputs help the contractile cells to grow and survive. This will assist in finding ways to manipulate the pathway to protect the heart (e.g. in patients with high blood pressure) or promote "healthy" growth (as in exercise) and reduce development of heart failure. Because the pathway is a target for cancer drugs that are toxic to the heart, the study will also aid in understanding how such drugs cause the heart to fail. This may help to identify patients who should not be treated with these drugs but receive alternative therapies. We may also be able to identify ways to treat cancer but avoid toxic effects on the heart.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use 360 rats and 6,020 mice 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?	Some animals will be subjected only to terminal anaesthesia administered via injection with removal of the heart immediately prior to death (i.e. non-recovery). We will breed genetically-altered mice with potential for heart problems. As far as possible, we will use systems to ensure that the highest level of severity is mild prior to experiments being initiated. All mice will be closely monitored for signs of ill health, and mice that become seriously ill will be humanely killed immediately. Ultrasound will be used to monitor the hearts of live animals (as in patients) over time. Mice will have hair removed from their chest (by shaving and with hair removal cream) and they will be under anaesthetic for the procedure. Mice will be given drugs that induce changes in the heart, and/or drugs used for cancer. Drugs will be delivered by a device implanted under the skin in a minor surgical procedure. We will study the effects of drugs affecting the ERK1/2 pathway at different stages of heart failure. Most animals will only develop mild heart failure, but (as in humans) there is a small,
	unpredictable risk of sudden death. Sometimes, we may need to assess the effects in heart failure models. These have increased risk of sudden death and morbidity, and can be severe. Suffering will be minimised by monitoring disease with ultrasound and humanely killing animals when disease becomes severe. We aim to restrict the number of mice categorised as severe to <20%. At the end of the experiments all animals will be killed. **A retrospective assessment of these predicted harms will be due by 05 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	Contractile cells of the heart (cardiomyocytes) do not divide and there are no cell lines that are representative of these cells. It is therefore necessary to use animals for their study. For studies of cardiomyocyte function within the intact heart, there are no non-animal alternatives. **A retrospective assessment of replacement will be due by 05 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	For cardiomyocytes, conditions are used to produce the greatest yield. The data from the cells are used to inform experiments with adult hearts. When appropriate, a professional statistician(s) will be consulted to ensure optimal experimental design to minimise animal numbers but ensure adequate precision and power, with the appropriate statistical analysis. In all cases, the minimum number of experiments will be performed to detect meaningful differences in responses, if they occur, at an appropriate level of statistical significance. We will breed genetically altered mice. Where possible, mice will be obtained from a supplier. Efficient management will ensure that we only produce enough mice for healthy maintenance and for the actual experiments. *In vivo* studies will require pilot studies to determine the numbers of animals required. These will be informed by published data where possible. Otherwise, they will be informed by data from cultured cells and *ex vivo* perfused hearts. The pilot studies will be initiated with small numbers of animals. Power calculations
	will be performed using pilot and existing data to determine the minimum numbers to detect meaningful differences in responses, if they occur, at an appropriate level of statistical significance. **A retrospective assessment of reduction will**

	be due by by 05 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.	Rats will generally be used for cells and heart perfusions because the heart size and numbers of cells means we will use substantially fewer animals. We have a large body of data on ERK1/2 signalling in this species. Mice will be used for *in vivo* studies because of the availability of genetically-altered models and the body of research already conducted with mice. Score sheets will be used to monitor health and well-being. If the humane endpoint is reached, the mouse will be killed. We will use genetically-altered mice and, where possible, we will use pre-existing lines, with preference for inducible and/or cardiospecific systems for gene expression/deletion. This will avoid confounding effects of, for example, kidney dysfunction or developmental defects. Echocardiography will be used to monitor cardiac function longitudinally in individual animals throughout the course of *in vivo* experiments. This reduces the number of animals required, brings the end-point of the study forward (restricting heart failure development), and improves the quality of the data. **A retrospective assessment of refinement will be due by by 05 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?